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Estrogen receptor 1 gene polymorphisms are associated with metabolic syndrome in postmenopausal women in China

Lingxia Zhao^{1,2}, Xuemei Fan^{1,2}, Lin Zuo³, Qiang Guo⁴, Xiaole Su⁵, Guangxia Xi², Ziyang Zhang¹, Jianlin Zhang¹ and Guoping Zheng^{1,6*}

Abstract

Background: Metabolic syndrome (MetS) includes obesity, diabetes, dyslipidemia and hypertension. Its incidence is rapidly increasing worldwide, particularly in postmenopausal women. Estrogens regulate glucose homeostasis and lipid metabolism via estrogen receptors 1 (ESR1) and 2 (ESR2). The current study aimed to elucidate associations of MetS with ESR1 and ESR2 gene polymorphisms in postmenopausal Chinese women.

Methods: This case-control study included 304 postmenopausal women (154 and 150 control and MetS patients, respectively). Clinical indicators related to MetS were assessed. Two ESR1 (PvuII and XbaI) and two ESR2 (RsaI and AluI) polymorphisms were evaluated by polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis.

Results: ESR1 polymorphisms were significantly different between MetS patients and healthy controls. G allele frequency for the XbaI polymorphism was significantly higher in patients than in control patients ($p = 0.004$, OR = 1.610, 95%CI 1.169–2.18). The haplotypes A–T ($p = 0.015$) and G–C ($p = 0.024$) showed significant differences. The minor alleles of the XbaI and PvuII gene polymorphisms in both homozygous and heterozygous forms showed associations with elevated waist circumference, fasting serum insulin and HOMA-IR. The minor G allele in homozygous and heterozygous forms of the RsaI and AluI gene polymorphisms showed associations with elevated total cholesterol and LDL-C.

Conclusions: In postmenopausal Chinese women, ESR1 polymorphism and the haplotypes A–T and G–C of XbaI–PvuII are associated with MetS, unlike ESR2 polymorphisms. Patients harboring the G allele of XbaI have elevated BMI, waist circumference, systolic and diastolic BP, FBG, HOMA-IR, total cholesterol, TG, LDL-C and NAFLD (%), and reduced HDL-C.

Keywords: Metabolic syndrome, Postmenopausal women, Estrogen receptors 1 and 2, Gene polymorphisms

Background

Metabolic syndrome (MetS) constitutes a threat to human health, with estimated prevalence rates of 17–40 and 16.3% in the Middle East and North Africa, respectively [1]. Its prevalence has also increased from 23.8% between 2000 and 2005, to 27% between 2010 and 2015 in China

[2]. MetS increases the risk of coronary artery disease, stroke, fatty liver disease, diabetes mellitus (DM), insomnia, and several cancers [3, 4]. Adult Treatment Panel III (ATP III) proposed that MetS encompasses at least three of the following ailments: abdominal obesity, high triglyceride (TG) levels, low high-density lipoprotein cholesterol (HDL-C) amounts, high blood pressure (BP), and high fasting blood glucose (FBG) [5]. All MetS variations increase the risk of cardiovascular disease (CVD). CVD incidence and mortality are significantly reduced in premenopausal women compared with men, but this difference

* Correspondence: zhengguoping2@126.com

¹Department of Biochemistry and Molecular Biology, Shanxi Medical University, Taiyuan, No.56, Xinjian South Road, Taiyuan, Shanxi 030001, People's Republic of China

⁶Centre for Transplantation and Renal Research, University of Sydney at Westmead Millennium Institute, Sydney, NSW 2145, Australia

Full list of author information is available at the end of the article



gradually diminishes after menopause [6], with a corresponding increase in MetS incidence [7].

