

## ORIGINAL ARTICLE

# Association between *ESR1* and *ESR2* gene polymorphisms and hyperlipidemia in Chinese Han postmenopausal women

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Estrogen was considered to be an important protective factor for cardiovascular diseases in women. Genetic association studies suggested that variations of *ESR1* and *ESR2* genes might have a potential role in lipid profile. Our study aimed to investigate the association of single-nucleotide polymorphisms (SNPs) of *ESR1* and *ESR2* with hyperlipidemia in Chinese Han postmenopausal women. A total of 443 postmenopausal women aged between 55 and 71 years were recruited from Shanghai, China for a case-control study (154 women with hyperlipidemia and 289 controls). We measured plasma estradiol concentration, glucose and lipid profile levels, evaluated their lifestyle and sequenced four SNPs, namely PvuII (rs2234693) and XbaI (rs9340799) of *ESR1* and 1082A>G (rs1256049) and 1730A>G (rs4986938) of *ESR2*. PvuII (rs2234693) and XbaI (rs9340799) showed significantly different distributions between cases and controls ( $P=0.002$  and  $P=0.023$ , respectively). In addition, haplotypes constructed from PvuII–XbaI were also associated with hyperlipidemia (global  $P=0.012$ ). Haplotypes T–A ( $P=0.005$ , odds ratio (OR) 1.52, 95% confidence interval (95% CI): 1.13–2.05) and C–G ( $P=0.010$ , OR 0.62, 95% CI: 0.43–0.89) had susceptible and protective effects, respectively. 1082A>G (rs1256049) and 1730A>G (rs4986938) showed no statistical association with hyperlipidemia. In conclusion, our results suggested that *ESR1* might have a potential role in hyperlipidemia risk, independent of age, estradiol level, body mass index and lifestyle in Chinese Han postmenopausal women.

*Journal of Human Genetics* (2010) 55, 50–54; doi:10.1038/jhg.2009.122; published online 20 November 2009

**Keywords:** Chinese; estrogen receptor; hyperlipidemia; single-nucleotide polymorphism

## INTRODUCTION

Incidence and mortality due to cardiovascular diseases (CVDs) are much higher in men than in women before menopausal age. However, the gender difference diminishes when women go into postmenopause.<sup>1,2</sup> This phenomenon is widely believed to result from the protective effects of estrogen. There are many possible mechanisms for the protective effects of estradiol on vasculature, including increased vasodilatation by its positive influence on endothelial nitric oxide (NO) synthase and subsequent production of NO.<sup>3</sup> Moreover, estradiol reduces the development of early lesions of atherosclerosis, through an improvement in the blood lipid profile, which reduces lipid deposits in the endothelium, with a decrease in total cholesterol and low-density lipoprotein cholesterol (LDL-C) and an increase in high-density lipoprotein cholesterol (HDL-C) in the plasma.<sup>4</sup> The physiological process of estrogen works by binding to estrogen receptors.<sup>5</sup> There are two known estrogen receptors, namely estrogen receptor- $\alpha$  (*ESR1*) and estrogen receptor- $\beta$  (*ESR2*), which are

transcription factors and are expressed in a wide range of tissues, including macrophages, adipose cells, vascular smooth muscle and vascular endothelial cells.<sup>6</sup> It was reported that fewer estrogen receptors were found in premenopausal women with atherosclerotic coronary arteries than in those with normal coronary arteries.<sup>7</sup> Recently, a genome-wide association study of plasma lipoprotein(a) levels that might involve multiple loci on chromosome 6q26–q27 in which the *ESR1* gene is located.<sup>8</sup> A number of independent studies that investigated the effects of single-nucleotide polymorphisms (SNPs) of *ESR1* and *ESR2* genes on estrogen-related characteristics had shown some interesting SNPs, such as PvuII (rs2234693) and XbaI (rs9340799) of *ESR1* and 1082A>G (rs1256049) and 1730A>G (rs4986938) of *ESR2* and so on. The PvuII (rs2234693) polymorphism may affect the binding of the transcription factor, resulting in the alteration of protein expression.<sup>9</sup> PvuII (rs2234693) and XbaI (rs9340799) of *ESR1* were reported to be associated with lipid

