Association of Pro12Ala Polymorphism in the Peroxisome Proliferative-Activated Receptor γ2 Gene with Obesity and Hypertension in Korean Women

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Summary This study examined whether the Pro12Ala polymorphism of the PPARγ2 gene is associated with obesity, hypertension and cardiovascular risk profiles in Korean adult women. We studied 129 Korean women (aged 42.71 ± 8.56 y) who were divided into 2 groups as a Pro12Pro homozygous group and a Pro12Ala heterozygous or Ala12Ala homozygous group based upon PPARγ2 genotype. Anthropometric parameters, blood pressure, abdominal fat area and blood lipid profiles were compared between the 2 groups, and the association of Ala allele frequency in PPARγ2 gene with obesity or hypertension was evaluated. Most anthropometric parameters and blood lipid profiles did not differ significantly between the genotypes. However, all variables of skinfold thickness, body circumference and abdominal fat area of Pro12Ala heterozygous were consistently higher compared to the Pro12Pro homozygous subjects without a significance differences. The hypertensive group had significantly higher (p=0.004) Ala12 allele frequency than the normotensive group whereas allele frequencies did not differ significantly between the obese group and non-obese group. Ala allele carriers had a significantly higher risk of hypertension than non-carriers in logistic regression analysis. There was no evidence that the Ala allele can be regarded as an independent risk factor for obesity. In conclusion, all variables related to obesity showed a consistently higher trend in Pro12Ala heterozygous subjects compared to Pro12Pro homozygous subjects. Pro12Ala heterozygous subjects showed an increasing trend of elevated blood pressure compared to Pro12Pro homozygous subjects. Ala12 variant as well as BMI and TG were regarded as independent risk factors for hypertension in our subjects.

Key Words PPARγ2, Pro12Ala polymorphism, obesity, hypertension, exercise

There is evidence of a genetic predisposition to obesity, and it is highly relevant to identify genetic risk factors for obesity that interact with the environment. This underlying relationship between obesity and genetic factors seem to be of great significance, influencing insulin sensitivity and metabolic function. Gene variants that regulate adipocyte metabolism may predispose individuals to develop obesity. One of the candidate genes is a nuclear hormone receptor called peroxisome proliferator-activated receptor-γ (PPARγ) which contains three isoforms of PPARγ1, PPARγ2, and PPARγ3 by alternative promoters and differential splicing (1). PPARγ2 is mainly expressed in adipocytes and regulates the transcription of many adipocyte-specific genes (2). A common variant of the human PPARγ2 gene is a substitution at the amino acid 12 codon of alanine for proline (Pro12Ala) (3). Previous studies were conducted to elucidate the relationship between this polymorphism and obesity and metabolic syndrome (4, 5). The Pro12Ala substitution of the PPARγ2 gene was associated with higher body mass index (BMI) in Caucasians (6) and in Taiwanese (7), and has been shown to influence the risk for type 2 diabetes and obesity in various ethnic populations. In contrast, Pro12Ala substitution in Finnish subjects was shown to be associated with lower BMI and improved insulin sensitivity (8). In carriers of the Ala12 allele, increased insulin sensitivity was reported for Caucasian (9) and Japanese (10) subjects. In the association between the Pro12Ala polymorphism and blood pressure, Douglas and Erdos (11) reported an association between an Ala allele and increased blood pressure values. and Stefanski et al. (12) reported that the Pro12Ala variant is associated with increased mean 24-h diastolic blood pressure in obese diabetic patients. However, Ostgren et al. (13) showed that the Pro12Ala polymorphism was associated with lower diastolic blood pressure in type II diabetic men, but not in women. In previous studies, the varying associations of the Pro12Ala polymorphism of the PPARγ2 gene with obesity, insulin sensitivity and blood pressure may be
likely resulting from differences in ethnic background and characteristics of subjects. It has not been sufficiently investigated yet whether the effect of an exercise intervention on adiposity and metabolic parameters is modified by the Pro12Ala polymorphism of the PPARγ2 gene.

This study examined whether the Pro12Ala polymorphism of the PPARγ2 gene is associated with obesity and cardiovascular risk profiles including visceral adiposity, blood pressure and blood lipids in Korean adult women. In addition, we compared the effect of an exercise intervention on the changes of obesity-related phenotypes according to the Pro12Ala polymorphism of the PPARγ2 gene.

