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Associations of the Estrogen Receptors 1 and 2 Gene Polymorphisms With the Metabolic Syndrome in Women

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Abstract

Background: Genetic variation of the estrogen receptor α (ESR1) and β (ESR2) has been associated with components of the metabolic syndrome.
Methods: The relationships of two ESR1 (rs2234693 and rs9340799) and three ESR2 (rs1271572, rs1256049, and rs4986938) polymorphisms with the metabolic syndrome were examined in 532 Caucasian female participants (median age 63.1 years) in the Women's Health Study. Most women (99.1%) were postmenopausal. The associations between ESR1 and ESR2 genotypes and haplotypes with the metabolic syndrome were evaluated. Effect modification by hormone therapy was also assessed.
Results: Genotype and haplotype distributions were similar between women with and without metabolic syndrome. We found no consistent associations between the genotypes and haplotypes tested and the metabolic syndrome, or its components, in logistic regression models. No effect modification by hormone therapy use was noted.
Conclusions: No association between these genetic variants in ESR1 and ESR2 and the metabolic syndrome was observed among these Caucasian women. Further investigation regarding the potential involvement of estrogen receptor genes and the metabolic syndrome may be warranted in other ethnic groups.

Introduction

Metabolic syndrome is a highly prevalent condition that is associated with substantially increased risk of type 2 diabetes mellitus and cardiovascular disease.[1,2] Although multiple definitions have been proposed, according to the Adult Treatment Panel III (ATP III) definition, the metabolic syndrome is characterized by three or more of the following: abdominal obesity, elevated triglycerides, low levels of high-density lipoprotein cholesterol (HDL-C), high blood pressure, and elevated fasting glucose.[3] Postmenopausal women have a high prevalence of metabolic syndrome,[4] and some studies suggest that the prevalence is higher in middle-aged women than middle-aged men.[5,6] In addition, metabolic syndrome may be associated with greater cardiovascular risk in women than in men.

Because the metabolic syndrome is a cluster of conditions, each of which has been associated with risk of cardiovascular disease (CVD), candidate genes previously implicated in the pathophysiology of CVD may represent potential candidates for metabolic syndrome. Prior genetic variation of the estrogen β receptor gene (ESR2) has been associated with risk of CVD, particularly myocardial infarction, in the Women's Health Study.[7] Furthermore, endogenous estrogen levels have been linked to several components of the metabolic syndrome, including glucose tolerance, lipid metabolism, and blood pressure.[8–10] Free estradiol levels were significantly higher among women with the metabolic syndrome than in women without metabolic syndrome in two separate studies.[10,11]

Estrogens exert their actions through two specific receptors, the estrogen receptor α (ER-α) encoded by ESR1 on chromosome 6q25.1 and ER-β encoded by ESR2 on chromosome 14q23.2. In animal models, ER-α knockout mice have insulin resistance, impaired glucose tolerance, and obesity, indicating that variation in estrogen receptor signaling may have relevant metabolic effects.[12] Studies in postmenopausal women have found associations between estrogen receptor genes (ESR1 or ESR2) and the metabolic syndrome components, particularly obesity and dyslipidemia.[13–16] Significant associations between ESR1 and ESR2 and metabolic

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syndrome have been reported in younger populations, as well as African American and Chinese populations[17–19]; however, postmenopausal or older women have not been specifically examined. Recently, ESR1, particularly intron 1 and introns 4–6, has been linked to type 2 diabetes mellitus.[16,20] On the basis of these prior reports, we tested the relationship between metabolic syndrome and two ESR1-estrogen receptor polymorphisms (rs2234693 and rs9340799) and three ESR2-estrogen receptor polymorphisms (rs1271572, rs1256049, and rs4986938) in a sample of 532 predominantly postmenopausal, Caucasian women.

Materials and Methods

Study design

Study participants were enrolled in the Women's Health Study (WHS), a recently completed, randomized, double-blinded, placebo-controlled trial of low-dose aspirin and vitamin E initiated in 1992 among 39,876 female, predominantly Caucasian, U.S. female health professionals, 45–89 years of age at study entry.[21,22] All participants were free of prior myocardial infarction, stroke, transient ischemic attacks, cancer, or any serious illness that might preclude participation at study entry.[21] Women enrolled in the WHS completed a baseline questionnaire, which included questions on demographics (age, race, marital status, and level of education), health characteristics/behaviors (height, weight, alcohol use, smoking status, physical activity, hormone therapy use), menopausal status (age at menopause and type of menopause), and past medical history (history of hypertension, diabetes mellitus, elevated cholesterol, and use of cholesterol drugs). Women were considered postmenopausal if they were either age 60 years or older or reported permanent cessation of menstrual periods due to natural menopause, complete oophorectomy, radiation, or chemotherapy. At baseline, participants also completed a 131-item semiquantitative food frequency questionnaire as previously described.[23] Nutrient intake assessments based on this food frequency questionnaire have been previously shown to be valid and reliable.[24]