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Received 30 August 2009; revised 29 September 2009; accepted 21 October 2009; published online 20 November 2009

profile,<sup>10–13</sup> Alzheimer's disease,<sup>14</sup> artery wall atherosclerosis,<sup>15</sup> atherosclerotic severity to hormone replacement therapy (HRT)<sup>16</sup> and pathogenesis of hypertension,<sup>17,18</sup> stroke,<sup>19</sup> CVDs<sup>20–23</sup> and type II diabetes.<sup>24,25</sup> 1082A>G (rs1256049) and 1730A>G (rs4986938) of *ESR2* might have a role in CVDs<sup>26,27</sup> and lipid profile.<sup>28,29</sup>

Most of the evidence was reported from the Western population; however, little was known about the SNP distributions of *ESRs* and their relationships with hyperlipidemia in Chinese women. In our study, we measured serum estrogen concentration and sequenced four representative SNPs, namely PvuII (rs2234693) and XbaI (rs9340799) of *ESR1* and 1082A>G (rs1256049) and 1730A>G (rs4986938) of *ESR2*. We aimed to investigate the association of SNPs of *ESR1* and *ESR2* genes with hyperlipidemia in a Chinese Han postmenopausal women population.

## MATERIALS AND METHODS

### Population and DNA preparation

A total of 443 Chinese Han postmenopausal women aged between 55 and 71 years were recruited from Shanghai, China, including 154 hyperlipidemia cases and 289 controls. Eligible candidates were defined as those who were stable residents in the area and without the following conditions: severe psychological disorders, physical disabilities, cancer, diabetes mellitus, CVDs, Alzheimer's disease or dementia, within 6 months; or currently diagnosed with tuberculosis, AIDs (acquired immune deficiency syndrome) or other communicable diseases. They did not take lipid-lowering medicines; neither did they undergo HRT. All cases were diagnosed according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria.<sup>30</sup> Controls were nonhyperlipidemic and were recruited from the same districts of Shanghai as were the cases. Main clinical data including age, sex, body mass index (BMI), waist circumference, systolic and diastolic blood pressures, smoking history, alcohol drinking and physical activity were recorded. Total cholesterol, HDL-C, LDL-C, triglyceride, glucose levels, C-reactive protein (CRP) and estradiol concentrations were determined from fasting blood samples of all participants.

We certified that all applicable institutional and governmental regulations with regard to the ethical use of human volunteers were followed during this research. A standard written informed consent, reviewed and approved by the ethics committee of the Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, was obtained from all participating subjects who were given a full explanation of the study.

High-molecular-weight genomic DNA was prepared from venous blood using the QuickGene 610L Automatic DNA/RNA Extraction System (Fujifilm, Tokyo, Japan).

### Genotyping

The minor allele frequencies of rs2234693, rs9340799, rs1256049 and rs4986938 were 0.402, 0.225, 0.299 and 0.128, respectively, in the Chinese Han population according to the dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP/>) and the hapmap human SNP database (<http://www.hapmap.org/>). We amplified a 470-bp genomic segment for the rs2234693 (T/C) and rs9340799 (A/G) polymorphisms (upstream primer 5'-CATGAACCACCATGCTCAGT-3', downstream primer 5'-TTACCTCTGCCGTCTGTTG-3'), a 475-bp genomic segment for the rs1256049 (A/G) polymorphism (upstream primer 5'-TTCTGAGCCGAGGTCGTAGT-3', downstream primer 5'-CCAGGACTTGTCCCACTC-3') and a 293-bp genomic segment for the rs4986938 (A/G) polymorphism (upstream primer 5'-CCGGCAGAGGACAGTAAAAG-3', downstream primer 5'-AGGCCATTGAGTGTGGAAAC-3'). PCR was performed using the GeneAmp 9700 System (Applied Biosystems, Foster City, CA, USA) in a 15 µl reaction, containing 10 ng genomic DNA, 1.2 U Taq polymerase, 0.25 µl of each primer (10 µM), 2.5 µl PCR buffer (10×, Qiagen, Valencia, CA, USA) and 1.5 µl dNTPs (each 2 mM). The amplification process began with an initial 10-min denaturation at 94 °C, followed by replication of 35 cycles of 30 s at 94 °C, 40 s at 60 °C and 40 s at 72 °C, and ended with an extension period at 72 °C for 7 min for both SNPs. The PCR products for sequencing were incubated with 0.1-U shrimp alkaline phosphatase at 37 °C for 60 min, followed by heat inactivation at 85 °C for 20 min. The treated PCR products were sequenced