**MATERIALS AND METHODS**

1. **Subjects**. We studied 129 Korean adults women (aged 42.7±8.56 y) who visited the Fitness Center at Keimyung University between May and August 2005. The study protocol was approved by the Institutional Review Boards of the Keimyung University. Informed consent was obtained from all study participants. Characteristics of the subjects are shown in Table 1. Subjects were divided into 2 groups as a Pro12Pro homozygous group and a Pro12Ala heterozygous or Ala12Ala homozygous group based upon PPARγ2 genotype. Anthropometric parameters, blood pressure, abdominal fat area and blood lipid profiles were compared between the 2 groups. Genotype frequencies were compared between obese and non-obese groups (based upon BMI 25 kg·m⁻²) or between hypertensive and normotensive groups (based upon BP 130/85 mmHg) (14), and the association of Ala allele frequency in PPARγ2 gene with obesity or hypertension was evaluated. In addition, 41 subjects were compared in changes of anthropometric parameters, blood pressure, abdominal fat area and blood lipid profiles after a 12-wk exercise intervention program across PPARγ2 genotypes. The exercise program at an individual intensity of 60% of the maximal heart rate or 1RM was performed for 30 min of walking and 1 set of resistance training (sit-up, bench press, push-up, trunk hyperextension, knee flexion and extension) 2 to 3 d each week. The exercise program did not include any specific dietary intervention; however, daily energy intake detailed by 3-d food records did not differ significantly between PPARγ2 genotypes.

2. **Anthropometric and body fat measurement**. BMI was calculated as weight divided by height squared (kg/m²), and percentage of body fat was estimated from the skinfold thickness of triceps, suprailliac crest, and thigh by Jackson’s (15) and Siri’s (16) equations. Skinfold thickness was measured using skinfold calipers (Skindex, USA). Waist circumference was measured on the mid-line between the lowest part of 12th rib and suprailliac crest by the WHO method (17). Waist hip ratio (WHR) was calculated as waist circumference/hip circumference. Abdominal fat area, subcutaneous fat area and visceral fat area on the level of L4–L5 were measured by computed tomographic scanning (Somatomertit, Siemens, Germany). Resting blood pressure

was determined as the average of three measures obtained on the left arm after subjects were seated quietly for 10 min using a random-zero sphygmomanometer (HICO, Japan) by an experienced technician.

3. **Determination of the PPARγ2 genotype**. The Pro12Ala substitution of the PPARγ2 gene was detected by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Briefly, DNA was extracted from lymphocyte after digestion by proteinase K and purification with phenol-chloroform. 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, 10X reaction buffer 3 μL (100 mM Tris-HCl, 400 mM KCl, 15 mM MgCl₂, pH 9.0). dNTP 2 μL. The sense primer was 5’-GCAATTTACGCCAGT-3’, and the antisense primer was 5’-GATATGGTGAGACAGTG-TATCATGGAAGGATCGCTTTCCG-3’. The PCR (Perkin Elmer, USA) started with denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1 min, 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 2% agarose gel and detected under polaroid MP-4 camera (Clifton Co., USA) after staining with ethidium bromide. The resulting band patterns were the Pro12Pro type (a single band of 270 bp), the Pro12Ala type (2 bands of 227 and 270 bp), and the Ala12Ala type. Blood parameters measurement. Blood samples were drawn from the antecubital vein after an overnight fast using Vacutainer blood-collection tubes (Becton Dickinson, Franklin Lakes, NJ), and serum samples were separated from whole blood by centrifugation at 1,000 × g for 15 min after blood was allowed to clot at room temperature for 30 min. Plasma samples were separated from whole blood, using EDTA as an anticoagulant immediately after blood sampling, by centrifugation at 1,000 × g for 15 min. These samples were stored at −80°C until assayed. Serum total cholesterol (TC) was determined by enzymatic methods using SIC-DIA L T-CHO Reagent kit (Shinyang Co., Korea), and triglyceride (TG) was determined using SICDIA L Reagent (Shinyang Co.), which adjusted for free glycerol. Serum high density lipoprotein cholesterol (HDL-C) was measured by the homogeneous enzymatic colorimetric method using an HDL-C plus kit (Roche Co., Germany), and low density lipoprotein cholesterol (LDL-C) was determined using the Friedewald equation (18) \[\text{LDL-C} = \text{TC} - \left(\frac{\text{HDL-C}}{\text{TG}/5}\right)\]. Serum levels of glucose and free fatty acid (FFA) were determined by enzymatic methods using GLU-HK (Asan Co., Korea) and NEFA-M reagent (Shinyang Co.).

5. **Statistical analysis**. The anthropometric and biochemical features are presented as mean and SD, or as median (interquartile range) for skewed variables. The distributions of continuous variables were assessed for normality and the logarithmic transformations of
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Table 1. Comparison of anthropometric parameters, blood pressure and blood lipid profiles between Pro12Pro homozygous group and a Pro12Ala heterozygous or Ala12Ala homozygous group in PPARγ2 gene polymorphism.

