0021-972X/00/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 85, No. 8
Printed in U.S.A.

Association of Estrogen Receptor-α Genotypes with Body Mass Index in Normal Healthy Postmenopausal Caucasian Women*

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ABSTRACT

Several lines of evidence suggest the importance of the estrogen receptor (ER) in determining body mass index (BMI). Our purpose was to investigate whether genetic polymorphisms at the restriction enzyme *Pvu*II site of the ER-α gene locus are associated with BMI variation. Data on BMI, age, and ER-α genotypes were obtained from 108 healthy midwestern U.S. postmenopausal Caucasian women. The study subjects were unrelated and aged 65 yr and over (mean age ± SD, 73.4 ± 5.1 yr), with an average BMI of 25.25 (SD, 4.04). The ER-α genotypes were obtained by PCR followed by restriction enzyme *Pvu*II digestion. We found that in our study subjects the ER-α genotypes are significantly associated with BMI (by ANOVA, $P = 0.04$), explaining about 6.2% of the BMI variation in our study sample. The allelic effects of this locus on BMI are approximately additive. In our sample, individuals of the PP and Pp genotypes have, respectively, 11.4% and 4.8% higher BMI than individuals of the pp genotype. There is a significant ER-α genotype by age interaction, so that in our sample PP individuals tend to gain weight with age, whereas Pp and pp individuals tend to lose weight with age. Therefore, the ER-α polymorphisms are associated with BMI variation in healthy postmenopausal Caucasian women aged 65 yr and over. Our result is consistent with some recent findings suggesting the potential effects of the ER on BMI. The importance of the ER-α genotypes in other populations and other age groups needs to be demonstrated. Although the results of the ER-α genotype by age interaction are obtained here from cross-sectional data, direct confirmation may come from longitudinal studies in which individuals are measured multiple times over several years. The importance of the ER-α genotypes on BMI should be confirmed by further studies using methods robust to the potential problem of population substructuring that may confound the conclusions of population association studies. (*J Clin Endocrinol Metab* **85:** 2748–2751, 2000)

BODY MASS INDEX (BMI; defined as the weight/height^2 ratio in the units of kilograms/meters^2) is an important index of morbid obesity (1–3). BMI is a complex quantitative trait (measured on continuous scales), and it is determined by multiple genetic and/or environmental factors (1–3). As obesity becomes an increasingly serious health problem in the western world (2–3), extensive genetic studies have been launched to search for genes underlying the BMI variation (4). Many candidate gene loci or genomic regions have been identified (4), although the results from different approaches and even those from the same approach may not be consistent with one another.

The polymorphisms of the gene for the α-type estrogen receptor (ER-α) are reportedly associated with ER expression, altered ER function, some disorders (*e.g.* breast cancer, hypertension, and spontaneous abortion), and bone mass density variation (for references, see Refs. 5–7). Several lines of recent evidence suggest a potential role for the ER in the determination of BMI: 1) a subtype of ER may have a role in ER-mediated responses in human adipose tissue (8); 2) a potent estrogen agonist/antagonist decreases fat body mass in aged female rats (9); 3) a selective ER modulator increases body mass (10); 4) the progesterone/estrogen receptor ratio correlates positively with BMI, which may reflect a correlation between body mass and serum estrogens (11); and 5) BMI is associated with ER level in breast cancer patients (12).

In this study we report an association of BMI with genotypes at the ER-α gene locus, quantify the magnitude of the ER-α genotypic effects on BMI variation, and reveal an interaction effect between the ER-α genotypes and age in determining BMI variation. To the best of our knowledge, this is the first study reporting genotypic effects of the ER-α locus on BMI variation.

Subjects and Methods

Subjects

The 108 subjects in this study came from the elderly women in a study of low dose, continuous, estrogen/progestin (LDE) recently completed in our center (5, 6, 13). They were unrelated, healthy, postmenopausal women from midwestern U.S. The data employed for this study were those measured at the time of study entry, *i.e.* before any subject had taken any medication. Their ages ranged from 65.0–87.4 yr, with a mean age ± SD of 73.4 ± 5.1 yr. Their height ranged from 1.430–1.717 m, with a mean height ± SD of 1.580 ± 0.060 m. Their weight ranged from 42.0–100.8 kg, with a mean ± SD of 63.4 ± 11.2 kg. Their BMI ranged from 17.15–39.04 kg/m^2, with a mean ± SD of 25.25 ± 4.04 kg/m^2. They were a relatively homogenous ethnic group, all Caucasians of European origin.

Received November 10, 1999. Revision received April 20, 2000. Accepted May 2, 2000.

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* This work was supported by grants from the NIH, the Health Future Foundation, and HuNan Normal University.