using an ABI Prism BigDye Terminator Cycle Sequencing Kit, version 3.1 (Applied Biosystems) on an ABI Prism 3100 sequencer (Applied Biosystems).

We conducted a duplicate quality control test (48 samples for each SNP), which reported 100% concordance.

### Statistical analysis

Hardy–Weinberg equilibrium testing and allele and genotype frequency analysis were carried out online on a robust and user-friendly software platform (<http://analysis.bio-x.cn/>) developed by our laboratory.<sup>31</sup> It was used to estimate linkage disequilibrium (LD) measured with standardized  $D'$  and to compare the discrepancies of allele, genotype and haplotype frequencies on SNPs between cases and controls using the  $\chi^2$ -test and the odds ratio (OR) test. Further genetic analysis procedures adjusted for age, estradiol level, BMI, smoking, alcohol consumption and exercise were assessed with a multivariate logistic regression model. For the additive model, homozygotes (1/1), heterozygotes (1/0) for the risk allele and homozygotes for the non-risk allele (0/0) were encoded to an ordered categorical variable for genotypes (2, 1 and 0). For rs2234693, TT=2, CT=1, CC=0; for rs9340799, AA=2, AG=1, GG=0; for rs1256049, AA=2, AG=1, GG=0; for rs4986938, AA=2, AG=1, GG=0. The dominant model was defined as 1/1+1/0 versus 0/0 and the recessive model as 1/1 versus 1/0+0/0. For rs2234693, TT+CT=2, CC=1 in the dominant model and TT=2, CT+CC=1 in the recessive model. Other SNPs were of similar analogy. The difference in quantitative traits, including BMI, triglycerides (Trig), cholesterol (Cho), HDL-C, LDL-C, CRP and estradiol concentration as the dependent variable, was analyzed by the Wilcoxon rank-sum test and the Kruskal–Wallis test, with genotype as the independent variable. Statistical analyses were carried out using the SPSS (SPSS, Chicago, IL, USA) program.

Power calculations were *post hoc* calculations performed on the G\*Power program.<sup>32</sup> All *P*-values reported were two-tailed. Statistical significance was defined as  $P < 0.05$ .

## RESULTS

A total of 443 Chinese Han postmenopausal women aged between 55 and 71 years were recruited from Shanghai, China, including 154 cases (mean age 60.1 years, s.d.=2.9) and 289 controls (mean age 59.3 years, s.d.=2.6). The clinical characteristics of participants are summarized in Table 1.

Genotype distributions of the four SNPs in cases and controls were in Hardy–Weinberg equilibrium ( $P > 0.05$ ). In power calculations using the G\*Power 3 program, we found that the sample size had >76% power for rs2234693, >60% for rs9340799, >68% for rs1256049 and >54% for rs4986938 to detect a weak gene effect (OR=1.3) with  $\alpha \leq 0.05$ .

We found significant differences in allele frequencies of PvuII (rs2234693) and XbaI (rs9340799) between cases and controls in Chinese Han postmenopausal women. The frequency of the T allele of PvuII (rs2234693) was significantly higher in patients (67.0%) than in controls (56.9%) ( $P=0.004$ , OR 1.54, 95% confidence interval (95% CI): 1.15–2.07), as was the A allele of XbaI (rs9340799) ( $P=0.023$ , OR 1.50, 95% CI: 1.06–2.14). These discrepancies remained in genotype distribution in additive, dominant and recessive models before or after adjusting for age, estradiol level, BMI, smoking, alcohol consumption and exercise (Table 2). Furthermore, model selection examination was performed by Akaike's information criterion, suggesting the propriety of the additive model for the two SNPs. We did not find any significant differences in either allele or genotype frequencies of 1082A>G (rs1256049) and 1730A>G (rs4986938) between cases and controls in additive, dominant and recessive models either before or after adjusting for age, estradiol level, BMI, smoking, alcohol consumption and exercise.