| Variables                      | Pro12Pro (n=115) | Pro12Ala or Ala12Ala (n=14) | p value | p value* |
|--------------------------------|------------------|-------------------------------|---------|----------|
| Age (y)                        | 42.23±8.82       | 46.71±4.41                   | 0.026   | —        |
| Height (cm)                    | 159.16±4.72      | 158.99±4.08                  | 0.855   | 0.399    |
| Body weight (kg)               | 61.82±8.09       | 64.73±11.45                  | 0.392   | 0.240    |
| BMI (kg·m⁻²)                   | 24.4±8.99        | 25.81±4.66                   | 0.244   | 0.054    |
| Body fat percent (%)           | 29.13±4.97       | 30.56±5.84                   | 0.429   | 0.418    |
| Skinfold of triceps (mm)       | 26.19±5.73       | 28.55±7.53                   | 0.064   | 0.225    |
| Skinfold of suprailiac (mm)    | 31.83±7.01       | 33.79±6.73                   | 0.236   | 0.643    |
| Skinfold of thigh (mm)         | 30.65±7.15       | 32.40±6.63                   | 0.429   | 0.513    |
| Waist circumference (cm)       | 77.60±6.75       | 82.03±11.27                  | 0.066   | 0.066    |
| Hip circumference (cm)         | 95.62±5.02       | 97.59±6.69                   | 0.272   | 0.045*   |
| WHR                            | 0.90±0.05        | 0.91±0.08                    | 0.318   | 0.824    |
| Total abdominal fat area (cm²) | 302.41 (256.63–325.24) | 329.00 (299.63–462.96)    | 0.091   | 0.052    |
| Subcutaneous fat area (cm²)    | 232.00 (181.40–252.00) | 245.45 (244.00–323.98)    | 0.062   | 0.079    |
| Visceral fat area (cm²)        | 73.54 (66.01–91.37) | 74.00 (42.93–128.78)   | 0.981   | 0.451    |
| Systolic blood pressure (mmHg) | 120.00 (110.00–130.00) | 132.50 (110.00–140.00)    | 0.080   | 0.009*   |
| Diastolic blood pressure (mmHg)| 80.00 (70.00–85.00) | 85.00 (70.00–90.00)   | 0.120   | 0.037*   |
| Glucose (mmol·L⁻¹)             | 6.73 (6.00–7.53)  | 7.01 (6.00–8.00)             | 0.868   | 0.963    |
| FFA (μEq·L⁻¹)                  | 4.66 (4.33–5.05)  | 4.72 (4.30–4.95)             | 0.868   | 0.963    |
| Total cholesterol (mmol·L⁻¹)    | 4.30 (3.85–4.88)  | 4.33 (3.79–4.79)             | 0.942   | 0.356    |
| HDL-C (mmol·L⁻¹)               | 1.40 (1.23–1.60)  | 1.41 (1.16–1.85)             | 0.985   | 0.874    |
| LDL-C (mmol·L⁻¹)               | 2.81 (2.32–3.21)  | 2.86 (2.30–3.21)             | 0.885   | 0.382    |
| TG (mmol·L⁻¹)                  | 0.78 (0.57–0.94)  | 0.72 (0.57–1.38)             | 0.746   | 0.349    |

Values are mean±SD, or as median (interquartile range) for skewed variables. *p<0.05. # ANCOVA after adjustment for age.

Table 2. Frequency of Pro12Ala polymorphisms and alleles with obesity or hypertension.

| Variables            | Obesity (n=43) | Non-obese (n=86) | Hypertensive (n=37) | Normotensive (n=92) | Chi-square value (p value) | Allele frequency | Chi-square value (p value) | OR (95% CI)   |
|----------------------|---------------|------------------|---------------------|---------------------|---------------------------|------------------|---------------------------|----------------|
| Genotype             |               |                  |                     |                     |                           |                  |                           |               |
| Pro12Pro             | 38 (88.4%)    | 72 (89.5%)       | 29 (78.4%)          | 86 (93.5%)          | 0.658 (0.720)             |                  |                           | 0.940         |
| Pro12Ala             | 5 (11.6%)     | 8 (9.3%)         | 7 (18.9%)           | 6 (6.5%)            |                           |                  |                           | 0.060         |
| Ala12Ala             | 0             | 1 (1.2%)         | 1 (2.7%)            | 0                   |                           |                  |                           | 0.004         |
| Chi-square value (p value) |           |                  |                     |                     |                           | 7.186 (0.028)    |                           | 0.963         |
| Allele frequency     |               |                  |                     |                     |                           |                  |                           | (0.319–2.913) |
| Pro                  | 0.940         |                  | 0.875               | 0.968               |                           |                  |                           |               |
| Ala                  | 0.060         |                  | 0.125               | 0.032               |                           |                  |                           |               |
| OR (95% CI)          | 0.963 (0.319–2.913) |                  | 0.233 (0.080–0.682) |                     |                           |                  |                           |               |

skewed variables (abdominal fat area, blood lipid profiles) were used in subsequent analyses. Differences in continuous variables between genotype groups were tested with Student’s t test when the distribution of the variable or of the logarithmically transformed variable approached a normal distribution and the variances of the variables were equal in the groups compared. Otherwise the Mann-Whitney U test was used. Additional analysis of differences was performed by analysis of covariance (ANCOVA) after being adjusted for age. The differences in changes of parameters after exercise intervention between genotype groups were tested with a similar process. The significance of deviations of observed genotype frequencies from those predicted by the Hardy-Weinberg equation were evaluated with χ² tests between obese and non-obese groups or between hypertensive and normotensive groups. A binary logistic regression model was used to test for the association of Pro12Ala genotypes and obesity and hypertension, controlling for the risk factors of age, BMI, LDL-C and TG by calculating the odds ratios (95% confidence interval) and corresponding p values. Significance was accepted at p<0.05.

