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Estrogen receptor 1 gene polymorphisms and decreased risk of obesity in women

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Abstract

Estrogen receptor α gene (*ESR1*) polymorphisms have been associated with several diseases, but whether they are associated with obesity is uncertain. To elucidate the role of genetic variation in the *ESR1* gene with body mass index (BMI), 543 white women (median age, 63 years) from the Women's Health Study were examined. Most were postmenopausal (99.3%). The relationships between rs2234693 and rs9340799 genotypes and their associated haplotypes with obesity (BMI ≥30 kg/m^2) and overweight (BMI ≥25 kg/m^2) were evaluated. Among women with the rs2234693 TT genotype, 18.3% were obese, whereas only 8.2% of those with the CC genotype were obese ($P = .04$). In a logistic regression model assuming additive inheritance, rs2234693 was associated with decreased odds of obesity (BMI ≥30 kg/m^2) (crude odds ratio = 0.63, 95% confidence interval = 0.44-0.90, $P = .01$). For rs9340799, only an inverse trend was observed for BMI ($P = .08$). Haplotypes that included the variant C allele were associated with a reduced risk of obesity (crude odds ratio = 0.65, 95% confidence interval = 0.44-0.94, $P = .02$ for C-G). The rs2234693 C allele of *ESR1* and its associated genotypes and haplotypes were inversely and consistently associated with obesity. One or more copies of the C allele were associated with decreased risk of obesity in white postmenopausal women.

1. Introduction

Obesity is a complex disease, which can be influenced by multiple genetic and environmental factors [1]. Recently, the Human Obesity Gene Map listed the estrogen receptor α gene (*ESR1*) as 1 of 127 possible candidate genes associated with obesity [2]. *ESR1* resides on chromosome 6q25 and is composed of 8 exons and 7 introns with a total size of 140 kilobases [3].

Estrogen receptor (ER)–α expression has been related both to menopausal status and adiposity in women [4,5]. Polymorphisms of *ESR1* have been also associated with variations in body mass index (BMI) and waist circumference [6-11]. In a Japanese study, the rs9340799 polymorphism, particularly the GG genotype, was associated with decreased whole-body and abdominal fat in older women [7]. In the Framingham Heart Study, 3 *ESR1* polymorphisms (rs2234693, rs9340799, and rs1801132) were associated with measures of adiposity in men, but not women [9]. A number of other small studies have found diverse results for the relationship between BMI and *ESR1* polymorphisms [6,8,10].

However, relatively few studies of genetic variation of *ESR1* and obesity in postmenopausal women have been conducted [10]. The purpose of this study was to examine the relationship of 2 polymorphisms in *ESR1* (rs2234693 and rs9340799) with risk of overweight or obesity in women.

2. Materials and methods

2.1. Subjects

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 white, US health professionals 45 to 89 years of age at study entry [12,13]. Before randomization, 28 524 participants provided an EDTA anticoagulant blood sample

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that was stored for genetic analysis. All participants were free of prior myocardial infarction, stroke, or any serious illness that might preclude participation at study entry [12]. Women enrolled in the WHS completed a baseline questionnaire, which included questions on demographics, health characteristics/behaviors (height, weight, alcohol use, diet, smoking status, physical activity, hormone therapy [HT] use), menopausal status, medical history of hypertension, diabetes mellitus, and elevated cholesterol. For those women 60 years or older, self-report of permanent cessation of menstrual periods due to natural menopause, complete oophorectomy, radiation, or chemotherapy was considered postmenopausal status. Yearly follow-up self-report questionnaires were used to update information [12].

Data on *ESR1* genetic variation were available for 543 white WHS participants who had previously been selected as control subjects for a nested case-control study of genetic variation of *ESR1* and cardiovascular disease. Of these, 539 (99.3%) were postmenopausal, with an additional 4 women who had biologically uncertain menopause. The study protocol was approved by the Brigham and Women's Hospital Institutional Review Board for Human Subjects Research.