In addition, the estimation of LD for all pairs of SNP markers showed that PvuII (rs2234693) and XbaI (rs9340799) of *ESR1* were in a relatively strong LD relationship with the standardized  $D'=0.94$ , as

well as 1082A>G (rs1256049) and 1730A>G (rs4986938) of *ESR2* with the standardized  $D'=0.99$ . We further analyzed the frequency distributions of haplotypes of the four SNPs above the common lowest frequency threshold (those with a frequency <0.03 will not be included in the analysis). Haplotypes constructed from PvuII-XbaI were associated with hyperlipidemia (global  $P=0.012$ ), and two of them, haplotype T-A ( $P=0.005$ , OR 1.52, 95% CI: 1.13–2.05) and C-G ( $P=0.010$ , OR 0.62, 95% CI: 0.43–0.89) with reference to all other haplotypes, were very significant. 1082A>G (rs1256049), 1730A>G (rs4986938) and haplotypes of 1082A>G–1730A>G showed no statistical association with hyperlipidemia (Table 3).

According to the NCEP ATP III criteria, hyperlipidemia is defined by four components that we were also concerned about. We specifically examined the relationship between the four SNPs and BMI, triglyceride (Trig), cholesterol (Cho), HDL-C (HDL), LDL-C (LDL), CRP and estradiol concentration in all postmenopausal women using the Wilcoxon rank-sum test and the Kruskal–Wallis test. No significant associations of the polymorphisms of *ESR1* or *ESR2* with metabolic quantitative traits were observed (Supplement 1).

**Table 1 Clinical characteristics of the participants**

	Hyperlipidemia	Control	P-value
Age (years)	60.1±2.9	59.3±2.6	0.003
Estradiol (pmol l <sup>-1</sup> )	15.7±7.6	15.4±7.0	0.706
Fasting blood glucose (mmol l <sup>-1</sup> )	4.9±0.6	4.9±0.6	0.966
HbA1C (%)	5.9±0.5	5.8±0.5	0.396
Systolic BP (mm Hg)	133±18	129±18	0.059
Diastolic BP (mm Hg)	82±9	80±10	0.065
BMI (kg m <sup>-2</sup> )	25.6±2.9	24.4±3.5	<0.001
Waist (cm)	85.9±7.5	81.6±9.2	<0.001
Cholesterol (mmol l <sup>-1</sup> )	5.0±1.2	4.6±0.6	<0.001
Triglycerides (mmol l <sup>-1</sup> )	2.6±1.3	1.3±0.4	<0.001
LDL (mmol l <sup>-1</sup> )	3.2±1.1	3.0±0.6	0.080
HDL (mmol l <sup>-1</sup> )	1.1±0.3	1.4±0.3	<0.001

Abbreviations: BMI, body mass index; BP, blood pressure; HbA1C, hemoglobin A1C; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Data are means ± s.d.

## DISCUSSION

Hyperlipidemia is the presence of increased or abnormal levels of lipids and/or lipoproteins in the blood and a strong risk factor for atherosclerosis, stroke, CAD and myocardial infarction (MI), as reported by epidemiological studies.<sup>30</sup> However, the risk has gender differences. Are women superior to men with regard to the risk of CVD? Research evidence suggests so. The incidence of CVD among women is low before menopause but steadily increases after the onset of menopause. This increase is believed to result partly from the loss of endogenous estrogen, which is involved in cardio-protective effects. Endogenous and exogenous estrogens decrease LDL-C, increase HDL-C levels and lead to an increment in mRNA for the LDL receptor.<sup>33</sup> Estrogen works by binding to estrogen receptors and carries out direct and indirect effects on cardiovascular tissues through mechanisms involving genomic and nongenomic pathways.<sup>34</sup> It was reported that fewer estrogen receptors were found in premenopausal women with atherosclerotic coronary arteries than in those with normal coronary arteries.<sup>7</sup> Recently, a genome-wide association study of plasma lipoprotein(a) suggested a complex genetic architecture of plasma lipoprotein(a) levels that might involve multiple loci on chromosome 6q26–q27, in which the *ESR1* gene is located.<sup>8</sup>