RESULTS

The genotype distribution of the Pro12Ala PPARγ2
polymorphism in the entire sample (n=129) was 89.1% (n=115), 10.1% (n=13), and 8.8% (n=1) for the Pro12Pro, Pro12Ala and Ala12Ala genotypes respectively. The Ala12 allele frequency was 0.058 and it was in Hardy-Weinberg equilibrium.

The comparison of anthropometric parameters, blood pressure and blood lipid profiles of subjects along with PPARγ2 genotypes are presented in Table 1. Because there was only one Ala12 homozygous subject, this subject was combined with the Pro12Ala heterozygous subjects and was compared with the Pro12Pro subjects for the comparison of variables in Table 1. Most anthropometric parameters and blood lipid profiles did not differ significantly between the genotypes; however, the Pro12Ala heterozygous subjects had a higher trend for total abdominal (p=0.091) and subcutaneous (p=0.062) fat area, waist circumference (p=0.066), systolic (p= 0.080) and diastolic (p=0.120) blood pressure than the Pro12Pro subjects. The Pro12Ala heterozygous subjects had significantly higher hip circumference (p=0.045), systolic (p=0.009) and diastolic (p=0.037) blood pressure than the Pro12Pro subjects after adjustment for age. All variables of skinfold thickness, body circumference and abdominal fat area of Pro12Ala heterozygous subjects were consistently higher as compared to the Pro12Pro homozygous subjects without significance differences.

Allele frequencies of Pro12Ala polymorphism did not differ significantly between the obese group and non-obese group (Table 2). However, the hypertensive group had significantly higher (p=0.004) Ala12 allele frequency than the normotensive group, and the prevalence of hypertension was higher in Ala12 allele than in non-carriers of the Ala12 allele.

A logistic regression model (Table 3) was used to adjust the odds ratio for obesity and the presence of the Ala allele, while controlling for other predictive variables. The Ala allele was analyzed as a categorical variable. Age, LDL-C and TG are given as continuous variables.

### Table 3. Logistic regression analysis for association of PPARγ2 Ala allele and other predictor variables with obesity.

| Variables | Odds ratio | 95% CI     | p value |
|-----------|------------|------------|---------|
| Ala allele| 1.188      | 0.288–4.892| 0.812   |
| Age       | 1.011      | 0.962–1.064| 0.659   |
| LDL-C     | 1.750      | 0.397–1.722| 0.460   |
| TG        | 4.257      | 1.698–10.668| 0.002   |

The PPARγ2 Ala allele is included as a categorical variable. Age, LDL-C and TG are given as continuous variables.

### Table 4. Logistic regression analysis for association of PPARγ2 Ala allele and other predictor variables with hypertension.

| Variables | Odds ratio | 95% CI     | p value |
|-----------|------------|------------|---------|
| Ala allele| 4.517      | 1.009–20.221| 0.049   |
| Age       | 1.053      | 0.990–1.121| 0.103   |
| BMI (≥25 kg·m⁻²) | 5.342   | 1.753–16.282| 0.003   |
| LDL-C     | 0.279      | 0.047–1.663| 0.161   |
| TG        | 4.232      | 1.371–13.066| 0.012   |

The PPARγ2 Ala allele and BMI are included as categorical variables. Age, LDL-C and TG are given as continuous variables.

### Table 5. Comparison of changes in anthropometric parameters, blood pressure and blood lipid profiles before and after exercise intervention according to PPARγ2 gene polymorphism (n=41).

| Variables                          | Pro12Pro (n=35) | Pro12Ala or Ala12Ala (n=6) | p value |
|------------------------------------|-----------------|-----------------------------|---------|
| ∆Body weight (kg)                  | 0.25±1.65       | −0.55±1.35                  | 0.245   |
| ∆BMI (kg·m⁻²)                      | 0.11±0.74       | −0.26±0.40                  | 0.178   |
| ∆Body fat percent (%)              | 0.53±1.91       | −0.47±0.64                  | 0.089   |
| ∆Skinfold of triceps (mm)          | 5.61±3.48       | 4.53±1.90                   | 0.307   |
| ∆Skinfold of abdomen (mm)          | 5.15±3.74       | 2.85±5.45                   | 0.353   |
| ∆Skinfold of thigh (mm)            | 4.68±4.81       | 4.40±2.48                   | 0.593   |
| ∆Waist circumference (cm)          | 1.35±2.56       | −0.13±1.15                  | 0.086   |
| ∆Hip circumference (cm)            | 0.79±1.75       | 1.13±1.38                   | 0.593   |
| ∆WHR                               | 0.01±0.02       | −0.01±0.02                  | 0.046*  |
| ∆Total abdominal fat area (cm²)    | 15.10±37.84     | 4.08±44.80                  | 0.576   |
| ∆Subcutaneous fat area (cm²)       | 4.73±28.74      | 0.41±36.84                  | 0.737   |
| ∆Visceral fat area (cm²)           | 8.67±11.99      | 4.94±10.41                  | 0.434   |
| ∆Systolic blood pressure (mmHg)    | 3.71±10.87      | 8.00±21.86                  | 0.966   |
| ∆Diastolic blood pressure (mmHg)   | 2.00±9.33       | 14.00±13.42                 | 0.052   |
| ∆FFA (μEq·L⁻¹)                     | −14.18±298.58   | 64.17±450.21                | 0.825   |
| ∆Total cholesterol (mmol·L⁻¹)      | −0.62±0.73      | −0.66±0.73                  | 0.854   |
| ∆HDL-C (mmol·L⁻¹)                  | −0.05±0.20      | −0.08±0.24                  | 0.618   |
| ∆LDL-C (mmol·L⁻¹)                  | −0.03±0.66      | 0.06±0.66                   | 0.658   |
| ∆TG (mmol·L⁻¹)                     | −0.34±0.74      | −0.73±1.12                  | 0.593   |