We evaluated data from a subset of women who were previously selected as controls for a nested case-control study of CVD within the WHS.[21] We excluded women with baseline diabetes ($n = 20$) and those with incomplete data for ESR1 and ESR2 genotypes, leaving 532 Caucasian women who were assessed for presence or absence of metabolic syndrome. In secondary sensitivity analyses using updated criteria that included diabetes as a metabolic syndrome component, we included the 20 women with type 2 diabetes mellitus (T2DM) at baseline. The Brigham and Women's Hospital Institutional Review Board approved the study protocol for Human Subjects Research.

Exposure variables

The primary outcome was metabolic syndrome status, which was defined according to a modified version of the National Cholesterol Education Program ATP III guidelines. The ATP III definition includes the presence of ≥3 of the following: increased waist circumference (≥88 cm for women), elevated blood pressure (>130 mmHg systolic or >85 mmHg diastolic) or treatment for high blood pressure (BP), abnormal glucose metabolism as identified by a fasting blood glucose level of 100 mg/dL or higher.[3] Due to the inability to measure baseline fasting blood sugar and waist circumference in this cohort, we used a modified definition of metabolic syndrome, which has been a previously validated and shown to predict cardiovascular outcomes in this cohort.[2] In addition, this modified definition resulted in nearly identical rates of metabolic syndrome among women in the WHS compared with National Health and Nutrition Examination Survey (NHANES) data using ATP III in the same time period.[2] Because waist circumference was not available at baseline, we used a cut point for obesity of body mass index (BMI) ≥26.7 kg/m^2. This value corresponded to the same percentile for BMI as did a waist circumference of 88 cm when it was measured at year 6 of follow up in the WHS. A Spearman correlation of 0.96 between self-reported and measured weights was found in validation study with a similar cohort of female health professionals.[25]

Because fasting glucose levels were not available, we used a diagnosis of diabetes during follow up to identify impairment of glucose metabolism. The diagnosis of diabetes was determined by self-report on the basis of annual questionnaires. The high validity of self-reported diabetes has been previously shown in the WHS.[26] Triglycerides and HDL-C levels were measured directly using stored baseline blood samples (Roche Diagnostics, Indianapolis, IN). We used self-reported blood pressure levels, and defined elevated blood pressure according to ATP III criteria: ≥130/85 mmHg. Self-reported blood pressure has been shown to be highly correlated with measured systolic and diastolic blood pressures in health professionals.[27]

Genotype determination

Two single-nucleotide polymorphisms (SNPs) in the ESR1 gene (rs2234693 and rs9340799) and three SNPs in the ESR2 gene (rs1256049, rs4986938, and rs1271572) were evaluated. Both ESR1 polymorphisms are in intron 1 and are separated by only 46 base pairs. The rs2234693 polymorphism is characterized by a T→C transition 397 nucleotides upstream in the intron (also known as *c.454-497T→C*) that obliterates the *Pvu*II restriction site. The T allele has previously been called the *p* allele, whereas the C allele has been called the *P* allele, denoting the absence of the *Pvu*II restriction site. The rs9340799 polymorphism marks an A→G transition 351 nucleotides upstream in intron 1 (also known as *c.454-351A → G*). Those with the G allele have an absent *Xba*I site that was previously called *X* in the literature, with the A allele denoted by *x*.

Of the three ESR2 polymorphisms, two were previously described by Rosencranz[28:] rs1256049 which represents a relatively rare G→A change at position 1082 in exon 5 (*Rsa*I restriction site, also known G1082A) and rs4986938 which is a G→A change at position 1730 in the 3′-untranslated region (3′-UTR) of exon 8 (A*lu*I restriction site, also known as G1730). Additionally, rs1271572, an A→C transposition in the promoter region, was selected due to possible functional status.

Genotype determination was performed using an ABI fluorescence-based allelic discrimination method (Applied Biosystems, Foster City, CA). Each 10-mL amplification

reaction volume contained 1× Taqman Universal Master Mix (Applied Biosystems, Foster City, CA) and 10 ng of template DNA. Amplification reactions were carried out in duplicates on an ABI 7900HT Sequence Detection System according to the manufacturer's specifications.

To confirm genotype assignment, two independent observers carried out scoring. Discordant results (1% of all scoring) were resolved by a joint reading, and, where necessary, a repeat genotyping.