In our study, we found a positive association between polymorphisms of *ESR1* and hyperlipidemia in Chinese Han postmenopausal

**Table 3 The estimated haplotype distributions in cases and controls**

SNPs	Haplotype	Case (freq)	Control (freq)	Global	
				P-value	P-value
<i>ESR1</i>	C–A	49.69 (0.169)	106.49 (0.193)	<b>0.012</b>	0.423
	C–G	47.31 (0.161)	131.51 (0.238)		
	T–A	193.31 (0.658)	311.51 (0.564)		
<i>ESR2</i>	A–G	93.98 (0.313)	173.98 (0.314)	0.460	0.982
	G–A	47.98 (0.160)	71.98 (0.130)		
	G–G	158.02 (0.527)	308.02 (0.556)		

Abbreviation: SNP, single-nucleotide polymorphism. Haplotypes are of at least 3% frequency in both case and control groups. P-values (<0.05) are in bold.

**Table 2 Allele and genotype distributions of the SNPs in hyperlipidemia and controls**

SNPs	Risk allele frequencies			Unadjusted OR (95% CI)	Genotype	Genotype distribution		Adjusted OR <sup>a</sup>		
	Risk allele	Control n (freq)	Hyperlipidemia n (freq)			Control n (freq)	Hyperlipidemia n (freq)	Additive model OR (95% CI)	Dominant model OR (95% CI)	Recessive model OR (95% CI)
<i>ESR1</i> PvuII	T	315 (0.569)	197 (0.670)	<b>1.54 (1.15–2.07)</b> $P_{\text{freq}}=0.004$	TT	94 (0.339)	67 (0.456)	<b>1.61 (1.19–2.18)</b> $P_{\text{add}}=0.002$	<b>2.28 (1.24–4.21)</b> $P_{\text{dom}}=0.008$	<b>1.73 (1.13–2.66)</b> $P_{\text{rec}}=0.012$
					CT	127 (0.458)	63 (0.429)			
					CC	56 (0.202)	17 (0.116)			
<i>ESR1</i> XbaI	A	438 (0.760)	253 (0.827)	<b>1.50 (1.06–2.14)</b> $P_{\text{freq}}=0.023$	AA	164 (0.569)	102 (0.667)	<b>1.70 (1.16–2.49)</b> $P_{\text{add}}=0.007$	<b>4.94 (1.03–23.7)</b> $P_{\text{dom}}=0.046$	<b>1.65 (1.08–2.53)</b> $P_{\text{rec}}=0.022$
					GA	110 (0.382)	49 (0.320)			
					GG	14 (0.049)	2 (0.013)			
<i>ESR2</i> 1082A>G	A	182 (0.316)	94 (0.307)	1.04 (0.77–1.41) $P_{\text{freq}}=0.789$	AA	30 (0.104)	11 (0.072)	0.94 (0.68–1.29) $P_{\text{add}}=0.692$	1.06 (0.70–1.59) $P_{\text{dom}}=0.796$	0.60 (0.28–1.29) $P_{\text{rec}}=0.191$
					AG	122 (0.424)	72 (0.471)			
					GG	136 (0.472)	70 (0.458)			
<i>ESR2</i> 1730A>G	A	72 (0.130)	48 (0.159)	1.27 (0.85–1.88) $P_{\text{freq}}=0.243$	AA	5 (0.018)	6 (0.040)	1.24 (0.83–1.85) $P_{\text{add}}=0.291$	1.18 (0.74–1.88) $P_{\text{dom}}=0.483$	2.47 (0.70–8.75) $P_{\text{rec}}=0.162$
					AG	62 (0.224)	36 (0.238)			
					GG	210 (0.758)	109 (0.722)			

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism. P<0.05 are in bold.

