Association of polymorphisms in the estrogen receptor α gene with body fat distribution

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OBJECTIVE: To examine whether polymorphisms of the estrogen receptor (ER) α gene are associated with body fat distribution.

DESIGN: Cross-sectional, epidemiological study of two single-nucleotide polymorphisms, a T → C (Pvu II) and an A → G (Xba I), in the first intron of the ER α gene.


MEASUREMENTS: The ER α genotypes (by automated fluorescent allele-specific DNA primer assay system), anthropometric variables, fat mass (FM) and percentage FM (%FM) (by dual-energy X-ray absorptiometry).

RESULTS: FM and waist were inversely associated with age (r = −0.630 and −0.504, respectively) in women with the GG genotype. On the other hand, waist circumference of the AA genotype was positively correlated with age (r = 0.231). Thus, for middle-aged women (40–59 y) with the AG or GG genotype body mass index (BMI), %FM, FM, waist, hip and waist-to-hip ratio (WHR) were larger than those with the AA genotype. In particular, FM and waist were greater by 20% and 9%, respectively, for the GG genotype, compared to the AA genotype. Alternatively, FM and waist were smaller by 18% and 6%, respectively, in older women with the GG genotype, compared to the AA genotype. No effect was found among the A → G polymorphisms for men.

CONCLUSION: No association was found between the ER α gene polymorphisms and body fat distribution in men. For women, the A → G polymorphism, in particular the GG genotype, may contribute to the development of upper-body obesity in middle-aged individuals, but may serve to decrease the whole-body and abdominal fat tissue of older individuals.


Keywords: estrogen receptor gene; polymorphism; body composition; waist; waist-to-hip ratio

Introduction

It has been found that body fat distribution is an important factor in coronary heart disease (CHD). In particular, a large waist circumference or waist-to-hip ratio (WHR) is closely associated with an increased prevalence of risk factors for CHD, for example, impaired glucose tolerance, insulin resistance, lipoprotein metabolic disorder and hypertension.1–3 Though, in general, upper-body or android-type obesity, with a large waist or WHR, is more frequently observed in men compared with women, this obesity phenotype is also observed fairly often in postmenopausal women.4 This is because estrogen deficiency during the normal menopausal transition accelerates the selective deposition of intra-abdominal fat.5 With respect to estrogen’s association to body fat distribution, several authors6–10 have reported that estrogen hormone replacement therapy had desirable effects on body fat distribution in postmenopausal women. These findings suggest that estrogen plays an important role in the modification of body fat distribution.

More recently, associations have been found between estrogen receptor (ER) α gene polymorphism and bone mineral density,11–18 pathogenesis of type II diabetes,19 and susceptibility to or age of onset of autoimmune diseases such as multiple sclerosis.20 The human ERα gene is located on chromosome 6p25.1, is comprised of eight exons, and spans
To our knowledge, little has been reported on the association between the ER gene polymorphisms and body fat distribution. In clinical settings for obesity treatment, understanding this association would be helpful not only for early preventative treatment of upper-body obesity but also for predicting the effects of estrogen replacement therapy on the modification of body fat distribution. The purpose of this study, therefore, was to examine whether the T→C (PvuII) and A→G (Xbal) polymorphisms of the ERz gene, alone or in combination, are associated with body fat distribution in a middle-aged to elderly Japanese population.

Methods

Subjects

There were 1110 women and 1128 men who participated in the first wave of examinations in the National Institute for Longevity Sciences–Longitudinal Study of Aging (NILS-LSA) from April 1998 to March 2000. There were randomly sampled, community-dwelling individuals aged 40–79 y, stratified by age and gender and living in the neighborhood of the NILS. Details of the NILS-LSA have been described elsewhere. The aim and design of the study were explained to each subject before they gave their written informed consent. The study was approved by the Committee of the Chubu National Hospital.

Determination of ERz genotypes

The ERz genotypes were determined in accordance with a study by Yamada et al. The ERz gene was analyzed with an automated fluorescent allele-specific DNA primer assay system (Toyobo Gene Analysis, Osaka, Japan). To determine the T→C (PvuII) genotype, the polymorphic region of the gene was amplified by polymerase chain reaction with allele-specific sense primers labeled at the 5′ end either with fluorescein isothiocyanate (5′-AGTTCCAATGTCTCCAGXGTG-3′) or with Texas red (5′-AGTTCCAATGTCTCCAGXCG-3′) and an antisense primer labeled at the 3′ end with biotin (5′-TCTGGAAACAGAGACAAAGC-3′). The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2.5 mmol/l MgCl2, and 1 U of DNA polymerase (rTaq; Toyobo) in rTaq buffer. The amplification protocol consisted of three parts: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 sec, annealing at 62.5 °C for 30 sec, and extension at 72 °C for 30 sec; and a final extension at 72 °C for 2 min.