Hormones have critical functions in MetS pathogenesis and progression. Estrogen is associated with many aspects of MetS [8] such as glucose tolerance, fat metabolism, and BP [9]. Estrogen exerts its physiological functions in combination with estrogen receptors (ERs). ERs are nuclear hormone receptors (transcription factors) found in macrophages, adipose cells, vascular smooth muscle cells, and vascular endothelial cells [10, 11]. Estrogen receptor- α (ESR1) and β (ESR2) represent two known estrogen receptors that have been assessed using transgenic ER α (ER α ^{-/-}) and/or ER β (ER β ^{-/-}) knockout mice [12, 13].

The subtypes of ER, ESR1, and ESR2 are distinct in structure and function (likely in carbohydrate and lipid metabolism) [14]. ESR1 and ESR2 are found on chromosomes 6q25.1 and 14q23.2, respectively [15]. Single nucleotide polymorphisms (SNPs) in ESR1, such as PvuII (rs2234693) and XbaI (rs9340799), are involved in MetS [16]. As for ESR2, the risk of MetS is determined by the RsaI (1082A > G) (rs1256049) and AluI (1730A > G) (rs4986938) polymorphisms [17]. ESR1 and ESR2 SNPs are associated with metabolic factors in Western populations [18]. Here we assessed associations of four representative ESR1 and ESR2 polymorphisms with MetS and related phenotypes in postmenopausal Han Chinese women.

Methods

Study population and laboratory measurements

This case control study was performed between January and November 2016 at the medical center of Shanxi Dayi Hospital (Taiyuan, China). Three hundred and four postmenopausal women aged 46–71 years were enrolled. Inclusion criteria were: ≥ 60 years old; permanent menstrual period discontinuation because of natural menopause.

At baseline, the subjects were assigned to 2 groups, including the control (154 healthy individuals; mean age of 63.0 ± 4.1 years) and MetS (150 MetS patients; mean age of 62.9 ± 4.8 years) groups. All subjects were Han Chinese and of the same ethnic group. All cases were diagnosed according to the Chinese type 2 diabetes Prevention Guide (2013) diagnostic guidelines [19]. MetS was reflected by ≥ 3 of the following parameters: waist circumference ≥ 85 cm; FBG ≥ 6.1 mM or 2 h post glucose-load blood glucose ≥ 7.8 mM or known diabetes; serum TG ≥ 1.70 mM; HDL-C < 1.04 mM; and BP $\geq 130/85$ mmHg or treated hypertension. Non-alcoholic fatty liver disease (NAFLD) diagnosis was performed by abdominal ultrasonography, as described previously [20]. Exclusion criteria were: severe psychological ailments, physical disability, any type of malignancy, or history of smoking, heart failure, myocardial infarction (MI), type I diabetes, kidney failure, and

chronic liver disease. The subjects were not undergoing hormone replacement.

Data collection

The main measurements and clinical data of the participants, including age, height, weight, body mass index (BMI), waist circumference, and systolic and diastolic BPs, were measured and recorded by specifically trained individuals. Waist circumference measurement was carried out in the horizontal plane at the midpoint between the lower border of ribs and the iliac crest. Systolic and diastolic BPs were obtained in right upper arm twice following a 15 min-rest with the patient sitting. Measurements were performed at 5 min intervals and averaged.

FBG, hemoglobin A1C (HbA1C), TG, total cholesterol (TC), HDL-C, low density lipoprotein (LDL-C; direct measurement), estradiol (E2), and fasting serum insulin levels in all participants were measured from fasting blood samples on an AutoAnalyzer (AU5400, Olympus, Tokyo, Japan), with reagents and calibrators provided by the manufacturer. Abdominal ultrasonography was conducted by specialized radiologists based on unified NAFLD diagnostic criteria. Insulin resistance (homeostasis model assessment of insulin resistance, HOMA-IR) was determined for each subject.

This study followed the Declaration of Helsinki, as well as institutional regulations regarding human subjects. Written informed consent was provided by all participants. The study protocol had approval from the ethics committee of the Shanxi Da Yi Hospital Institute for Biological Sciences, Chinese Academy of Sciences.

