Comparison of Body Fat Distribution and Blood Lipid Profiles according to Trp64Arg Polymorphism for the β3-Adrenergic Receptor Gene in Korean Middle-Aged Women

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Summary The aim of this study was to determine whether there was an association between body fat distribution, blood lipid profiles, and β1-adrenergic receptor gene polymorphism in Korean middle-aged women. Subjects were grouped according to BMI as obese (≥25 BMI, n=95) or non-obese (BMI<25, n=93). The Trp64Arg mutation of the β1-adrenergic receptor gene was detected by PCR-RFLP. Skinfold thickness, body circumference, intra-abdominal fat area by CT, and blood lipid profiles were also measured. Data were compared using ANOVA, Bonferroni t-test, and Chi-square. Significance for statistical analyses were set at p<0.05. In the obese group, 63.16% were Trp64Trp homozygotes and 36.84% were Trp64Arg heterozygotes, compared to 80.65% who were Trp64Trp homozygotes and 19.35% who were Trp64Arg heterozygotes in the non-obese group. These results indicated a significant (χ²=4.943, p<0.05) difference between the two groups. Frequency of the Arg64 allele in the obese group (16.84%) showed a significant (χ²=4.185, p<0.05) difference as compared to the non-obese group (9.68%). Skinfold thickness and body circumference of the Trp64Arg heterozygote group showed a consistent increase as compared to the Trp64Trp homozygote group. Visceral fat area and VSR of Trp64Arg heterozygote group showed a higher tendency than Trp64Trp homozygotes in the obese group, but these differences were not statistically significant. In conclusion, the Trp64Arg polymorphism of the β3-adrenergic receptor gene is associated with obesity in middle-aged Korean women, but it is difficult to suggest the prominent association of the Trp64Arg polymorphism of the β3-adrenergic receptor gene with prevalence of abdominal obesity or dyslipidemia in Korean middle-aged women.

Key Words obesity, β3-adrenergic receptor gene, Trp64Arg polymorphism, body fat distribution

The obese phenotype is under the control of genetic and environmental influences. Much insight has been gained regarding obesity-related genetic factors (1), and the advent of advanced molecular biological techniques has accelerated the search for the specific genes influencing human obesity. Several candidate genes have been associated with energy expenditure, body fat distribution, and lipid metabolism (2). Among these genes, the β3-adrenergic receptor gene regulates lipolysis in adipose tissue, and contributes to population variations in energy expenditure and body fat distribution (3). The β3-adrenergic receptor is quite different from β1 and β2 receptor subtypes in that it is relatively resistant to desensitization and may maintain signaling during periods of sustained sympathetic stimulation (4). A missense mutation of the β3-adrenergic receptor gene, results in replacement of tryptophan by arginine at codon 64 (Trp64Arg). This single nucleotide polymorphism (SNP) has been associated with morbid obesity and insulin resistance (5). The Trp64Arg polymorphism in the β3-adrenergic receptor gene was shown to be associated decreased energy expenditure in Pima Indians (5), prevalence of obesity in Finns (6), weight gain in French (7), and an increase of BMI and lack of intervention effects in Japanese (8, 9). However, this purported association has been controversial and results have been contradictory. Several studies have failed to show on association between the Trp64Arg polymorphism in the β3-adrenergic receptor gene and the obesity phenotype in other populations, including Aymara natives from Chile (10), Mexican-Americans (11), Chinese (12), and Finns (13).

These inconsistent results might be the result of the differences in gender, race, disease history, body fat lev-
MATERIALS AND METHODS

1. Subjects. Subjects participating in this study were recruited from 188 female volunteers aged 30–50 y who visited the Fitness Center at Keimyung University between February and May 2004. The study protocol was approved by the Institutional Review Boards of the Keimyung University. Informed consent was obtained from all the study participants. Subjects were divided into an obese (≥25 kg/m²) group (n=95) and a non-obese (<25 kg/m²) group (n=93) based upon BMI. Characteristics of the subjects are shown in Table 1.

2. Phenotype measurement. Anthropometric and body fat measurement: Body mass index (BMI) was calculated as weight (kg)/height² in m², and percentage of body fat was estimated from the skinfold thickness of triceps, suprailiac crest, and thigh by Jackson’s and Siri’s equations (14, 15). Body circumference was measured at chest, waist, hip, upper arm, thigh, calf, wrist, and ankle. Waist circumference was measured on mid-line between the lowest part of 12th rib and supra iliac crest by WHO’s method (16). Waist hip ratio (WHR) was calculated as waist circumference/hip circumference. Skinfold thickness of triceps, biceps, subcapular, thorax, abdomen, suprailiac crest, axillary, and thigh were measured by skinfold caliper (Skindex, USA). Abdominal fat area, subcutaneous fat area, visceral fat area, and the ratio of visceral fat area to subcutaneous area (VSR) on the level of L4–L5 were measured by computerized tomographic scanning.