Statistical analysis

The distribution of baseline characteristics according to the metabolic syndrome status was examined. On the basis of nonparametric distribution, all continuous variables were examined by the Wilcoxon rank-sum test, whereas the chi-squared test was used for categorical variables. We calculated genotype and allele frequencies and performed a Hardy–Weinberg equilibrium test using chi-squared analysis. The association between ESR1 and ESR2 genotypes and the metabolic syndrome was also examined using the chi-squared test. In addition, crude and multivariate logistic regressions were performed to investigate the relationship between genotypes and metabolic syndrome. Additional adjustment for age at randomization, age at menopause, smoking status (never, past and current), physical activity (rarely/never to <1/week and 1–3/week to >4/week), and hormone replacement therapy use (yes/no) was also performed. A number of covariates (alcohol and red wine consumption; total intakes of carbohydrate, protein, saturated, monounsaturated and polyunsaturated fat [all of them adjusted by energy]; educational level; marital status and income] which could be, associated with metabolic syndrome or had a p value chi-squared test <0.2 were evaluated. According to these criteria, the following covariates were included in the regression model using the forward stepwise method[29]: alcohol consumption (≤3 drinks/month, 1 drink/week to ≥1 drink/day), total fat adjusted for energy intake (continuous), and educational level (≤3 years of college, ≥4 years of college).

All analyses were conducted assuming an additive dominant and recessive mode of inheritance. Potential interactions between hormone use and ESR1 and ESR2 genotypes were tested using a formal interaction term (genotype × hormone use). Subgroup analysis stratified by hormone replacement

Table 1. Baseline Characteristics of 532 Apparently Healthy Caucasian Women With and Without Metabolic Syndrome in the Women's Health Study

Characteristics	*Metabolic syndrome*[b] *(n = 133)*	*No metabolic syndrome*[b] *(n = 399)*	*P*
Median age (IQR)[a]	63.9 (59.4–67.7)	62.8 (57.4–67.9)	0.39
Median age at menopause (IQR)[a]	49 (45–52)	49 (44–52)	0.53
Median BMI (IQR)	28.5 (26.6–30.2)	23.7 (21.7–25.8)	<0.0001
BMI ≥ 26.7 kg/m^2 (%)	73.7	16.0	<0.0001
Blood pressure ≥ 130/85 mmHg (%)	82.7	17.3	<0.0001
Diabetes during follow up (%)	9.0	1.3	<0.0001
Triglycerides ≥ 150 mg/dL	79.0	23.6	<0.0001
HDL < 50 mg/dL (%)	90.2	32.1	<0.0001
Hormone use (%)			
Never	36.1	32.8	
Past	29.3	21.1	
Current	34.6	46.1	0.04
Smoking status (%)			
Never	43.6	41.6	
Past	33.8	37.8	
Current	22.6	20.6	0.70
Alcohol (%)			
Rarely/never	51.1	45.4	
1–3 drinks/month	12.8	15.3	
1–6 drinks/week	23.3	28.1	
≥1 drink/day	12.8	11.3	0.54
Exercise (%)			
Rarely/never	42.9	38.1	
<1/week	18.8	18.8	
1–3/week	29.3	29.1	
≥4/week	9.0	14.0	0.47

[a]IQR is interquartile range (25th to 75th percentile).

[b]Metabolic syndrome was defined as ≥3 with the following criteria: BMI ≥26.7 kg/m^2, triglycerides ≥150 mg/dL, high-density lipoprotein-cholesterol ≤50 mg/dL, blood pressure ≥130/85 mmHg, self-reported diabetes diagnosis at follow-up. *P* values were obtained from the Wilcoxon rank-sum test (nonparametric) for continuous variables and chi-squared for categorical variables.

Abbreviations: IRQ, interquartile range; BMI, body mass index; HDL, high-density lipoprotein.

therapy use was also performed. The association of each component of the metabolic syndrome with ESR1 and ESR2 polymorphisms was also performed. Pairwise linkage disequilibrum (LD) was examined as described by Devlin and Risch.[30] Haplotype frequencies were calculated with HAPSTAT software (http://www.bios.uncedu/~lin/ software). Only haplotypes with a frequency of 10% or higher were considered in our analyses. For each odds ratio, we calculated 95% confidence intervals (CIs). A two-tailed p value of 0.05 was considered to represent a statistically significant result. All statistical analyses were conducted with the use of SAS software (version 9.1; SAS institute, Cary, NC).