Genomic DNA extraction and genotyping

Venous blood (2 ml) specimens were obtained in EDTA anticoagulation tubes and used for DNA extraction. Genomic DNA extraction from 200 μ L whole blood was carried out with the TIANamp Blood DNA Kit (Tiangen Biotech, China), as instructed by the manufacturer.

The PvuII (c454-397 T > C) and XbaI (c454-351A > G) polymorphisms in ESR1 as well as the RsaI (1082A > G) and AluI (1730A > G) polymorphisms in ESR2 were assessed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). A 1374 base pair (bp) fragment was amplified for the PvuII (c454-397 T > C) and XbaI (c454-351A > G) polymorphisms using the following primer pair: upstream, 5'-CTGCCACCCTATCTGTATCTTTCTATTCTCC-3'; downstream, 5'-TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA-3'. A 476-bp fragment for the RsaI (1082A > G) polymorphism was amplified using the following primer pair: upstream, 5'-TTCTGAGCCGAGGTCGTAGT-3'; downstream, 5'-CCAGGACTTTGTTCCCACTC-3'. A 391-bp fragment for the AluI (1730A > G) polymorphism was amplified with the following primer pair: upstream, 5'-GCACCTTTTGTCC

CCATAGTAAC-3'; downstream, 5'-CTATGGCTTCC TCACACCGAC-3'. PCR was carried out on an ABI Prism 7500 sequence system (Applied Biosystems, USA) in 25 μ l reactions, containing 5 μ l genomic DNA, 10 μ l of Taq PCR Master mix (2 \times), 2 μ l of each primer, and 8 μ l PCR buffer (Tiangen Biotech, China), as follows: 94 °C (4 min); 35 cycles of 94 °C (30 s), 57 °C (40 s) and 72 °C (40 s); 72 °C (7 min). Digestion of PCR products was performed overnight using XbaI (expected fragments of 1374, 393, and 981 bp), PvuII (expected fragments of 1374, 438, and 936 bp), RsaI (expected fragments of 476, 183, and 293 bp), and AluI (expected fragments of 391, 144, and 247 bp) (Biolabs, New England) at 37 °C. All the digested fragments were electrophoresed on 2% agarose gels containing Goldview. The genotypes of 10% specimens were verified by direct sequencing.

Statistical analysis

SPSS 20.0 was employed for statistical analyses. Normally distributed continuous data are mean \pm standard deviation (SD). Genotype differences were assessed in MetS patients by analysis of variance. Baseline features between the MetS and control groups were evaluated by Student *t*-test. Comparisons among multiple groups were performed by ANOVA with Bonferroni correction. Hardy–Weinberg equilibrium test, allele genotype frequency assessment, and haplotype evaluation were carried out online with the SHEsis platform. Genotype frequency, allele frequency, and haplotype were analyzed by the chi-square test and

odds ratio (OR) assessment. Multivariable logistic regression was performed to adjust ORs for possible confounders. $p < 0.05$ indicated statistical significance. The function `pwr.chisq.test()` in the R package was used to calculate statistical power.

Results

Demographic and clinical variables

The MetS and control groups included postmenopausal women, with similar ages in both groups. All clinical parameters in Table 1 showed statistically significant differences between the MetS and control groups. Elevated BMI values and waist circumferences were observed in MetS patients, who also showed higher mean systolic and diastolic BPs compared with controls. In addition, the MetS group had markedly higher FBG, hemoglobin A1c, fasting serum insulin and HOMA-IR levels, and worse lipid profile. Furthermore, prevalence of nonalcoholic fatty liver disease was far higher in MetS patients compared with controls.

Allele frequencies and genotype distribution

Genotype distribution for each of the four SNPs was compatible with the Hardy–Weinberg equilibrium ($p > 0.05$) in MetS and control patients. The Chi-square test of XbaI genotype's frequencies in the case and control groups can be used to calculate statistical power. The test power was calculated (power = 1), indicating that the allele frequencies and genotype distributions of MetS patients and controls (Table 2) led to credible conclusions.