Blood parameters: Blood samples were drawn from the antecubital vein after an overnight fast, and serum and plasma were stored at −70°C. Serum total cholesterol was determined by enzymatic methods using a SICDIA L-T-CHO Reagent kit (Shinyang Co., Korea), and TG was determined using SICDIA L TG Reagent (Shinyang Co.) adjusted for free glycerol. Serum HDL-C was measured by the homogeneous enzymatic colorimetric method using an HDL-C plus kit (Roche Co., Germany), and LDL-C was determined using the Friedewald equation. Serum levels of glucose and free fatty acid were determined by enzymatic methods using GLU-HK (Asan Co., Korea) and NEF A-M reagent (Shinyang Co.).

3. Genotyping. The Trp64Arg mutation of the β2-adrenergic receptor gene was detected by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Briefly, DNA was extracted from lymphocytes after digestion by proteinase K and purification with phenol-chloroform (17). Polymerase chain reaction (PCR) was performed in a final volume of 30 µL containing 300 ng DNA, Taq DNA polymerase 0.3 µL (Bioneer Co., Korea), 50 pm antisense primer 0.5 µL, 50 pm sense primer 0.5 µL, 10× reaction buffer 3 µL (100 mM Tris-HCl, 200 mM KCl, 15 mM MgCl₂, pH 9.0), 1 µL dNTP 2 µL. The sense primer was 5′-CCAGTGGGCTGCCAGGGG-3′, and the antisense primer was 5′-GCCAGTGCCGCCCAACGGG-3′. The PCR (Perkin Elmer, USA) started with denaturation at 96°C for 5 min, followed by 35 cycles of denaturation at 96°C for 40 s, annealing at 65°C for 30 s, and extension at 72°C for 30 s, with a final elongation at 72°C for 10 min. The amplified product was digested at 60°C for 3 h with 5 U BstN1 (Promega Co., USA). The fragments were resolved on a 3% agarose gel and detected under a Polaroid MP-4 camera (Clifton Co., USA) after staining with ethidium bromide.

4. Statistical analysis. Values are reported as the mean and standard deviation. The significance of deviations of observed genotype frequencies from those predicted by the Hardy-Weinberg equation were evaluated with χ² tests. Significant differences of dependent variables between genotypes were assessed using ANOVA and Bonferroni t-test. p<0.05 was considered statistically significant.

RESULTS

Obese and non-obese groups exhibited a significant (χ²=4.943, p<0.05) genotype difference such that 63.16% were Trp64Trp homozygotes and 36.84% were Trp64Arg heterozygotes in the obese group compared to 80.65% who were Trp64Trp homozygotes and 19.35% who were Trp64Arg heterozygotes in the non-obese group. The frequency of the Arg64 allele in the obese group (16.84%) showed a significant (χ²=4.185, p<0.05) difference as compared to that in the non-obese group (9.68%) (Table 2). We could not find Arg64Arg homozygote subjects in either group.

For the analysis of entire body fat distribution, we measured skinfold thickness and body circumference. No variables of skinfold thickness and body circumference were significantly different between the Trp64Trp homozygote group and the Trp64Arg heterozygote group (Tables 3 and 4). But these results for the...
Trp64Arg heterozygote group showed a consistent increase as compared to the Trp64Trp homozygote group.

Serum levels of triglyceride, HDL-C, fasting glucose and FFA showed a significant \( (p<0.05) \) difference between the obese and non-obese groups. In obese or non-obese individuals, blood levels of lipid profiles showed no significant difference between the Trp64Arg heterozygote group and the Trp64Trp homozygote group (Table 5).

In obese subjects, the visceral fat area and VSR of the Trp64Arg heterozygote group were higher than for the Trp64Trp homozygote group, but these differences did not reach statistical significance (Table 6).

**DISCUSSION**

The \( \beta_1 \)-adrenergic receptor gene is expressed in abdominal adipose tissue, and regulates a lipid metabolism and body temperature (18). Catecholamines binding to \( \beta_1 \)-adrenergic receptors activate the cascade of stimulatory G-proteins, adenylate cyclase, formation of cAMP, and ultimately protein kinase and hormone-sensitive lipase. Thus, stimulation of \( \beta_1 \)-adrenergic receptors causes hydrolysis of triglyceride into fatty acids and glycerol by activation of hormone-sensitive lipase (19). The Trp64Arg polymorphism of the \( \beta_1 \)-adrenergic receptor gene has been associated with depression of lipolysis and morbid obesity in some (5, 6, 8), but not all (11, 20) studies. To determine whether this relationship exists in Koreans, we related body composition as measured by CT, skinfold thickness and body circumferences to the \( \beta_1 \)-adrenergic receptor genotype. We found that a significantly higher percentage (37%) of middle-aged Korean obese women had the Trp64Arg polymorphism when compared to non-obese women (19%). These results together with recent findings in Pima Indians (5), Mexicans (21), Japanese (22), Chinese (23), Finns (6), and Caucasians (7) suggest that this polymorphism for the \( \beta_1 \)-adrenergic receptor gene mutation may be involved in a genetic predisposition for obesity in Korean middle-aged women. However, some other previous studies have not supported an associa-
Our results demonstrate that the Trp64Arg polymorphism of the β1-adrenergic receptor gene is associated with obesity in Korean middle-aged women, but it is difficult to suggest the prominent association of the β1-adrenergic receptor gene mutation may not effect the development of susceptibility to insulin resistance in Korean middle-aged women.