To determine the A→G (Xbal) genotype, the polymorphic region of the gene was amplified by polymerase chain reaction with a sense primer labeled at the 5′ end with biotin (5′-CTGTTTCCCCAGAGACCCGTGAG-3′) and allele-specific antisense primers labeled at the 3′ end either with fluorescein isothiocyanate (5′-CCAATGCTCACTCCCAACTXATA-3′) or with Texas red (5′-CCAATGCTCACTCCCAACTXCA-3′). The reaction mixture (with the exception of the primers) and the amplification protocol (with the exception that the annealing temperature was 65 °C) were identical to those used for genotyping the T→C (PvuII) polymorphism.

Amplified DNA was incubated in a solution containing streptavidin-conjugated magnetic beads in the wells of a 96-well plate at room temperature. The plate was placed on a magnetic stand, and the supernatants were then collected from each well, transferred to the wells of a 96-well plate containing 0.01 M NaOH, and measured for fluorescence with a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 and 538 nm, respectively, for fluorescein isothiocyanate and at 584 and 612 nm, respectively, for Texas red.

The T→C and A→G polymorphisms were determined in 2228 subjects (1108 women, 1120 men) and in 2235 subjects (1107 women, 1128 men), respectively.

Anthropometric variables

Body weight was measured to the nearest 0.01 kg, using a digital scale, height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, and body mass index (BMI) was calculated as weight (kg) divided by height squared (m²). Waist circumference and WHR were used as the indices for body fat distribution in this study. WHR was calculated as a ratio of waist circumference measured at the level of the umbilicus to hip circumference.

Body composition by dual-energy X-ray absorptiometry

Whole-body fat mass (FM), fat-free mass (FFM) and percentage FM (%FM), assessed by dual-energy X-ray absorptiometry (QDR-4500; Hologic, Madison, OH, USA), were used as the indices for determining body composition. Transverse scans were used to measure FM and FFM, and pixels of soft tissue were used to calculate the ratio (R value) of mass attenuation coefficients at 40–50 keV (low energy) and 80–100 keV (high energy), using software version 1.3Z.
Physical activity, smoking status, menstrual status and hormonal replacement therapy
A detailed interview with questionnaire sheets revealed work time and leisure time physical activities of the subjects. Amount of physical activity was calculated as a product of the metabolic-equivalent (MET) by duration in minutes. Smoking status, menstrual status and hormonal replacement therapy were examined by a medical doctor. Menopause was defined as the absence of menses for at least 12 months by a questionnaire.

Biochemical assays of blood
An antecubital blood sample was drawn from each subject after an overnight fast. Serum total cholesterol and triglycerides were determined enzymatically, serum high-density lipoprotein cholesterol was measured by the heparin-manganese precipitation method and fasting plasma glucose was assayed by a glucose oxidase method. Plasma insulin was measured in duplicate by radioimmunoassay. Serum low-density lipoprotein cholesterol was estimated according to the Friedewald formula.

Data analysis
To examine whether age may influence the relation between the ERα gene polymorphisms and body fat distribution, we subdivided each gender group into two age groups: middle-aged (40–59 y) and older (60–79 y). Furthermore, middle-aged women were categorized by menopause status. Values are expressed as mean ± standard error (s.e.) in the tables and figures. Allele frequencies were estimated by the gene-counting method, and the χ² test was used to identify significant departures from Hardy–Weinberg equilibrium. The distribution of haplotypes for the T→C and A→G polymorphisms was calculated according to the method by Thompson et al. The data were compared by one-way analysis of variance and the Tukey–Kramer post hoc test. When a significant difference exists, analysis of covariance was used with age, smoking status, menstrual status, hormonal replacement therapy and physical activities as covariates. The relations between age and both FM and waist were tested by correlation analysis. In each statistical analysis, probability values below 0.05 were regarded as significant. The data were analyzed with the Statistical Analysis System (SAS), release 6.12.