Table 1 Patient clinico-biochemical features

Variables	Metabolic syndrome ($n = 150$)	No metabolic syndrome ($n = 154$)	P
Age (years)	63.0 \pm 4.1	62.9 \pm 4.8	0.751
BMI (kg/m ²)	25.4 \pm 2.9	22.5 \pm 3.0	<0.001
Waist circumference (cm)	88.3 \pm 5.2	81.9 \pm 2.5	0.001
FBG (mM)	7.1 \pm 1.4	5.2 \pm 0.7	<0.001
HbA1C (%)	6.7 \pm 0.9	5.2 \pm 0.4	0.003
Systolic blood pressure (mmHg)	148.1 \pm 6.9	122.1 \pm 4.9	<0.001
Diastolic blood pressure (mmHg)	92.3 \pm 4.5	71.8 \pm 5.7	0.002
Triglycerides (mM)	2.4 \pm 1.1	1.8 \pm 1.2	<0.001
Total cholesterol (mM)	5.1 \pm 1.0	4.6 \pm 1.1	<0.001
HDL-C (mM)	1.0 \pm 0.1	1.3 \pm 0.1	<0.001
LDL-C (mM)	3.1 \pm 0.9	2.8 \pm 0.6	0.008
NAFLD (%)	110(73.3)	33 (21.4)	<0.001
Fasting serum insulin (μ IU/mL)	14.3 \pm 7.2	9.2 \pm 5.0	<0.001
Estradiol (pg/ml)	23.2 \pm 7.2	27.0 \pm 7.2	<0.001
HOMA-IR	3.5 \pm 1.8	2.1 \pm 1.3	<0.001

Abbreviations BMI body mass index, FBG fasting blood glucose, HbA1C hemoglobin A1C, HDL high density lipoprotein, LDL low-density lipoprotein, NAFLD nonalcoholic fatty liver disease

Data are mean \pm SD. Comparisons were performed by *t*-test
 $p < 0.05$ indicates a significant difference from the normal control

Table 2 Allele frequencies and genotype distributions of ESR1 and ESR2 gene polymorphisms in the metabolic syndrome (MetS) and control groups

Variables	MetS n (%) (n = 150)	Control n (%) (n = 154)	P	OR(95% CI) ^a
ESR1				
XbaI (A > G)				
A allele	131 (43.7)	171 (55.5)		
G allele	169 (56.3)	137 (44.5)	0.004	1.610 (1.169, 2.18)
1 (AA)	29 (19.3)	45 (29.2)		
2 (AG)	73 (48.7)	81 (52.6)	0.894	1.044 (0.556, 1.960)
3 (GG)	48 (32.0)	28 (18.2)	0.109	2.660 (0.874, 3.829)
PvuII (T > C)				
T allele	143 (47.7)	157 (52.3)		
C allele	158 (51.3)	150 (48.7)	0.223	1.219 (0.887, 1.676)
1 (TT)	31 (20.7)	38 (24.7)		
2 (TC)	79 (52.7)	84 (54.5)	0.012	2.440 (1.216, 4.897)
3 (CC)	40 (26.7)	32 (20.8)	0.038	2.288 (1.045, 5.009)
ESR2				
RsaI (B5) (1082A > G)				
A allele	101 (33.7)	99 (32.1)		
G allele	199 (66.3)	209 (67.9)	0.689	1.071 (0.764, 1.503)
1 (AA)	20 (13.3)	18 (11.7)		
2 (AG)	61 (40.7)	63 (40.9)	0.863	1.074 (0.476, 2.426)
3 (GG)	69 (46.0)	73 (47.4)	0.834	0.851 (0.412, 2.044)
AluI (B8) (1730A > G)				
A allele	100 (33.3)	96 (31.2)		
G allele	200 (66.7)	212 (68.8)	0.568	1.104 (0.786, 1.552)
1 (AA)	21 (14.0)	20 (13.0)		
2 (AG)	58 (38.7)	56 (36.4)	0.225	0.592 (0.254, 1.382)
3 (GG)	71 (47.3)	78 (50.6)	0.172	0.567 (0.251, 1.281)

Abbreviations: CI confidence interval, OR odds ratio, NA not available

OR OR value of rectified BMI and waist circumference for every allele

^a95% of the 95% CI for the OR CI

p < 0.05 indicated statistical significance

G allele frequency in the XbaI polymorphism was markedly elevated in MetS patients compared with control individuals (*p* = 0.004; OR = 1.610, 95%CI 1.169–2.18).