2.2. Exposure variables

Baseline BMI (self-reported weight in kilograms divided by the square of self-reported height in meters) was analyzed as a continuous and categorical variable, according to the World Health Organization (WHO) criteria as follows: less than 25 kg/m^2 (normal), 25 to less than 30.0 kg/m^2 (overweight), and at least 30 kg/m^2 (obesity) [14]. The category of "nonobese" (BMI <30 kg/m^2) was also examined. Self-reported weight was highly correlated (r = 0.96) in a similar population of female health professionals [15].

2.3. Genotype determination

Two single nucleotide polymorphisms in the *ESR1* gene (rs2234693 and rs9340799) were chosen based on prior associations for cardiovascular disease in the literature [8,16]. Both of these polymorphisms are in the first intron of *ESR1* gene, 397 and 351 base pairs upstream of exon 2. The rs2234693 polymorphism is characterized by a T→C transition (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 (also known as *c.454-351A>G*). Those with the G allele have an absent *Xba*I site that has previously been called *X* in the literature, with the A allele denoted by *x*. This polymorphism has previously also been called *IVS1-354A/G*.

Genotype determination was performed using 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) and 10 ng of template DNA. Amplification reactions were carried out in duplicates on ABI 7900HT Sequence Detection System according to the manufacturer's specifications.

To confirm genotype assignment, 2 independents observers carried out scoring. Discordant results (1% of all scoring) were resolved by a joint reading and, where necessary, another genotyping.

2.4. Statistical analysis

The baseline characteristics were examined according to BMI categories based on the WHO criteria [14]. Based on nonparametric distributions, all continuous variables were examined by Kruskal-Wallis test or Wilcoxon rank-sum [17], whereas χ^2 test was used for categorical variables. Genotype and allele frequencies were calculated, and Hardy-Weinberg equilibrium was tested using χ^2 analysis. As prespecified in our analysis plan, the association between *ESR1* genotypes and adiposity was examined first using BMI as a continuous variable, then according to WHO BMI criteria, in 3 categories (BMI <25 kg/m^2, 25-29.9 kg/m^2, ≥30 kg/m^2), obesity (BMI <30 kg/m^2 vs ≥30 kg/m^2), and overweight (BMI <25 kg/m^2 vs ≥25 kg/m^2) [17]. General linear regression models were used to determine whether BMI varied according to *ESR1* genotypes. In addition, logistic regression was used to examine the relationship between dichotomous outcomes: obese vs nonobese as well as overweight vs normal. Additional adjustment for age at randomization, age at menopause, smoking status (never, past, and current), physical activity (rarely/never, <1/wk, 1-3/wk and ≥4/wk), and HT use (yes/no) was also performed. All analyses were conducted assuming an additive mode of inheritance. Possible associations between several covariates (alcohol and red wine consumption; energy-adjusted total intakes of carbohydrate, protein, and saturated, monounsaturated, and polyunsaturated fats; educational level; marital status; and income) were evaluated and considered in the regression model. We considered all potential confounders with *P* value (from likelihood ratio) less than .2 or with some pathophysiology link with BMI. Potential interactions between HT use and *ESR1* genotypes were tested using a formal interaction term (genotype ∗ HT) as well as in analyses stratified by HT. Pairwise linkage disequilibrium (LD) was examined as described by Devlin and Risch [18]. We also planned our haplotype analyses to offer insight into the relation between the 2 polymorphisms together and our outcome (obesity and overweight), which is not available from analyzing individual polymorphisms [9]. Haplotype frequencies were estimated from genotype data using the PHASE v2.1 algorithm [19,20]. For each odds ratio (OR), we calculated 95% confidence intervals (CIs). A 2-tailed *P* value of .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).

3. Results

Baseline characteristics of the 543 women are shown in Table 1. The median age was 63 years, and most (99.3%) were postmenopausal. The prevalence of BMI of at least 25 kg/m^2 was 46%.

The allele frequencies for rs2234693 were 54% for T and 46% for C alleles, whereas for rs9340799, the frequencies were 66% for A and 34% for G alleles.