Results
Physical and biochemical blood characteristics of the subjects are shown for each gender in Table 1. No difference was found in BMI, waist, hip or WHR between genders, whereas %FM and FM were significantly greater in women than in men. The distributions of ERα genotypes with regard to the T→C and A→G SNPs were in Hardy–Weinberg equilibrium for the subjects (see Table 2). There was no difference in the
distribution between genders or between age groups. The distribution of haplotypes for the T→C and A→G polymorphisms in all study subjects was as follows: T/A, 61.9%; T/G, 0.2%; C/A, 28.6%; and C/G, 9.3%. The T→C and A→G SNPs were in linkage disequilibrium (pairwise linkage disequilibrium coefficient, D′ (D/Dmax), of 0.97; standardized linkage disequilibrium coefficient, r, of 0.40; P<0.0001, χ² test).

Table 1 Physical and biochemical blood characteristics of subjects (n = 2238)

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>1110</td>
<td>1128</td>
</tr>
<tr>
<td>Age(y)</td>
<td>59.3 ± 0.3</td>
<td>59.2 ± 0.3</td>
</tr>
<tr>
<td>Body mass index(Kg/m²)</td>
<td>22.9 ± 0.1</td>
<td>22.9 ± 0.1</td>
</tr>
<tr>
<td>Percent fat mass(%)</td>
<td>31.5 ± 0.2</td>
<td>21.3 ± 0.1</td>
</tr>
<tr>
<td>Fat mass(Kg)</td>
<td>16.8 ± 0.1</td>
<td>13.5 ± 0.1</td>
</tr>
<tr>
<td>Fat-free mass(Kg)</td>
<td>35.7 ± 0.1</td>
<td>48.7 ± 0.2</td>
</tr>
<tr>
<td>Waist(cm)</td>
<td>83.7 ± 0.3</td>
<td>84.2 ± 0.3</td>
</tr>
<tr>
<td>Hip(cm)</td>
<td>90.7 ± 0.2</td>
<td>91.1 ± 0.2</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.92 ± 0.002</td>
<td>0.92 ± 0.002</td>
</tr>
<tr>
<td>Total cholesterol(mg/dl)</td>
<td>226.9 ± 1.1</td>
<td>212.2 ± 1.0</td>
</tr>
<tr>
<td>Triglycerides(mg/dl)</td>
<td>109.2 ± 1.8</td>
<td>133.9 ± 2.0</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol (mg/dl)</td>
<td>66.0 ± 0.5</td>
<td>57.4 ± 0.4</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol (mg/dl)</td>
<td>139.4 ± 1.1</td>
<td>131.8 ± 1.0</td>
</tr>
<tr>
<td>Glucose(mg/dl)</td>
<td>100.7 ± 0.6</td>
<td>105.8 ± 0.2</td>
</tr>
<tr>
<td>Insulin (µ/ml)</td>
<td>8.4 ± 0.2</td>
<td>8.3 ± 0.2</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± s.e.

T→C polymorphisms (PvuII)
For both genders, no significant difference was found in any variables among the T→C polymorphisms with the exception of BMI of the older men (table not shown). Means ± s.e. of BMI of the older men were 22.9 ± 0.2, 22.5 ± 0.2 and 22.0 ± 0.3 kg/m² in the TT, TC and CC genotypes, respectively (TT > CC, P = 0.025).
A → G polymorphism (XbaI)

For middle-aged women with the AG and/or GG genotypes, BMI, %FM, FM, waist, hip and WHR were greater than in the middle-aged women with the AA genotype (Table 3). In particular, those with the GG genotype had a 9% greater BMI, a 20% greater FM and a 9% larger waist compared with the AA genotype. Fasting insulin was significantly higher in the individuals with the AG genotype (8.3 ± 0.3 μU/ml), compared with the AA genotype (7.4 ± 0.2 μU/ml) (see Figure 1). No difference was found in plasma lipids and fasting blood glucose among the genotypes. When the analysis of covariance with age, smoking status, menstrual status, hormonal replacement therapy and physical activities as covariates was used, these results remained essentially unchanged with the exception of hip circumference and WHR (P < 0.1). Significant differences (P < 0.05) were still observed in BMI (AA 22.3 ± 0.3, AG 23.7 ± 0.4, GG 24.4 ± 1.0: AG and GG > AA), %FM (AA 29.9 ± 0.4, AG 31.9 ± 0.6, GG 33.1 ± 1.5: AG and GG > AA), FM (AA 18.0 ± 0.4, AG 18.2 ± 0.6, GG 19.1 ± 1.5: AG and GG > AA), waist (AA 81.3 ± 0.7, AG 85.5 ± 1.1, GG 88.2 ± 2.5: AG and GG > AA) and fasting insulin (AA 7.3 ± 0.2, AG 8.4 ± 0.4, GG 8.0 ± 0.9: AG > AA) among the genotypes. For older women with the GG genotype, %FM, FM and waist were smaller by 10, 18 and 6%, respectively, compared to the older women with the AA and/or AG genotypes. These results were also unchanged when the analysis of covariance was used. Significant differences (P < 0.05) were still observed in %FM (AA 32.1 ± 0.5, AG 33.7 ± 0.7, GG 27.5 ± 1.8: AA and AG > GG) and FM (AA 16.4 ± 0.4, AG 18.1 ± 0.6, GG 13.4 ± 1.6: AA and AG > GG) among the genotypes. No difference was found in any variables among the genotypes in men (table was not shown).