The subjects were chosen randomly to enter the LDE study according to the following entry and exclusion criteria. The principal entry criterion was a spinal bone mineral density (BMD) less than 0.90 g/cm^2 measured by dual energy x-ray absorptiometry with a Norland XR-26 scanner (software version 2.2.1; Norland Corp., Ft. Atkinson, WI). The average BMD z-score for all study subjects was −0.182 (SD, 0.834). The BMD z-scores are standard normal values obtained by adjusting BMD measurements for age with general referent population data provided by the scanner manufacturer. Low BMD values were selected for the study subjects with an aim to investigate the ability of low dose, continuous, estrogen/progestin to increase or preserve BMD in elderly women with relatively low BMD. Exclusion criteria were previous hip fracture, estrogen replacement or treatment with calcitonin in the previous 6 months, any treatment with bisphosphonates or fluoride, treatment with corticosteroids for more than 6 months duration, any corticosteroid treatment within the previous 6 months, and excessive cigarette smoking, defined as more than 10 cigarettes/day. Treatment with thyroid hormone was accepted provided the subject was euthyroid, and serum TSH was normal. Good health and absence of major organ system disease were documented by clinical examination and blood chemistry profile. The study was reviewed and approved by the Creighton University institutional review board. Each subject provided written informed consent before entering the study.

Of the 108 study subjects who were analyzed for BMI, 21 did not have body scans at the baseline before entering the LDE study. Of the remaining 87 subjects who did have body scans at the baseline, 8 had prosthetics, such as hip or knee replacements, which render body measurements invalid. Whole body scans by the Norland XR-26 scanner can give results for body mass composition as bone mass, fat mass, and lean mass. Therefore, there were 79 individuals who had fat mass measurements.

Genotyping

DNA was isolated from whole blood using the Puregene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN). The oligonucleotide primers used to determine the *Pvu*II polymorphisms within the ER-α gene were those used by Yaich (14) (forward, 5′-CTGCCACCCTATCTGTATCTTTTCCTATTCTCC-3′; reverse, 5′-TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA-3′). After an initial denaturing at 94 C for 5 min, PCR was conducted through 30 cycles of the following steps: denaturation at 94 C for 30 s, annealing at 62 C for 20 s, and polymerase extension at 72 C for 90 s. After cycling, a final extension at 72 C for 10 min was performed. After amplification, the PCR product of approximately 1.3 kb was digested with restriction endonuclease *Pvu*II, and electrophoresed in a 1.5% agarose gel. This PCR product contains a part for intron 1 and exon 2 of the ER-α gene. The *Pvu*II restriction fragment length polymorphism is a commonly employed marker for genetic analyses of the ER gene, and it is located in intro I of the ER-α gene. Gels were stained with ethidium bromide, visualized under UV light, and photographed. The absence of the *Pvu*II site was designated the P allele, and the presence of this restriction enzyme cutting site was designated the p allele.

Statistical analyses

All statistical analyses were conducted using SAS (15). The genotype frequencies were tested against Hardy-Weinberg ratios by $\chi 2$ tests. Normality of the BMI data within each of the three ER-α genotypes was tested by graphic methods (16). Bartlett's test (16) was performed to test the homogeneity of variances in BMI for the individuals of the three ER-α genotypes. One-way ANOVA was performed to test the association between BMI variation and the ER-α genotype (Fig. 1). Multiple regression analysis was performed for BMI. The regressor variables were age, ER-α genotype, and the interaction between them modeled as the product of these two factors. Outlier diagnosis for the regression analysis was performed by the R-Student statistic (15). The assumptions of the regression analysis were inspected with residual plot analyses. In multiple regression analyses, the three ER-α genotypes were coded numerically, with 0 for PP, 1 for Pp, and 2 for pp, respectively. This coding reflects the number and the amount of additive effects of the p allele in an individual. This coding can be justified by the finding (Fig. 1) that the within-locus allelic effects at the ER-α locus are approximately additive,

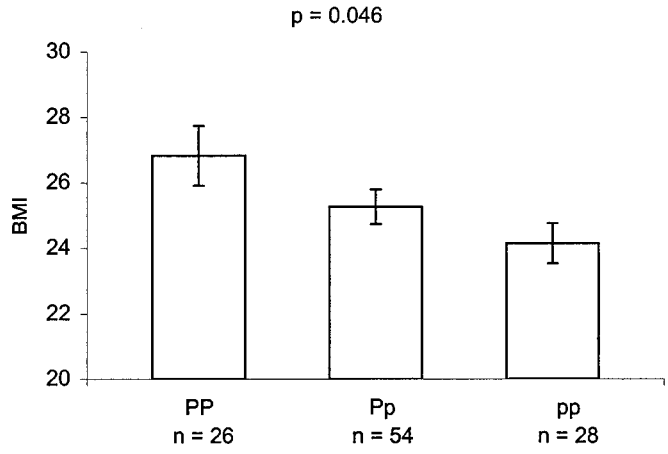

FIG. 1. Distribution of BMI in the three ER-α genotypes in the study subjects. The mean and SE of BMI for each ER-α genotype are plotted. The *P* value of the one-way ANOVA for testing the ER-α genotype effects on BMI is given, as is the sample size (n) for each ER-α genotype.