Results

The baseline characteristics of the 532 healthy women, without known cancer, CVD (myocardial infarction, revascularization, or ischemic stroke), or diabetes, according to the metabolic syndrome status are shown in Table 1. Overall, the prevalence of the metabolic syndrome was 25%. Among those with metabolic syndrome, 73.3% had BMI ≥26.7kg/m^2 compared with 16.6% of women without metabolic syndrome, $p < 0.001$ (Table 1).

Allele frequency did not differ according to metabolic syndrome status for ESR1 or ESR2 polymorphisms (Table 2). The rs2234693 genotype (ESR1) and the rs4986938 genotype (ESR2) frequencies were in Hardy–Weinberg equilibrium ($p = 0.06$ and $p = 0.22$, respectively). No associations were observed between the metabolic syndrome and any of the ESR1 or ESR2 genotypes (Table 2).

In logistic regression analyses, no significant associations were found with the metabolic syndrome for any polymorphisms using additive (Table 3) dominant or recessive genetic models (data not shown). Further adjustment provided null findings. No effect modification by hormone use was found in stratified analyses or in tests of formal interaction terms between hormone use and ESR1 or ESR2 genotypes. We also assessed the association with each individual component of the metabolic syndrome and we did not find any significant associations (data not shown).

Table 2. Allele and Genotype Distribution According to Presence or Absence of Metabolic Syndrome in 532 Apparently Healthy Caucasian Women in the Women's Health Study

	Metabolic syndrome	*No metabolic syndrome*	*P*[b]
ESR1			
rs2234693[a]			
TT	28.2	32.6	
TC	46.8	44.9	
CC	25.0	22.6	0.65
T	0.52	0.55	
C	0.48	0.45	0.35
rs9340799			
AA	46.4	48.2	
AG	38.4	37.6	
GG	15.2	14.3	0.93
A	0.66	0.67	
G	0.34	0.33	0.69
ESR2			
rs1271572[a]			
TT	35.0	31.4	
TC	48.0	55.5	
CC	17.1	13.1	0.31
T	0.58	0.60	
C	0.41	0.41	0.95
rs1256049			
AA	93.8	91.8	
AG	5.4	7.7	
GG	0.8	0.50	0.66
A	0.97	0.96	
G	0.03	0.04	0.55
rs4986938			
AA	32.8	35.1	
AG	51.2	50.5	
GG	16.0	14.4	0.86
A	0.58	0.60	
G	0.42	0.40	0.59

[a]rs2234693 and rs4986938 genotype distributions were in Hardy–Weinberg equilibrium.
[b]*P* values chi-squared test.

Table 3. Odds Ratios for the Metabolic Syndrome in 532 Apparently Healthy Caucasian Women in the Women's Health Study According to ESR1 and ESR2 Genotypes

	Age-adjusted odds ratio (95% CI)	*P*[b]	*Multivariate adjusted odds ratio*[a] *(95% CI)*	*P*
ESR1				
rs2234693	1.14 (0.86–1.5)	0.36	1.12 (0.85–1.49)	0.43
rs9340799	1.06 (0.80–1.41)	0.67	1.03 (0.78–1.38)	0.82
ESR2				
rs1271572	1.01 (0.74–1.38)	0.96	1.04 (0.76–1.42)	0.81
rs1256049	0.79 (0.39–1.63)	0.53	0.71 (0.34–1.47)	0.35
rs4986938	1.09 (0.81–1.47)	0.58	1.06 (0.78–1.44)	0.71

Each polymorphism in separate model.
[a]Multivariate adjustment by age, hormone therapy use, exercise, smoking status, and educational level.
Abbreviation: CI, confidence interval.

Moderate LD was found between rs1256049 and rs4986938 (normalized Lewontin D' values = 0.77), whereas weak LD was observed for the other two ESR2 associations. Strong LD was observed between the ESR1 polymorphisms (normalized Lewontin $D' = 0.96$).

ESR1 and ESR2 haplotype distributions are shown in Table 4. In logistic regression analyses based on those haplotypes with ≥10% of frequency, no significant associations were found with metabolic syndrome (Table 5). Additional formal interaction terms between hormone use and ESR1 or ESR2 haplotypes did not alter our results (data not shown). Furthermore, in sensitivity analyses, we included women with diabetes mellitus at baseline and did not find any significant change in our findings.