Table 4 compares age, FM, waist and WHR among the genotypes in middle-aged women.

**Table 3** Physical status and Physical activities of women according to age group and A→G genotype (n = 1107)

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Number(%)</th>
<th>AA</th>
<th>AG</th>
<th>GG</th>
<th>AA</th>
<th>AG</th>
<th>GG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle-aged</td>
<td>376(68.2%)</td>
<td>156(28.3%)</td>
<td>19(3.5%)</td>
<td></td>
<td>362(65.1%)</td>
<td>174(31.3%)</td>
<td>20(3.6%)</td>
</tr>
<tr>
<td>Older</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as means ± s.e.

AG>AA (P = 0.0015), GG>AA (P = 0.0181).

AG>AA (P = 0.0316), GG>AG (P = 0.0061).

AG>AA (P = 0.0023), GG>AA (P = 0.0089).

AG>GA(P = 0.0059), GG>AA (P = 0.0024).

AG>AA (P = 0.0084).

AG>AA (P = 0.0018), GG>AG (P = 0.0316).

AG>GG (P = 0.0232).

AG>AA(P = 0.0323), AG>GG (P = 0.0221).

**Figure 1** Comparison of fasting insulin levels among A→G genotypes in middle-aged women.
whereas no difference was found in postmenopausal women. These results also remained essentially unchanged with the exception of WHR (P < 0.1) when the analysis of covariance with age and smoking status as covariates was used. Significant differences (P < 0.05) were still observed in FM (AA 15.8 ± 0.5, AG 18.0 ± 0.7, GG 20.7 ± 1.6: GG > AA) and waist (AA 80.2 ± 0.7, AG 83.7 ± 1.3, GG 88.5 ± 3.2: GG > AA) among the genotypes.

**Combination of the T → C and A → G polymorphisms**
To determine whether the T → C and A → G polymorphisms synergistically influence body fat distribution, we compared the variables by combined genotypes (table not shown). Because of the small number of subjects, TT/AG (n = 1), TT/GG (n = 0) and TC/GG (n = 5) were excluded from this analysis.

For middle-aged women with the CC/GG genotype, the mean values of BMI, FM and waist were significantly larger by 8%, 18% and 8%, respectively, compared to those with the TT/AA genotype. But the differences between the CC/GG and TT/AA genotypes were similar to the differences between the A → G polymorphism (see Table 3). The physical activities did not differ between the CC/GG and TT/AA genotypes. For older women with the CC/GG genotype, the mean values of %FM and FM were significantly lower by 12% and 17%, respectively, compared to those with the TT/AA genotype. These results also indicate that the effects of the combined genotypes on %FM and FM are not different from the A → G polymorphism alone. For both middle-aged and older men, no difference was found in any variables among the combined genotypes.

**Relation of age with FM and waist**
Figure 2 shows the relations between age and both FM and waist in combined data from middle-aged and older women. A significant and inverse correlation (r = –0.630, P < 0.001) was found between FM and age in the group with the GG genotype. Waist circumference was positively associated with age (r = 0.231, P < 0.001) in the group with the AA genotype, whereas an inverse association (r = –0.504, P < 0.001) was found in the group with the GG genotype.

**Discussion**
Although we have reported in the previous study that the A → G polymorphism of the ERα gene may be associated with a greater BMI in middle-aged women, little is known about the association between the ERα gene polymorphisms and body fat distribution or body composition. Rankinen et al. reported in the eighth update of the human obesity gene map that 174 studies found positive associations of obesity phenotype with 58 candidate genes, but no more than one study by Speer et al. showed any association between ERα gene polymorphisms and obesity phenotype. According to the study, 29 subjects (23 women and 6 men) with android-type obesity, 69 and 31% had the AG and GG genotypes, respectively, and the AA genotype was not found.