Values are mean±SD. *p<0.05. ∆: difference between before and after exercise intervention.
possible, while age, LDL-C and TG were included in the model as continuous variables. There was no evidence that the PPARG2 Ala allele can be regarded as an independent risk factor for obesity.

A logistic regression analysis with hypertension as the dependent variable and Ala allele, age, BMI, LDL-C and TG as the independent variables showed that carriers of the polymorphism Pro12Ala of the PPARG2 gene had a significantly higher risk of hypertension than non-carriers. This analysis showed that the Ala allele, BMI (&gt; 27 kg·m⁻²) and TG are significant risk factors for hypertension (Table 4).

The changes in most variables did not differ after a 12-wk exercise intervention across groups of the PPARG2 genotypes. However, the Pro12Ala heterozygous subjects showed a significantly greater (p = 0.046) reduction in WHR than the Pro12Pro homozygous subjects, and showed a highly decreasing trend for body fat percent (p = 0.089) and waist circumference (p = 0.086) (Table 5). We did not find evidence of the association between PPARG2 gene polymorphism and the response to exercise intervention.

**DISCUSSION**

The frequency of the Ala12 allele of 0.058 in PPARG2 polymorphism in our subjects was a similar trend to the frequencies reported in other East Asian populations of 0.035 for Korean (19), 0.039 for Chinese (20), 0.040 for Taiwanese (7), 0.041 for Japanese (21), whereas this frequency is much less than the frequencies reported in the Caucasian population of 0.11–0.13 (6, 22, 23) (Table 6).

In the present study, there was no evidence to suggest that the Pro12Ala polymorphism was a predisposing factor for obesity in female adults, as allele frequencies were not significantly different between the obese and non-obese group. Additionally, there was no significant difference in BMI, the percentage of body fat or other anthropometric parameters related to obesity between Pro12Ala heterozygous subjects and Pro12Pro homozygous subjects. Kim et al. (19) reported that body weight, BMI, and WHR are significantly higher in Ala allele carriers than in non-carriers, which is inconsistent with our results. Masud and Ye (5) reported that BMI was significantly higher in Ala allele carriers compared with Pro allele homozygous through a meta-analysis using data from 30 independent studies. Previous studies have shown that the Pro12Ala substitution in the PPARG2 receptor is associated with obesity in several populations, such as Spanish (24), Caucasian (6), native Javanese in Indonesia (25) and Swedish middle-aged men (26).

This inconsistency seems due to a small number and different BMI of subjects of our study as compared to the previous studies. The discrepancies among these studies may therefore be due to racial differences in the Ala allele frequency or distribution in BMI. Masud and Ye (5) reported that the allele was associated with significantly higher BMI among subjects with BMI of greater than 27, but no significant association was found among subjects with BMI of less than 27. The only other study of Korean subjects (19) showed higher
BMI values (mean values 25.59) than our subjects.

In addition, the other previous studies showed that the Pro12Ala polymorphism of PPARγ2 gene is not associated with obesity in French (27), Japanese (28) and Danish (29) subjects. Moreover, logistic regression analysis for obesity showed no association with the Pro12Ala polymorphism of PPARγ2 gene in our study. Therefore, we could not confirm the association of the Pro12Ala polymorphism of PPARγ2 gene with obesity in our subjects.

We also found no significant difference in blood lipid profiles between Pro12Ala heterozygous subjects and Pro12Pro homozygous subjects. Previous studies have shown conflicting results regarding the association of the Pro12Ala polymorphism of PPARγ2 gene with blood lipid profiles. Beamer et al. (6) found that subjects with the Ala allele had lower HDL-C and higher TG levels compared with Pro12Pro homozygous subjects, and Meirhaeghe et al. (30) observed higher levels of TC, LDL-C and apolipoprotein B in subjects with the Ala allele compared with Pro12Pro homozygous subjects. These findings may result from the Ala isoform of PPARγ2 being less affective at activating target genes including lipoprotein lipase (LPL) in vitro (8, 31). However, Deeb et al. (8) found that Ala12Ala homozygous individuals had significantly higher HDL-C and lower TG compared with Pro12Pro homozygous and Pro12Ala heterozygous subjects in an elderly Finnish population. Swarbrick et al. (32) observed no differences for TC or LDL-C in obese or lean subjects. Therefore, we cannot suggest an association of the Pro12Ala polymorphism of PPARγ2 gene with blood lipid profiles.