Median BMI did not differ by genotype for either polymorphism (data not shown). A trend toward an inverse association between BMI and rs2234693 genotypes (P = .09) was observed across BMI categories (≥30 kg/m^2, 25-<30 kg/m^2, <25 kg/m^2) (data not shown). Of those with the TT genotype, 18.3% were obese, compared with 13.5% of those with TC and only 8.2% of those with CC genotypes (Table 1). When obese women were compared with nonobese women (<30 kg/m^2), the inverse association between BMI and rs2234693 genotype was more evident (P = .04) (data not shown). We found no evidence for any significant association with BMI categories and rs9340799 genotypes.

No significant association between BMI as a continuous variable and *ESR1* genotypes was observed in general linear regression models (data not shown). In logistic regression analyses, women with the rs2234693 polymorphism had a reduced risk of obesity (age-adjusted OR = 0.63 [95% CI, 0.44-0.90] for BMI ≥30 kg/m^2 compared

Table 1
Baseline characteristics according to BMI in 543 apparently healthy white women in the WHS

Characteristics	BMI (kg/m^2)			
	Normal (<25)	Overweight (25-<30)	Obese (≥30)	*P*
Age[a] (IQR)	63 (57-68)	64 (59-69)	61 (57-65)	.03
Age at menopause[a] (IQR)	49 (45-52)	49 (44-52)	49 (45-52)	.75
Total fat[b] (IQR)	57.5 (50.0-64.9)	58.6 (50.1-65.3)	59.9 (53.3-69.7)	.12
Saturated fat[b] (IQR)	19.4 (16.1-22.7)	19.6 (17.0-22.7)	20.6 (18.3-24.8)	.06
Hormone use (%)				.004
Never	32.1	31.3	46.0	
Past	19.5	27.8	28.4	
Current	48.5	40.9	25.7	
Smoking status (%)				.41
Never	41.3	44.3	48.7	
Past	35.8	38.6	36.5	
Current	22.9	17.1	14.9	
Alcohol (%)				.05
Rarely/never	43.3	46.0	62.2	
1-3 drinks/mo	13.7	17.1	10.8	
1-6 drinks/wk	30.4	23.9	23.0	
≥1 drink/d	12.6	13.1	4.1	
Exercise (%)				.001
Rarely/never	35.5	35.8	54.1	
<1/wk	19.1	18.2	23.0	
1-3/wk	28.7	36.9	17.6	
≥4/wk	16.7	9.1	5.4	
Total cholesterol ≥240 mg/dL	50.0	36.1	13.9	.28
Hypertension, %[c]	39.8	39.8	20.5	<.0001
Diabetes, %	17.7	47.1	35.3	.003
Genotype distribution rs2234693[d]				
TT	54.4	27.2	18.3	.10
TC	52.5	34.0	13.5	
CC	54.9	36.9	8.2	
rs9340799				.65
AA	52.5	31.5	16.0	
AG	55.1	32.7	12.2	
GG	54.4	35.4	10.1	

P values were obtained from Kruskal-Wallis (nonparametric) for continuous variables and χ^2 for categorical variables. Exact *P* values were considered for genotype association. IQR indicates interquartile range.

[a] 25th to 75th percentile.

[b] All macronutrients (in milligrams per day) were adjusted for total energy intake.

[c] Hypertension defined as physician diagnosis of hypertension or reported blood pressure of greater than 140 mm Hg systolic or greater than 90 mm Hg diastolic blood pressure.

[d] rs2234693 genotype distribution was in Hardy-Weinberg equilibrium.

Table 2
Odds ratios for obesity and overweight in 543 apparently healthy white women in the WHS, according to *ESR1* genotypes (additive model)

	OR[b] (OR, 95% CI, *P*)		Adjusted OR[c] (OR, 95% CI, *P*)	
Overweight[a]		*P*		*P*
rs2234693				
Normal	Referent (1.0)		Referent (1.0)	
Overweight	0.99 (0.79-1.26)	.97	0.96 (0.75-1.24)	.75
rs9340799				
Normal	Referent (1.0)		Referent (1.0)	
Overweight	0.94 (0.74-1.19)	.62	0.89(0.69-1.15)	.39
Obese[a]		*P*		*P*
rs2234693				
Nonobese	Referent (1.0)		Referent (1.0)	
Obese	0.63(0.44-0.90)	.01	0.57(0.39-0.84)	.005
rs9340799				
Nonobese	Referent (1.0)		Referent (1.0)	
Obese	0.73 (0.51-1.04)	.08	0.69(0.46-1.04)	.07

[a] Overweight women (25.0-> 30.0 kg/m^2) were compared with women with normal BMI (<25.0 kg/m^2). Obese women (≥30.0 kg/m^2) were compared with nonobese women (BMI <30.0 kg/m^2).