Discussion

Although prior studies[13,17–19] have suggested an association between several polymorphisms in the ER-α and ER-β genes and metabolic syndrome or its components, we found no consistent relationship between the tested ESR1 and ESR2 polymorphisms and metabolic syndrome in Caucasian postmenopausal women. Two prior studies have found significant associations between specific ESR1 and the ESR2 polymorphisms and metabolic syndrome in younger or middle-aged women, as well as in specific ethnic populations.[17,19] Gallagher et al., using a family-based approach, found that several polymorphisms of ESR1, including the ones that we tested, were associated with metabolic syndrome or its components in African Americans.[17] Specifically, the rs9340799 *G* allele was associated with increased risk of metabolic syndrome, but not with individual metabolic traits. In contrast, the rs2234693 *C* allele was associated with reduced insulin sensitivity.[17] The relationships between the components of metabolic syndrome and estrogen receptor genes were also evaluated in The Study of Women's Health Across the Nation (SWAN) Genetics Study, a community-based sample of perimenopausal African-American, Caucasian, Chinese, and Japanese women aged 42–52 years, who were not using exogenous hormones.[19] Statistically significant relationships between ESR2 rs1256030 and metabolic syndromeas well as HDL-C were observed in Chinese women,[18] but not in Caucasian women or other ethnic groups.

Similar to the lack of association among Caucasian women reported by the SWAN study,[19] we did not observe any association of the tested ESR1 and the ERS2 genotypes

Table 4. ESR1 and ESR2 Haplotype Distributions According to Metabolic Syndrome Status

Haplotype frequency %	*Metabolic syndrome*	*No metabolic syndrome*	*P*[a]
ESR1			
T-A	50.6%	54.5%	
C-G	33%	32.5%	
C-A	15%	12.4%	0.05
ESR2[b]			
T-G-G	30.9%	29.7%	
G-G-A	34.3%	29.2%	
G-G-G	23.9%	26.7%	0.001

[a]*P* permuted over 100 iterations.
[b]G represents the major allele at each site: rs1271572, rs1256049, rs4986938; A denotes minor allele at sites rs1256049 and rs4986938; and T denotes minor allele at rs1271572. Remaining possible haplotypes with ≤10% frequency were not considered.

Table 5. Odds Ratios for Metabolic Syndrome in 532 Apparently Healthy Caucasian Women in the Women's Health Study, According to ESR1 and ESR2 Haplotype, and Assuming an Additive Model

	Odds ratio, unadjusted (95% CI)	*P*
ESR1 (rs2234693, rs9340799)		
T-A	Referent (1.0)	
C-A	1.27 (0.82–1.96)	0.28
C-G	1.08 (0.79–1.48)	0.64
ESR2 (rs1271572, rs1256049, and rs4986938)		
T-G-G[a]	Referent (1.0)	
G-G-G	0.85 (0.55–1.31)	0.46
G-G-A	1.13 (0.79–1.60)	0.51

Remaining possible haplotypes with ≤10% frequency were not considered.

[a]G denotes major allele at each site: rs1271572, rs1256049, and rs4986938; A denotes minor allele at sites rs1256049 and rs4986938; and T denotes minor allele at rs1271572.

Abbreviations: OR, odds ratio; CI, confidence interval.

and haplotypes with metabolic syndrome or its components in our sample of Caucasian postmenopausal women. The lack of significant findings in our study could be partly due to differences in sample size, age, and race/ethnicity. The prevalence of metabolic syndrome in our population was similar to that of women in NHANES during the same time period.[31] However, since that time period, rates of metabolic syndrome have increased markedly in the United States.[1] In our study, we had the ability to detect a risk ratio of greater than 1.60 with a minor allele frequency of 0.50, and a risk ratio greater than 2.30 with a minor allele frequency of 0.05 (assuming 80% power, and additive model, and α of 0.05) Thus, we cannot rule out a modest risk of metabolic syndrome associated with the polymorphisms/haplotypes tested. We did not have other ESR1 or ESR2 loci available and thus cannot exclude the possibility that examination of different polymorphisms/loci might obtain different results.

The candidate gene approach relies on prior knowledge of biological pathways and associations of the candidate gene with the phenotype of interest. In recent years, genome-wide association studies of common, complex diseases have become available, and have provided insights in the underlying pathophysiological mechanisms of several common disorders. Unfortunately, to date, no large genome-wide association investigations have been conducted in relation to metabolic syndrome, thus, highlighting the need for large-scale, prospective studies in this important clinical condition. In this context, in addition to the candidate gene set described here, the Women's Genome Health Study project[32] will eventually include full genome-wide scan data (estimated completion end of 2008). Thus, more detailed results regarding other potential genetic predispositions to metabolic syndrome are expected in future analyses. Of note, the present investigation (the study population[21] and the ESR1-ESR2 genotyping) was carried out prior to the initiation of the Women's Genome Health Study project.

Further investigation of the ESR1 and the ESR2 gene variations and the metabolic syndrome, particularly in other cohorts with different age, gender, and ethnicity, is warranted.

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Author Disclosure Statement

No competing financial interests exist.

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