However, all variables related to obesity showed a consistently higher trend in Pro12Ala heterozygous subjects compared to Pro12Pro homozygous subjects. In particular, hip circumference after adjustment for age showed a significantly (p=0.045) higher value, and abdominal subcutaneous fat area showed an increasing trend (p=0.062) in Pro12Ala heterozygous subjects as compared to Pro12Pro homozygous subjects. This result may be explained by the differences in PPARγ expression between subcutaneous and visceral fat (33), a different fat distribution and the increase of PPARγ2 mRNA in subcutaneous adipose tissue in women (34). Sanchez et al. (24) suggested that the Pro12Ala polymorphism of the PPARγ2 gene may promote peripheral deposition of adipose tissue.

Interestingly, Pro12Ala heterozygous subjects showed significantly higher blood pressure compared to Pro12Pro homozygous subjects after being adjusted for age. Moreover, the Ala12 variant with BMI and TG was regarded as an independent risk factor for hypertension in our subjects. It is well established that between 30% and 50% of blood pressure variation in a population is determined by genetic factors (35), and PPARγ is a key component for blood pressure homeostasis (36). Previous studies in obese and type 2 diabetic patients reported an association between the Pro12Ala variant and blood pressure (11, 12). However, Pischon et al. (37) suggested that Pro12Ala and Ala12Ala variants are not associated with a risk of the occurrence of coronary heart disease. Ostgren et al. (13) showed that the Pro12Ala variant was associated with lower diastolic blood pressure in type 2 diabetic men, but not in women, where no association was found. Temelkova-Kurtchsev et al. (38) suggested that the Ala12Ala genotype of the PPARγ2 gene may protect from early atherosclerosis in subjects at risk for diabetes. Al-Shali et al. (39) suggested that the PPARγ2 Pro12Ala variant may be related to less atherosclerosis, as measured by intima media thickness in a cross-sectional study. One of the explanations for this discrepancy may be the lack of control for the use of antihypertensive medications by many of the analyzed patients.

The main factors related to the association between the Pro12Ala polymorphism of the PPARγ2 gene and higher blood pressure has been suggested by the reduced function of the PPARγ (12). Sugawara et al. (40) showed that stimulation of PPARγ leads to suppression of type 1 angiotension II receptor gene expression in rat vascular smooth cells. Our results support this, as significant association of BMI with hypertension was found. A positive relationship of BMI to blood pressure in normal subjects is well described (41). Therefore, it is possible that blood pressure increase is related the increase of BMI with the presence of the Pro12Ala variant.

Luan et al. (42) proposed that PPARγ2 is one of the mediators of the gene-environment interactions. Nicklas et al. (43) found the differences of body weight and insulin sensitivity change after an exercise and diet intervention according to the PPARγ2 gene polymorphism. However, it still remains unclear whether this polymorphism affects the response of metabolic markers to physical activity. Lindl et al. (44) reported that subjects with the Ala12Ala genotype lost more body weight than subjects having the Pro12Pro genotype. Kim et al. (19) suggest that the PPARγ2 gene polymorphism does not significantly affect serum biochemical parameters or outcomes of a weight loss program. Therefore, we cannot suggest the evidence of an association between PPARγ2 gene polymorphism and the response to exercise intervention.

In conclusion, although we cannot suggest an association of the Pro12Ala polymorphism of the PPARγ2 gene with obesity, all variables related to obesity showed a consistently higher trend in Pro12Ala heterozygous subjects compared to Pro12Pro homozygous subjects. Pro12Ala heterozygous subjects showed a increasing trend of blood pressure compared to Pro12Pro homozygous subjects as well. The Ala12 variant, BMI and TG were regarded as independent risk factors for hypertension in our subjects. We did not find an association between PPARγ2 gene polymorphism and the response to an exercise intervention.

REFERENCES

1) Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre A-M, Saladin R, Najib J, Laville M, Fruchart J-C, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H, Auv-
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2. Werman A, Hollenberg A, Solanes G, Bjørbaek C, Vidal-Puig AJ, Flier JS. 1997. Ligand-independent activation domain in the N-terminus of peroxisome proliferator-activated receptor-γ: Differential activity of PPARγ-1 and -2 isoforms and influence of insulin. J Biol Chem 272: 18779–18789.

3. Yen CJ, Beaamer BA, Negri C, Silver K, Brown KA, Yarnall DP, Burns DK, Roth J, Shuldiner AR. 1997. Molecular scanning of the human peroxisome proliferator-activated receptor-γ (PPARγ) gene in diabetic Caucasians: Identification of a Pro12Ala PPARγ2 missense mutation. Biochem Biophys Res Commun 241: 270–274.