The absence of this association between this polymorphism and blood lipid markers and body fat distribution in this study could be due to differences in nutritional and lifestyle factors among subjects. Hao et al. (23) suggested no significant association of Trp64Arg polymorphism of the β1-adrenergic receptor gene with risk factors of diabetes, hypertension, or dyslipidemia, and Oksanen et al. (37) reported no significant association between the β1-adrenergic receptor genotype and the level of the serum cholesterol, HDL-C, triglyceride, glucose or insulin in Finns. Hence, previous studies have found inconsistent results regarding an association between the Trp64Arg polymorphism of the β1-adrenergic receptor gene and obesity-related risk factors of metabolic disease.

In our study, although skinfold thickness and body circumference of Trp64Arg heterozygotes showed a consistent increase as compared to Trp64Trp homozygotes in obese and non-obese groups, no variables of skinfold thickness or body circumference were significantly different. Importantly, visceral fat area, WHR, and VSR were not significantly different between Trp64Trp homozygotes and Trp64Arg heterozygotes. In addition, we could not find a significant difference of lipid profile levels between Trp64Arg heterozygotes and Trp64Trp homozygotes, and β1-adrenergic receptor gene mutation may not effect the development of susceptibility to insulin resistance in Korean middle-aged women.

Table 5. Results of blood lipid profiles in both groups.

| Items      | Non-obese | Obese   |
|------------|-----------|---------|
|            | Trp/Trp   | Trp/Arg | Trp/Trp   | Trp/Arg   |
| Total-C    | 4.43      | 4.22    | 4.73      | 4.81      |
| (mmol/L)   | 0.66      | 0.37    | 1.02      | 0.92      |
| TG         | 1.17      | 0.93    | 1.49       | 1.34      |
| (mmol/L)   | 0.59      | 0.34    | 0.80      | 0.51      |
| HDL-C      | 1.62      | 1.59    | 1.43*     | 1.55      |
| (mmol/L)   | 0.38      | 0.28    | 1.30      | 0.28      |
| LDL-C      | 2.39      | 2.30    | 2.64      | 2.75      |
| (mmol/L)   | 0.54      | 0.60    | 0.83      | 0.04      |
| Fasting glucose | 3.52    | 3.44    | 3.55      | 4.02*     |
| (mmol/L)   | 0.72      | 0.56    | 0.93      | 1.04      |
| FFA        | 643.35    | 761.28  | 751.48*   | 685.94*   |
| (μmol/L)   | 188.20    | 217.29  | 242.68    | 218.37    |

Values are mean and SD.
*p<0.05 compared to homozygotes of non-obese group.
#p<0.05 compared to heterozygotes of non-obese group.

Table 6. Results of abdominal fat area and WHR in both groups.

| Items         | Non-obese | Obese   |
|---------------|-----------|---------|
|               | Trp/Trp   | Trp/Arg | Trp/Trp   | Trp/Arg   |
| Total fat     | 238.64    | 283.35  | 408.28*   | 334.84    |
| (cm²)         | 64.15     | 24.62   | 80.62     | 93.33     |
| Subcutaneous fat | 176.76  | 227.34  | 312.86*   | 225.87    |
| (cm²)         | 52.81     | 36.12   | 76.81     | 60.25     |
| Visceral fat  | 56.76     | 51.66   | 88.70     | 95.18     |
| (cm²)         | 23.74     | 18.13   | 35.30     | 49.01     |
| VSR           | 0.34      | 0.24    | 0.30      | 0.44      |
|               | 0.15      | 0.10    | 0.16      | 0.22      |
| WHR           | 0.86      | 0.85    | 0.91*     | 0.91*     |
|               | 0.05      | 0.05    | 0.05      | 0.05      |

Values are mean and SD.
*p<0.05 compared to homozygotes of non-obese group.
#p<0.05 compared to heterozygotes of non-obese group.
Trp64Arg polymorphism of the β1-adrenergic receptor gene with prevalence of abdominal obesity or dyslipidemia in Korean middle-aged women.

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