[b] Adjustment by age.

[c] Multivariate adjustment by age at randomization, age at menopause, hormones use, exercise, educational level, alcohol consumption, smoking status, and total fat adjusted for energy intake.

with BMI <30 kg/m^2). Additional adjustment slightly strengthened the association with obesity (Table 2). For rs9340799, there was only a trend in the same direction when comparing obese with nonobese women (*P* = .08). No association was found between overweight and *ESR1* polymorphisms.

In stratified analyses, we explored potential effect modifications by hormone use. The rs2234693 polymorphism was associated with decreased risk of obesity among women who did not use HT (*P* = .002), but not for HT users (*P* = .98) (Fig. 1). However, very few hormone users were obese (n = 16). No significant association between obesity and rs9340799 according to HT use was found (Fig. 2). The

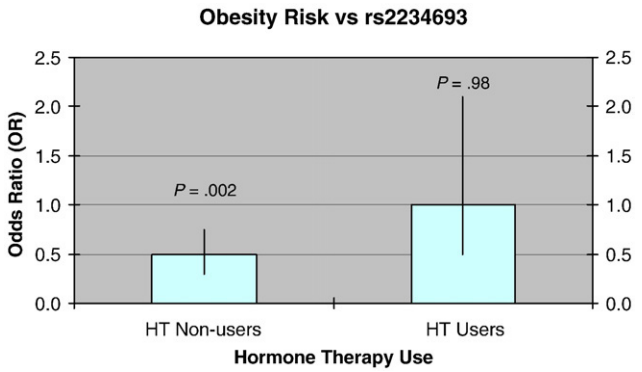

Fig. 1. Obese women (≥30.0 kg/m^2) were compared with women with BMI less than 30.0 kg/m^2, according to HT use. *P* values were obtained from multivariate logistic regression (adjusted for age at randomization, age at menopause, exercise, educational level, alcohol consumption, smoking status, and total fat adjusted for energy intake).

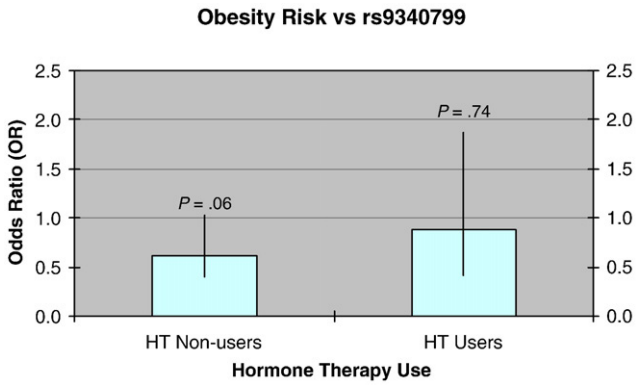

Fig. 2. Obese women (≥30.0 kg/m^2) were compared with women with BMI less than 30.0 kg/m^2, according to HT use. *P* values were obtained from multivariate logistic regression (adjusted for age at randomization, age at menopause, exercise, educational level, alcohol consumption, smoking status, total fat adjusted for energy intake).

interaction term between hormone use and s2234693 was of borderline significance (*P* interaction = .13), whereas for rs9340799, there was no suggestion of an interaction (*P* for interaction term = .41).

The polymorphisms tested were in LD (normalized Lewontin *D*' = 0.96). Of 4 possible haplotypes, 1 haplotype (T-G) had a frequency of less than 5%. The most common haplotype, T-A, had a frequency of 52%; and the C-A and C-G haplotypes had frequencies of 13% and 33%, respectively. In logistic regression analyses adjusting for the same covariates used in the genotype models, a decreased risk of obesity among women with the C-G haplotype was observed (OR = 0.65 [95% CI, 0.44-0.94], *P* = .02) (Supplemental table 1). Furthermore, adjustment resulted in only minor changes in risk estimates.