<sup>a</sup>Adjusted with age, estradiol level, BMI, smoking, alcohol consumption and exercise.

women. Women who carried the PvuII T allele of *ESR1* had an increased risk of hyperlipidemia. After adjusting for age, estradiol level, BMI, smoking, alcohol consumption and exercise, genotype distribution was still significantly different between cases and controls in additive, dominant and recessive models. Positive associations were also found at XbaI of *ESR1*, which was close to and at LD with PvuII. Recently, Demissie *et al.*<sup>35</sup> reported that women with the PvuII T allele had a higher small LDL particle concentration. Our results were consistent with those of previous studies and suggested that *ESR1* had an important role in lipid profile. In addition, further analysis on haplotypes in our study validated the positive association of PvuII–XbaI with hyperlipidemia. Although the above associations could be eliminated after strict Bonferroni's correction ( $\times 22$ ), the results still had marginal significance. A larger sample with enhanced detecting power and comprehensive meta-analysis should be helpful in understanding the association between polymorphisms of *ESR1* and hyperlipidemia.

A possible mechanism to influence *ESR1* expression is the altered binding of transcription or splicing of mRNA. The PvuII SNP of *ESR1*, along with XbaI, is located upstream of exon2 of the *ESR1* gene. Herrington *et al.*<sup>9</sup> reported that the PvuII T allele eliminated a functional binding site for the transcription factor B-myb, which suggested that the presence of this allele was likely to result in a lower *ESR1* transcription. In the absence of estrogen receptors, estrogen signaling is likely to undertake negative feedback and, therefore, estrogen actions are decreased. This finding was supported by investigations on myocardial infarction,<sup>22</sup> osteoporosis,<sup>36</sup> shorter stature<sup>37</sup> and later age at menopause.<sup>38</sup> In addition, it was reported that estrogen repressed the transcription of the hepatic lipase gene.<sup>39</sup> Therefore, decreased estrogen activity involved with the T allele probably results in elevated hepatic lipase activity and consequently increases the mass and percentage of LDL as reported.<sup>35</sup>

In previous studies, Shearman *et al.*<sup>21</sup> reported that the PvuII C allele was predisposed to MI and stroke<sup>40</sup> in men, which was seemingly contradictory to our finding that the T allele increased the risk of hyperlipidemia in women. However, the results were not as completely conflicting as they seemed. Schuit *et al.* reported that T-allele women carriers had substantial increased risk of MI, and they explained the discrepancy between men and women with a reasonable hypothesis. In men, compared with postmenopausal women of the same age, estradiol levels were approximately three times higher. In the presence of steady and sufficient estradiol levels, men might not be influenced by an altered *ESR1* expression. However, a sharp estrogen deficiency after menopause, accompanied by a lower *ESR1* expression caused by the PvuII T allele, might lead to a higher susceptibility to CVDs.<sup>22</sup> Recently, a study further showed the possible mechanism involved with adiponectin. The T allele of PvuII was associated with low serum levels of adiponectin, which might lead to a high risk of CVD in postmenopausal women.<sup>41</sup> Moreover, Hayashi *et al.*<sup>42</sup> reported that only women with a TT genotype had increased arterial stiffness, whereas men did not. Ferrero *et al.*<sup>43</sup> reported that women homozygous for the T allele treated with coronary stent implantation had a higher risk of in-stent restenosis than did men. Lamon-Fava *et al.*<sup>44</sup> reported that postmenopausal women carrying the T allele had lower HDL-C and higher plasma TG. Our results were consistent with this interesting gender-specific effect of PvuII SNP. During the postmenopausal period, when the protective effect of estrogen is lost, women with the T-allele polymorphism and a lower efficient *ESR1* may be more vulnerable to lipid metabolism. On the other hand, men are not disturbed by lower *ESR1*, and are even more sensitive to a higher estrogen effect, namely, with the C allele, such as in CAD.<sup>20</sup> Interestingly, this effect is probably driven not only endogenously but

also exogenously by phytoestrogens. It was reported that soy protein intake, accompanied by soy-isoflavone, had a positive impact on hyperglycemia and metabolic syndrome in men, but had a negative impact in women.<sup>45</sup>