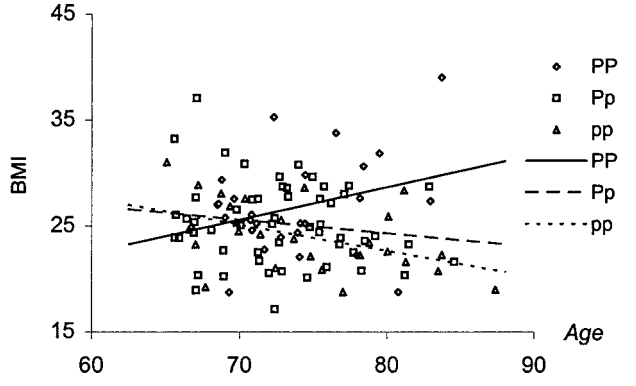

FIG. 2. Different dynamics of BMI with aging for the three ER-α genotypes in the study subjects. Plotted are the three lines of the regression of BMI on age for each of the three ER-α genotypes.

so that pp has the smallest BMI, Pp has an intermediate BMI, and PP has the highest BMI. The proportion of the total BMI variance that is attributable to the ER-α genotype is obtained with the VARCOMP procedure in SAS (15). As a significant ER-α genotype by age interaction was found, separate regression analyses were performed for BMI within each of the three ER-α genotypes, with age as the regressor variable. The three regression lines, the BMI and age data for each individual within each of the three genotypes, were plotted in Fig. 2 to demonstrate intuitively the interaction between age and ER-α genotypic effects. Among the 79 subjects who had fat mass measurements, we performed statistical analyses for body fat mass similar to the analyses we performed for BMI.

Results

The ER-α genotype frequencies were in close agreement with Hardy-Weinberg ratios (by χ^2 test, $P = 0.997$). There were 26 individuals with the PP genotype, 54 with the Pp genotype, and 28 with the pp genotype. The P allele frequency was 0.49, and the p allele frequency was 0.51.

The analyses did not reveal any significant violation of the assumptions of ANOVA and regression analyses. For example, the *p* value was 0.22 for Bartlett's test for homogeneity of variances of BMI for the three ER-α genotypes. In our

study sample of 108 elderly Caucasian women, ANOVA revealed significant ER-α genotypic effects ($P = 0.04$) for BMI, explaining about 6.2% of the BMI variation. In our study subjects, on the average, individuals of the pp genotype had the lowest BMI [24.11 $\pm$ 0.63 ($\pm$SE) kg/m^2], individuals of the Pp genotype had intermediate BMI (25.26 $\pm$ 0.53 kg/m^2), and individuals of the PP genotype had the highest BMI (26.85 $\pm$ 0.91 kg/m^2). Thus, ER-α genotypic effects on BMI are approximately additive (Fig. 1). In our study subjects, individuals with Pp and PP genotypes had, respectively, approximately 4.8% and 11.4% higher BMI than those with the pp genotype. There was a significant ($P = 0.01$) ER-α genotype by age interaction, as revealed by the partial regression coefficient for the interaction term in the multiple regression analysis (Table 1). This interaction signals that the dynamics of BMI with aging were not the same for the three ER-α genotypes and that the differences among genotypes depends on the specific age or age ranges of the subjects. With the caveat of cohort effects, Fig. 2 clearly shows that individuals of the PP genotype tend to gain weight with age, whereas those of Pp and pp genotypes tend to lose weight with age. It is also clear (Fig. 2) that, due to the ER-α genotype by age interaction, individuals of the PP genotype tended to have higher BMI than individuals of Pp and pp genotypes beyond the age of about 70 yr, whereas below the age of about 70 yr, the Pp and pp genotypes tended to have higher BMI than PP individuals. However, overall in our sample, PP individuals had, on the average, the highest BMI despite the ER-α genotype by age interaction. This is because most of the study subjects were elderly women over 70 yr old (Fig. 2).

Although we found that fat mass tended to be greatest for the PP genotype, intermediate for the Pp genotype, and the smallest for the pp genotype (Fig. 3), consistent with the findings in Fig. 1 for BMI, the test result was not significant ($P = 0.50$). It is not clear whether the lack of significance is due to sample size differences or other factors. Other analyses of fat mass also showed patterns of results similar to those for BMI, but none achieved statistically significant results. Larger samples will be needed to examine genotypic effects of the ER-α locus on fat mass.