4. Ek J, Urhammer SA, Sørensen TI, Andersen T, Auwerx J. 1999. Homozygosity of the Pro12Ala variant of the peroxisome proliferator-activated receptor-γ (PPARγ2): Divergent modulating effects on body mass index in obese and lean Caucasian men. Diabetologia 42: 892–895.

5. Masud S, Ye S. 2003. Effect of the peroxisome proliferator-activated receptor-γ gene Pro12Ala variant on body mass index: A meta-analysis. J Med Genet 40: 773–780.

6. Beaamer BA, Yen CJ, Andersen RE, Gavrilova O, Rumberger JM, Durcan MJ, Yarnall DP, Hawkins AL, Grillin CA, Burns DK, Roth J, Reitman M, Shuldiner AR. 1998. Association of the Pro12Ala variant in the peroxisome proliferator-activated receptor-γ gene with obesity in two Caucasian populations. Diabetes 47: 1806–1808.

7. Lei HH, Chen MH, Yang WS, Chiu MC, Chen MC, Tai TY, Chuang LM. 2000. Peroxisome proliferator-activated receptor gamma 2 Pro12Ala variant is strongly associated with increased body mass in the Taiwanese. Metabolism 49: 1267–1270.

8. Deeb SS, Fajas I, Nemoto M, Pihlajamaki J, Mykkänen L, Kuusiisto J, Laakso M, Fujimoto W, Auwerx J. 1998. Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 20: 284–287.

9. Stumvoll M, Wahl HG, Lobel K, Becker R, Machicao F, Jacob S, Haring H. 2001. Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-gamma2 gene is associated with increased antilipolytic insulin sensitivity. Diabetes 50: 876–881.

10. Harra K, Okada T, Tobe K, Yasuda K, Mori Y, Kadowaki H, Hagara R, Akanuma Y, Kimura S, Ito C, Kadowaki T. 2000. The Pro12Ala polymorphism in PPAR gamma 2 may confer resistance to type 2 diabetes. Biochem Biophys Res Commun 271: 212–216.

11. Douglas JA, Erdos MR. 2001. The peroxisome proliferator-activated receptor-γ pro12Ala variant. Association with type 2 diabetes and trait differences. Diabetes 50: 886–890.

12. Stefanski A, Majkowska L, Ciechanowicz A, Frankow M, Safranow K, Parczewski M, Moleda P, Pilarska K. 2006. Association between the Pro12Ala variant of the peroxisome proliferator-activated receptor-gamma2 gene and increased 24-h diastolic blood pressure in obese patients with type II diabetes. J Human Hypertens 20: 684–692.

13. Ostgren CJ, Lindblad U, Melander O, Melander A, Groop L, Rastam L. 2003. Peroxisome proliferator-activated receptor-gamma2Pro12Ala polymorphism and the association with blood pressure in type 2 diabetes: Skaraborg hypertension and diabetes project. J Hypertens 21: 1657–1662.

14. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Spertus JA, Costa F. 2005. Diagnosis and management of the metabolic syndrome. Circulation 112: 2735–2752.

15. Jackson AS, Pollock ML, Ward A. 1980. Generalized equations for predicting body density of women. Med Sci Sports Exerc 12: 175–182.

16. Siri WE. 1961. Body composition from fluid spaces and density: Analysis of methods. In: Techniques for Measuring Body Composition (Brozek J, Henschel A, eds), p 223–244. National Academy of Sciences National Research Council, Washington DC.

17. WHO. 1999. Report of a WHO Consultation on Obesity: Preventing and Managing. In the Global Epidemic. Geneva.

18. Friedewald WT, Levy RI, Fredrickson DS. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18: 499–502.

19. Kim KS, Choi SM, Shin SU, Yang HS, Yoon Y. 2004. Effects of peroxisome proliferator-activated receptor-γ Pro12Ala polymorphism on body fat distribution in female Korean subjects. Metabolism 53: 1538–1543.

20. Fu M, Chen H, Li X, Li J, Wu B, Cheng L, Cai M, Fu Z. 2002. Association of Pro12Ala variant in peroxisome proliferator-activated receptor-gamma2 gene with type 2 diabetes mellitus. Zhonghua Yi Xue Yi Chua Xue Za Zhi 19: 234–238.

21. Mori H, Ikegami H, Kawaguchi Y, Seino S, Yokoi N, Takeda J, Inoue I, Seino Y, Yasuda K, Hanafusa T, Yamagata K, Awata T, Kadowaki T, Haru K, Yamada N, Gotoda T, Iwasaki N, Iwamoto Y, Sanke T, Nanjo K, Oka Y, Matsutani A, Maeda E, Kasuga M. 2001. The Pro12Ala substitution in PPARgamma is associated with resistance to development of diabetes in the general population: Possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. Diabetes 50: 891–894.