Genotype distributions of the XbaI, PvuII, RsaI and AluI polymorphisms, and allele frequencies of the PvuII, RsaI and AluI polymorphisms were comparable between MetS patients and controls (*p* > 0.05).

Haplotype distributions of the four SNPs

LD assessment of all SNP pairs revealed XbaI and PvuII of ESR1 were associated, as well as RsaI (B5) and AluI (B8) of ESR2 (Additional file 1: Table S1). Haplotype distributions of the 4 SNPs (PvuII, XbaI, RsaI and AluI) are shown in Table 3. The exclusion criteria was set as frequency < 0.03.

ESR1 and ESR2 haplotype frequency distributions were greater than 3%. Analysis showed that XbaI–PvuII based haplotypes were associated with MetS (global *p* = 0.022), while those constructed from RsaI–AluI had no significant associations with MetS (*p* > 0.05). The haplotypes A–T (*p* = 0.015; OR = 0.638, 95%CI 0.443–0.919) and G–C (*p* = 0.024; OR = 1.512, 95%CI 1.055–2.168) obtained from XbaI–PvuII showed significant differences.

Associations of genotypes with clinical characteristics

Associations of the XbaI polymorphism with MetS components are shown in Table 4. Patients harboring the G allele in both homozygous and heterozygous forms (i.e. GG or AG) had elevated BMI, waist circumference,

Table 3 Estimated haplotype distributions in the metabolic syndrome (MetS) and control groups

SNP	Haplotype	MetS (freq.)	Control (freq.)	Global <i>p</i> -value	<i>p</i> -value
ESR1	A-C	65.15 (0.217)	76.73 (0.249)	0.022	0.351
XbaI-PvuII	A-T	65.85 (0.220)	94.27 (0.306)		0.015
	G-C	93.85 (0.313)	71.27 (0.231)		0.024
	G-T	75.15 (0.250)	65.73 (0.213)		0.279
ESR2	A-A	37.99 (0.127)	25.20 (0.082)	0.271	0.070
1082A > G-1730A > G	A-G	63.01 (0.210)	73.8 (0.240)		0.383
	G-A	62.01 (0.207)	70.8 (0.230)		0.489
	G-G	136.99 (0.457)	138.20 (0.44)		0.845

Abbreviation: SNP single-nucleotide polymorphism

Haplotypes have frequencies $\geq 3\%$ in the MetS and control groups

$p < 0.05$ indicated statistical significance

systolic and diastolic BPs, FBG, HOMA-IR, TC, TG, LDL-C and NAFLD (%), and lower HDL-C. Among subjects with the PvuII polymorphism, those harboring the minor C allele in both homozygous and heterozygous forms (i.e. CC or TC) showed elevated BMI, fasting serum insulin and HOMA-IR (Additional file 1: Table S5). Associations of XbaI, PvuII and MetS were confirmed by multivariable regression analysis (Table 5). Among subjects with the RsaI polymorphism, those harboring the minor G allele in both homozygous and heterozygous forms showed elevated TG, LDL-C, fasting serum insulin and HOMA-IR (Additional file 1: Table S3). Among subjects with the AluI polymorphism, those carrying the minor G allele in both homozygous and

heterozygous forms (AG or GG) showed elevated TC and LDL-C (Additional file 1: Table S4).