4. Discussion

In these mostly postmenopausal women, one or more copies of the variant C allele of *ESR1* rs2234693 and its associated haplotypes were associated with decreased risk of prevalent obesity. This association appeared strongest among women who were not using postmenopausal HT.

Although there has been controversy about the relationship between *ESR1* polymorphisms and BMI, our findings are similar to several other reports [7,8,10]. Differences in representation by sex, menopausal status, and use of HT may explain some differences across studies. The Framingham Heart Study investigated the association between the *ESR1* polymorphisms and BMI as well as the relationship with waist circumference in 1763 unrelated men and women (mean age, 56 years) [9]. In this population, men homozygous for the rs2234693 C allele had lower waist circumference (99.3 cm) than TT homozygotes (99.8 cm) and heterozygotes (100.6 cm) (*P* > .004). Similar results were also observed for the rs9340799 polymorphism. Although no

association was observed for women, this may have been due to a smaller sample size in women, as well as fewer postmenopausal women [9]. A Brazilian case-control study of 295 subjects (mean age, 44 years), investigated the association of *ESR1* polymorphisms with premature coronary artery disease and some cardiovascular risk factors [8]. In this study, the rs9340799 polymorphism, but not rs2234693, was associated with lower BMI; but results were not presented separately for men and women [8]. Okura et al [7] also found a similar inverse association with adiposity parameters and the rs9340799 polymorphism in 2238 middle-aged and elderly Japanese population. Older women (60-79 years) homozygous for the variant (GG) had a lower whole-body fat mass and waist circumference compared with those homozygous for wild type [7]. No difference was found for rs2234693, with the exception of a slightly lower mean BMI among older men (60-79 years) with the CC genotype (TT>CC, $P = .03$) [7]. Although some previous studies included postmenopausal women in their analyses, few have studied the association between *ESR1* and adiposity only in this population [10]. Deng et al [10] examined the association of the *ESR1* rs2234693 genotypes and BMI in a small sample of 108 healthy postmenopausal white women. In contrast to our study, women with TC and CC genotypes had, respectively, 4.8% and 11.4% higher mean BMI than those with the TT genotype [10]. A similar but nonsignificant trend was also observed for the rs9340799 genotype, which is not unexpected given the tight LD between these 2 polymorphisms.

Estrogen receptor–α expression has been related to menopausal status and obesity [4]. Meza-Munoz et al [4] found lower levels of ER-α and progesterone receptors in adipose tissue of postmenopausal than premenopausal women. Nonobese menopausal women had lower levels of ER-α expression in adipose tissue ($P < .03$) than postmenopausal obese women. In postmenopausal women, the predominant source of estrogens is adrenal steroids converted to bioactive estrogens in fat tissue [21]. It has been proposed that ER-α determines the tissue sensitivity to estrogen [22]. In addition, *ESR1* variation may influence endogenous estradiol levels. In postmenopausal women, the TA haplotype of rs2234693 and rs9340799 was associated with an allele dose-dependent decrease in estradiol levels [23]. In our study, although we had limited power in hormone users, we found a suggestion that the inverse association between *ESR1* polymorphisms and obesity was most pronounced among postmenopausal women who were not using exogenous estrogen therapy. Exogenous hormone use might obscure an *ESR1* effect.

This study has several strengths and limitations. It is one of the largest reported studies of *ESR1* polymorphisms and obesity in postmenopausal women. Of note, our analyses were restricted to white participants and thus cannot necessarily be applied to other racial or ethnic groups. Although we have observed an inverse association between these polymorphisms and obesity in women, we have no direct evidence of causative relationship for these particular polymorphisms. We had limited power to detect interactions or perform subgroups analyses. However, the current results do replicate findings of some prior groups.

In conclusion, we found a significant inverse association between the *ESR1* rs2234693 polymorphism and prevalent obesity in women. Carriers of the rs2234693-C minor allele had a 38% lower prevalence of obesity. The mechanisms by which the *ESR1* gene might be related to obesity deserve further exploration.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2009.01.003.

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