Little was known about the association of 1082A>G (rs1256049) and 1730A>G (rs4986938) of *ESR2* with hyperlipidemia. Results of previous studies with regard to the association between these two SNPs and CVDs were controversial. They were reported as being associated with deep-vein thrombosis<sup>26</sup> and CVDs<sup>27</sup> in Caucasians. In another study, mean LDL and total cholesterol concentrations in premenopausal or postmenopausal women with higher estrogen exposure were lower in those with the GA genotype for 1082A>G than in those with the AA genotype in Brazilians of European descent.<sup>29</sup> Conversely, Domingues-Montanari *et al.*<sup>46</sup> reported that no association with MI was observed for 1082A>G or 1730A>G in the Spanish population. In this study, we did not find any association of 1082A>G (rs1256049) and 1730A>G (rs4986938) with hyperlipidemia. According to power calculation, false-negative results cannot be avoided adequately, unless the effect of the SNP is strong enough to detect, such as  $OR \geq 1.4$ , but the real effect is probably weaker. In addition, population heterogeneity may be a factor, especially for a complicated metabolic disorder. Further studies with more tag SNPs chosen in different gene blocks of *ESR2* are necessary.

According to the NCEP ATP III criteria, hyperlipidemia is defined by four components: triglyceride, total cholesterol, HDL-C and LDL-C. Estrogen receptor genes and estrogen have important roles in lipid profile.<sup>10,13</sup> We examined the relationship of four SNPs with BMI, Cho, Trig, HDL, LDL, CRP and estradiol distribution tested by the Kruskal–Wallis test. However, none showed significant association with any of the metabolic quantitative traits. We assumed that the susceptibility to hyperlipidemia, based on *ESR1* gene polymorphisms, was reflected from a comprehensive lipid profile, and not separately from each component. Variations of the *ESR1* gene changed not only a single character, such as triglyceride or total cholesterol, but also affected the general risk above a threshold for hyperlipidemia, and even further for other diseases.<sup>47</sup> The main effect and discrepancy became undetectable when divided into pieces of characters. A larger sample to deliver enhanced statistical power should be helpful.

Genetic association studies are often disturbed by population stratification. In our study, all participants were of Chinese Han origin and 55–71-year-old Shanghai local residents. Therefore, our sample could be considered ethnically homogeneous.

In summary, to our knowledge, this was the first study to investigate the association of *ESR1* and *ESR2* gene polymorphisms with hyperlipidemia risk in the Chinese Han postmenopausal women population. Our study suggested that postmenopausal women who carried the T allele of PvuII (rs2234693) or the A allele of XbaI (rs9340799), or the T–A haplotype of PvuII–XbaI of the *ESR1* gene, had increased risk of hyperlipidemia independent of their age, estradiol level, BMI, smoking, alcohol consumption and physical activity. We hope that this study will be a reference point for further studies on hyperlipidemia and CVDs in Asian and other ethnic populations.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### ACKNOWLEDGEMENTS

We appreciate the contribution of all of the members participating in this study, as well as that of the doctors who helped us in the diagnosis. This study was supported by grants from the National Natural Science Foundation of China, the national S973 and 863 Programs, Chinese Nutrition Society (05015),

Dannon Institute, Shanghai-Unilever Research and Development Fund (06SU07007), Shanghai Municipality Science & Technology Commission (05JC14090,07DJ14005, Shanghai Leading Academic Discipline Project (B205), the Knowledge Innovation Program of Shanghai Institutes for Biological Sciences, the Chinese Academy of Sciences (2007KIP210,2009KIP305) and the Chinese Academy of Sciences (KSCX2-YW-R-01, KSCX2-YW-N-034).

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