Regarding XbaI, the control group showed significantly different FBG, TG, TC, HDL-C and LDL-C among the three genotypes (all $p < 0.05$). In MetS patients, BMI, TG, TC and LDL-C were markedly different (Additional file 1: Table S2). As for RsaI (B5), the control group showed significant differences in fasting insulin and HOMA-IR among the three genotypes (all $p < 0.05$); in MetS patients, LDL-C and HOMA-IR were significantly different (Additional file 1: Table S3). As for AluI (B8), BMI and TC significantly differed among the three genotypes in control patients (all $p < 0.05$); in MetS patients, age and TG significantly differed (Additional file 1: Table S4). Regarding

Table 4 Associations of XbaI genotypes with various clinical characteristics

Variables	AA (n = 74)	AG (n = 154)	GG (n = 76)
Age (years)	62.14 ± 4.24	63.24 ± 4.85	63.11 ± 3.75
BMI (kg/m ²)	22.97 ± 2.99	23.98 ± 3.40*	24.71 ± 3.18*
Waist circumference (cm)	84.26 ± 5.36	84.92 ± 5.13	86.07 ± 4.96*
FBG (mM)	4.95 ± 0.88	5.21 ± 1.41*	5.70 ± 1.10*#
HbA1C (%)	5.89 ± 1.00	5.94 ± 1.05	6.09 ± 0.98
Systolic blood pressure (mmHg)	132.31 ± 14.39	134.15 ± 13.96	139.05 ± 14.12*#
Diastolic blood pressure (mmHg)	79.95 ± 11.96	81.14 ± 11.39	85.86 ± 10.69*#
Triglycerides (mM)	1.84 ± 0.92	2.08 ± 1.20*	2.38 ± 1.16*
Total cholesterol (mM)	4.29 ± 1.20	4.72 ± 0.83*	5.53 ± 1.12*#
HDL-C (mM)	1.25 ± 0.26	1.15 ± 0.21*	1.06 ± 0.20*#
LDL-C (mM)	2.74 ± 0.64	2.83 ± 0.67	3.39 ± 0.83*#
NAFLD (%)	28 (37.8)	70 (45.5)	45 (59.2)*
Fasting serum insulin (μIU/mL)	11.15 ± 5.68	11.54 ± 6.23	12.54 ± 8.31
Estradiol (pg/ml)	26.82 ± 6.99	24.66 ± 7.48	24.49 ± 7.67
HOMA-IR	2.47 ± 1.32	2.72 ± 1.77*	3.20 ± 1.70*

Measurement data are mean ± SD, and were assessed by one way ANOVA with post-hoc SNK test

*Significant difference from patients harboring AA ($p < 0.05$)

#Significant difference from patients harboring AG ($p < 0.05$). Enumeration count data were described as rate, and assessed by the chi-square test

Table 5 Multivariable logistic regression analysis with MetS as the dependent variable

Risk factor	OR (95%CI)	P
BMI	6.541 (2.537,16.864)	< 0.001
Triglycerides	2.386 (1.238,4.599)	0.009
XbaI		
AA (reference)	–	–
AG	1.505 (0.819,2.764)	0.188
GG	2.330 (1.161,4.679)	0.017
PvuII		
TT (reference)	–	–
TC	2.235 (1.120,4.461)	0.023
CC	2.679 (1.225,5.855)	0.014

Abbreviations: BMI body mass index, CI confidence interval, OR odds ratio
 $p < 0.05$ indicated statistical significance

PvuII, estradiol levels were significantly different among the three genotypes in control patients (all $p < 0.05$); in MetS patients, age, BMI, systolic BP, HDL-C and HOMA-IR were significantly different (Additional file 1: Table S5).