One of the major findings of the present study is to find the association of the A → G polymorphism or the combination of the T → C and A → G polymorphisms with not only a greater BMI but also larger %FM, FM, waist circumference and WHR in middle-aged women. Moreover, the results of Table 4 reveal that for premenopausal women, the effect of the ERα gene polymorphisms on body fat distribution (FM and waist) was more significant than for postmenopausal women. These observations suggest that the greater FM of individuals with the gene mutation were due to the selective fat accumulation at the abdomen, especially the intra-abdominal cavity. Further studies on the association between the gene polymorphisms and amount of intra-abdominal fat are needed to clarify the above speculation.

An increasing fasting insulin is induced by an excess accumulation of abdominal fat. In addition, Cooke et al. found that knocked-out ERα caused adipocyte hyperplasia and hypertrophy in white adipose tissue, and is accompanied by insulin resistance and glucose intolerance in rats. On the basis of the above studies, we expected that fasting

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**Table 4 Comparison of age, fat mass and waist among the A → G genotypes in middle-aged women according to menstrual status (n = 536)**

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal (n = 277)</th>
<th>Postmenopausal (n = 259)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA (69.3%)</td>
<td>AG (26.7%)</td>
</tr>
<tr>
<td>Number(%)</td>
<td>192 (74(26.7%))</td>
<td>74 (26.7%)</td>
</tr>
<tr>
<td>Age(y)</td>
<td>46.0 ± 0.2</td>
<td>45.5 ± 0.4</td>
</tr>
<tr>
<td>Fat mass(Kg)</td>
<td>15.9 ± 0.3</td>
<td>17.4 ± 0.6</td>
</tr>
<tr>
<td>Waist(cm)</td>
<td>80.0 ± 0.6</td>
<td>82.8 ± 1.1</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.87 ± 0.004</td>
<td>0.89 ± 0.008</td>
</tr>
</tbody>
</table>

Data are expressed as means ± S.E.

*GG>AA (P = 0.0005).
*GG>AA (P = 0.0021).
*GG>AA (P = 0.0025), GG>AG (P = 0.0406).
insulin level in the GG genotype would be highest of all the genotypes. However, as illustrated in Figure 1, fasting insulin level in the GG genotype did not always indicate the highest values. Recently, Snijder et al reported that fasting insulin level was positively correlated with waist circumference but was inversely associated with hip circumference. Subjects with the GG genotype have not only larger waist but also larger hip compared with the AG genotype. Thus, their fasting insulin levels might be influenced by the two opposite functions with each other.

Regardless of the strong association between the A→G polymorphism and BMI, FM, waist or WHR in middle-aged women (see Table 3), the T→C polymorphism alone was not associated with any variables. Moreover, additive and synergistic effects of the genotypes were not apparent with regard to body fat distribution or body composition in the subjects. These results suggest that the A→G polymorphism plays an important role in body fat distribution and body composition in middle-aged women, but the T→C polymorphism does not.

Figure 2 shows that the waist circumference of women with the AA genotype increased with age, which is expected. This indicates a natural (normal) change in body fat distribution of middle-aged and older women. In contrast, results of the GG genotype revealed that FM and waist size were inversely associated with age. Consequently, our data suggest that (1) middle-aged women with the GG genotype presented with a larger FM and waist, and (2) older women with the GG genotype presented with a smaller FM and waist, compared with the AA genotype, despite the observation in the middle-aged women (see Table 3). Estrogen plays an important role in maintaining desirable fat distribution in premenopausal women. Therefore, when a functional change of the ERα was induced by the gene mutation, the estrogen sensitivity is deteriorated, which possibly caused the android-type fat distribution in middle-aged and premenopausal women with the GG genotype (see Table 4). On the other hand, the smaller FM and waist of older women with the GG genotype have been possibly induced by some specific effects of the gene mutation; however, the mechanisms cannot be explained by the data from this study.

Both the T→C and A→G polymorphisms are found in intronic regions. Intronic changes in gene sequence may have an impact on the expression of other genes by influencing the transcription and/or stability of mRNA of those genes. Thus, further studies on the relations of ERα gene polymorphisms and body fat distribution are needed to validate the findings of this study.

Our data raise the possibility that the A→G polymorphisms of the ERα gene, especially the GG genotype, contribute to development of the android-type fat distribution in middle-aged and premenopausal women. In older women, this gene polymorphism may serve to decrease whole-body and abdominal fat tissue.

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