Discussion

To the best of our knowledge, this is the first report on the association of the ER-α genotypes with BMI variation that suggests a potential role for the ER-α genotypic effects on BMI for women. The *Pvu*II restriction fragment length polymorphism is located in intro I of the ER-α gene. The mutation at the *Pvu*II restriction site itself is thus unlikely to be of functional importance. However, our results suggest that there is a functional mutation in the ER gene that is significant for BMI variation. Therefore, if our results are robust upon further studies in different populations, further detailed molecular genetic studies of the ER gene are warranted to identify the functional mutation.

The findings from this study were consistent with previous evidence suggesting a potential role of ER-α in determining BMI. The genomic location of the ER-α gene is on 6q25.1 in the human genome (http://www3.ncbi.nlm.nih.gov/Omim). Interestingly, this genomic location coincides with the syntenic location in the human genome (6q25-q27) that harbors an important quantitative trait locus (QTL) for BMI, as suggested by QTL mapping in mice (4, 17). As stated in the introduction, gene-searching results of different studies using various approaches and even those using the same approach (including QTL mapping studies) are often inconsistent. Therefore, our results here serve at least as a valuable confirmatory study for the importance of the genomic region 6q25-q27. Most importantly, genomic regions identified in QTL mapping studies are generally large (*e.g.* ~10–30 cM in length). There are usually dozens of genes in such a genomic region and which one may be important is usually not clear without extensive further studies. Therefore, our results are important in explicitly suggesting that the ER-α gene may be the gene important for BMI variation in the genomic region 6q25-q27 identified in the previous QTL mapping study.

The significant ER-α genotype by age interaction is detected here from cross-sectional data; direct confirmation may come from longitudinal studies in which individuals are measured multiple times over several years. The finding of an ER-α genotype by age interaction suggests that appropriate sampling and/or statistical analysis to control for the age of the study subjects may be necessary to detect ER-α

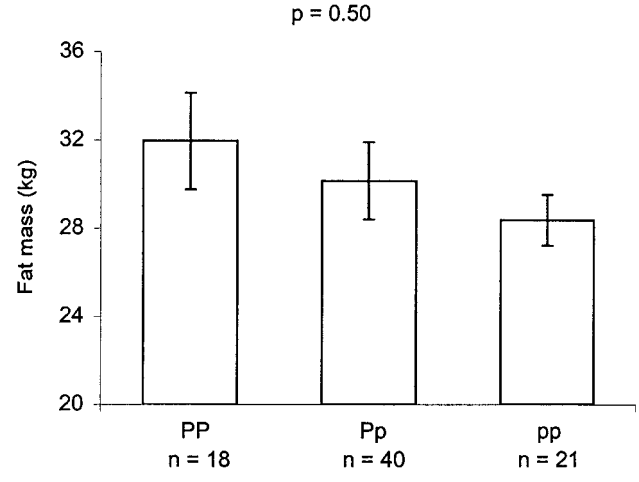

FIG. 3. Distribution of fat mass in the three ER-α genotypes in the study subjects. The mean and SE of fat mass for each ER-α genotype are plotted. The P value of the one-way ANOVA for testing the ER-α genotype effects on fat mass is given, as is the sample sizes (n) for each ER-α genotype.

TABLE 1. Multiple regression analyses of BMI

	Intercept	Age	ER	Age × ER	Adjusted r^2
BMI	10.86 (0.30)	0.21 (0.13)	17.50 (0.02)	−0.254 (0.01)	0.10

Note, the numbers reported are the partial regression coefficients and their associated significance levels (P values, numbers in *parentheses*). ER, The genotypes at the *Pvu*II site of the ER-α gene.

genotypic effects. The ER-α genotype by age interaction implies that the magnitude and the direction of the ER-α genotypic effect depends on the age of the subjects. Therefore, in future studies to substantiate the ER-α genotypic effects on BMI in other populations and/or other age groups, care should be taken to control for the age of the study subjects. Either study subjects within a narrow age range should be collected and analyzed, or BMI should be adjusted for the ages of the study subjects by statistical methods such as multiple regression for each genotype respectively. Although significant associations of the ER-α genotypes with BMI were detected in our sample, the magnitudes of the ER-α genotypic effects on BMI were relatively small. Nevertheless, the genetic effects were significant, so that different genotypes can differ by up to about 11.4% of the average BMI, a magnitude that can potentially have significant clinical implications and applications.

Significant genetic results generated by regular population association studies that are not family based may be plagued by the problem of population admixture (18–21). Therefore, future research should be conducted to eliminate this possibility using approaches such as the transmission disequilibrium test (22). As various genotypes at the ER-α locus may have different effects at different ages, the ages of the study subjects should be considered and properly controlled or adjusted in future studies.

Acknowledgments

C. T. Parrish helped with the genotyping for this study. We are grateful to the two anonymous reviewers for their constructive comments that helped to improve this manuscript.

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