Discussion

Changing lifestyles, socioeconomic status, and dietary habits have likely contributed to a rapid increase in the incidence of MetS, which is currently considered a major threat to human health worldwide [21]. High MetS prevalence is found among postmenopausal women [22]. Associations of several polymorphisms in the ESR1 and ESR2 genes with MetS or related components have been described [23]. These associations were also reported in the Study of Women's Health Across the Nation (SWAN) trial of perimenopausal African-American, Caucasian, Chinese, and Japanese women between 42 and 52 years old [24]. The current study revealed positive associations of ESR1 polymorphisms with MetS in postmenopausal Chinese Han women. Subjects harboring the G allele of XbaI, or the A-T and G-C haplotypes of XbaI–PvuII polymorphisms of ESR1, showed elevated risk of MetS. Similar results were obtained in studies of women of different ages [25, 26]. Two ESR2 subtypes, including RsaI and AluI, were not significantly associated with MetS in this study.

ERb^{-/-} mice show normal or improved glucose tolerance and insulin release compared with wild type counterparts [27]. Meanwhile, ERa^{-/-} animals display glucose intolerance and insulin resistance [12]. ESR1 and ESR2 are widely distributed in various organs and tissues related to sugar metabolism. Associations of ESR1 polymorphisms and (T2DM) were demonstrated [28]. ESR2 polymorphisms are associated with MetS in Chinese and Japanese populations; in addition, SNPs in the ESR1 show associations with insulin sensitivity in Asian

women [24]. ER-α can exert co-modulatory functions with insulin receptor substrate 1 (IRS1) to modulate insulin signaling [29]. Previous studies suggested an inhibitory effect of ERb on glucose transporter four (GLUT4) expression, and revealed that unchecked ERb activity could result in diabetes [25, 30]. As shown above, patients harboring the minor alleles of XbaI and PvuII gene polymorphisms, in both homozygous (GG and CC) or heterozygous (AG and TC) forms, had higher FBG, fasting serum insulin and HOMA-IR. Meanwhile, those carrying the RsaI polymorphism of ESR2 (homozygous or heterozygous) had lower fasting serum insulin and HOMA-IR.

Estrogens have critical functions in lipoprotein metabolism. An imbalance between ESR1 and ESR2 in the adipose tissue could therefore affect the development of metabolic diseases. Epidemiological studies showed hyperlipidemia is a significant risk factor for atherosclerosis, stroke, CAD, and MI [31]. In mice, ERb absence inhibit triglyceride accumulation, maintains normal insulin signaling in the liver and skeletal muscle, and ameliorates whole body insulin sensitivity as well as glucose tolerance [32]. ESR1 polymorphisms are associated with LDL particle size, reduced LDL cholesterol, and TC in adolescent female individuals [33]. SNPs in ESR1 show associations with the degree of adiposity, BMI, and waist circumference [34]. The PvuII T allele of ESR1 and associated genotypes and haplotypes are associated with risk of hyperlipidemia in postmenopausal Chinese Han females [35]. In addition, women with the PvuII T allele show elevated amounts of small LDL particles [36]. Furthermore, postmenopausal women with the T allele have reduced HDL-C and elevated TG, and are more vulnerable to lipid metabolism disorders [37]. These findings were corroborated by several other reports showing associations of ESR1 polymorphisms with BMI; however, associations of ESR2 polymorphisms with hyperlipidemia remain unclear. A study showed that homozygotes for the AluI (rs4986938) of ESR2 have higher BMI, serum TG and apolipoprotein B, and reduced HDL-C [38]. In mice, ESR2 deficiency is associated with abnormal vascular function and hypertension [39]. ESR2 polymorphisms are also associated with hypertension in postmenopausal Japanese women [40]. As shown above, positive associations were obtained of the G allele of XbaI polymorphism of the ESR1 gene with elevated TG, TC, LDL-C levels, as well as lower HDL-C, in postmenopausal Chinese women. The RsaI and AluI polymorphisms of ESR2 exhibited associations with TG, TC and LDL-C amounts.

Conclusion

Currently, MetS incidence is higher in postmenopausal women compared with age—matched males. Interventions

to halt the rapidly increasing MetS incidence are of great clinical interest. Several studies have assessed associations of ESR1 and ESR2 with MetS or related components in different populations, but have often yielded conflicting findings. Furthermore, very few such studies have evaluated Chinese subjects. We here demonstrated associations of ESR1 and ESR2 polymorphisms with MetS and related components, especially obesity, and lipid and glucose metabolism, in postmenopausal Chinese Han females. Meanwhile, the G allele frequency of the XbaI polymorphism was markedly elevated in MetS patients compared with the control group. The RsaI and AluI polymorphisms of the ESR2 gene were not associated with MetS. In addition, XbaI and PvuII polymorphisms of ESR1 were associated with some MetS components. The current findings could include biases related to sample size, age, and ethnicity. Nevertheless, the present data could provide valuable insights into MetS epidemiology, particularly in Han Chinese individuals, providing a basis for the development of novel therapeutic interventions.

Additional files

Additional file 1: Table S1. Linkage disequilibrium tests (D' and r^2). **Table S2.** Relationship between XbaI genotypes and clinical indicators within each group. **Table S3.** Relationship between RsaI (B5) genotypes and clinical indicators in each group. **Table S4.** Relationship between AluI (B8) genotypes and clinical indicators of each group. **Table S5.** Relationship between PvuII genotypes and clinical indicators in each group. **Figure S1.** Genotype validation by direct sequencing for PvuII and XbaI. **Figure S2.** Genotype validation by direct sequencing for RsaI. **Figure S3.** Genotype validation by direct sequencing for AluI. (DOCX 430 kb)

Abbreviations

ATP III: Adult Treatment Panel III; BMI: Body mass index; BP: Blood pressure; CVD: Cardiovascular disease; DM: Diabetes mellitus; E2: Estradiol; ER: Estrogen receptor; ESR: Estrogen receptor; FBG: Fasting blood glucose; GLUT4: Glucose transporter four; HbA1C: Hemoglobin A1C; HDL-C: High-density lipoprotein cholesterol; HOMA-IR: Homeostasis model assessment of insulin resistance; IRS1: Insulin receptor substrate 1; LDL-C: Low density lipoprotein; MetS: Metabolic syndrome; MI: Myocardial infarction; NAFLD: Non-alcoholic fatty liver disease; SNPs: Single-nucleotide polymorphisms; SWAN: Study of Women's Health Across the Nation; T2DM: Type 2 diabetes mellitus; TC: Total cholesterol; TG: Triglyceride

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Availability of data and materials

All data supporting the findings reported in this work are at the medical center of Dayi Hospital Affiliated to Shanxi Medical University, but not available to the public. However, all data can be obtained from the authors upon reasonable request, with approval from the Shanxi Dayi Hospital ethics committee.

Authors' contributions

ZLX and ZGP supervised data generation and analysis, and drafted the manuscript. FXM and XGX were responsible for data collection. GQ contributed to study design and carried out statistical analyses. ZL and SXL interpreted the acquired data. ZZY and ZJL performed PCR. All authors were involved in study conception and design, as well as data acquisition. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The current study of human subjects was approved by the Shanxi Dayi Hospital Medicine Institutional Review Board (YXLL-2015-022). Written informed consent was obtained from each participant before enrolment.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interest.

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Author details

¹Department of Biochemistry and Molecular Biology, Shanxi Medical University, Taiyuan, No.56, Xinjian South Road, Taiyuan, Shanxi 030001, People's Republic of China. ²Department of Endocrinology Medicine, Dayi Hospital Affiliated to Shanxi Medical University, Taiyuan, Shanxi 030032, People's Republic of China. ³Department of Physiology, Shanxi Medical University, Taiyuan, Shanxi 030001, People's Republic of China. ⁴Department of Medical Statistics, School of Public Health of Shanxi Medical University, Taiyuan, Shanxi 030001, People's Republic of China. ⁵Renal Division, Shanxi Medical University Second Hospital, Shanxi Kidney Disease Institute, Taiyuan, Shanxi 030001, People's Republic of China. ⁶Centre for Transplantation and Renal Research, University of Sydney at Westmead Millennium Institute, Sydney, NSW 2145, Australia.

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