22. Kolehmainen M, Uusitupa M, Alhuva E, Laakso M, Vidal H. 2003. Effect of the Pro12Ala polymorphism in the peroxisome proliferator-activated receptor (PPAR) gamma 2 gene on the expression of PPARgamma target genes in adipose tissue of massively obese subjects. J Clin Endocrinol Metab 88: 1717–1722.

23. Schaffler A, Barth N, Schmitz G, Zietz B, Palitzsch KD, Scholmerich J. 2002. Frequency and significance of Pro12Ala and Pro115Gln polymorphism in gene for peroxisome proliferator-activated receptor gamma regarding metabolic parameters in a Caucasian cohort. Endocrine 14: 369–373.

24. Sanchez JLG, Rios MS, Perez CF, Laakso M, Larrad MTM. 2002. Effect of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ gene on adipo-posit, insulin sensitivity and lipid profile in the Spanish population. Eur J Endocrinol 147: 495–501.

25. Dunuwijt CW, Nagata M, Moriyama H, Harra K, Yasuda H, Nakayama M, Kotani R, Yamada K, Sakata M, Kurohara M, Wiyono P, Asdje H, Sakaue M, Taniguchi H, Yokono K. 2005. A possible association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor-γ2 gene with obesity in native Javanese in Indonesia. Diabetes Metab Res Rev 21: 465–469.
26) Rosmond R, Chagnon M, Bouchard C. 2003. The Pro12Ala PPARY2 gene missense mutation is associated with obesity and insulin resistance in Swedish middle-aged men. *Diabetes Metab Res Rev* 19: 159–163.

27) Ghoussaini M, Meyre D, Lobbens S, Charpentier G, Clement K, Charles M-A, Tauber M, Weill J, Froguel P. 2005. Implication of the Pro12Ala polymorphism of the PPARG-gamma 2 gene in type 2 diabetes and obesity in the French population. *BMC Med Genet* 6: 11.

28) Yamamoto Y, Hirose H, Miyashita K, Nishikai K, Saito I, Taniyama M, Tomita M, Saruta T. 2002. PPARY2 gene Pro12Ala polymorphism may influence serum level of an adipocyte-derived protein, adiponectin, in the Japanese population. *Metabolism* 51: 1407–1409.

29) Larsen TM, Larsen LH, Torekov SK, Ek J, Black E, Toubro S, Astrup A, Sorensen TIA, Hansen T, Pedersen O. 2005. Novel variants in the putative peroxisome proliferator-activated receptor-y promoter and relationships with obesity in men. *Obesity Res* 13: 953–958.

30) Meirhaeghe A, Fajas L, Helbecque N, Cottel D, Auwerx J, Vidal H. 2001. Gene expression in visceral and subcutaneous adipose tissues: Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest* 99: 2416–2422.

31) Vidal H. 2001. Gene expression in visceral and subcutaneous adipose tissues. *Ann Med* 33: 547–555.

32) Vidal-Puig AJ, Considine RV, Jimenez-Linan M, Werman A, Pories WJ, Caro JF, Elier JS. 1997. Peroxisome proliferator-activated receptor gene expression in human tissues: Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest* 99: 2416–2422.

33) Ward R. 1995. Familial aggregation and genetic epidemiology of blood pressure. In: Hypertension: Pathophysiology, Diagnosis, and Management (Laragh J, Brenner B, eds), p 67–88. Raven Press. New York.

34) Barroso I, Gurnell M, Crowley VE, Agostini M, Schwabel JW, Soos MA, Maslen G, Williams TDM, Lewis H, Schafer AJ, Chatterjee VK, O’Rahilly S. 1999. Dominant negative mutations in human PPARY associated with severe insulin resistance, diabetes mellitus and hypertension. *Nature* 402: 880–883.

35) Pischon T, Pui JK, Manson JE, Hu FB, Rexrode KM, Hunter D, Rimm EB. 2005. Peroxisome proliferator-activated receptor-y2 P12A polymorphism and risk of coronary heart disease in US men and women. *Arterioscler Thromb Vasc Biol* 25: 1654–1658.

36) Temelkova-Kurktschiev T, Hanefeld M, Chinetti G, Zawadzki C, Haulon S, Kubaszek A, Koehler C, Leonhardt W, Staels B, Laakso M. 2004. Ala12Ala genotype of the peroxisome proliferator-activated receptor y2 protects against atherosclerosis. *J Clin Endocrinol Metab* 89: 4238–4242.

37) Al-Shali KZ, House AA, Hanley AJ, Khan HM, Harris SB, Zinnman B, Mamakeesick M, Fenster A, Spence JD, Hegele RA. 2004. Genetic variations in PPARG encoding peroxisome proliferator-activated receptor gamma associated with carotid atherosclerosis. *Stroke* 35: 2036–2040.

38) Sugawara A, Takeuchi K, Urano A, Ikeda Y, Arima S, Kudo M, Sato K, Taniyama Y, Ito S. 2001. Transcriptional suppression of type 1 angiotensin II receptor gene expression by peroxisome proliferator-activated receptor-y in vascular smooth muscle cells. *Endocrinology* 142: 3125–3134.

39) Brown CD, Higgins M, Donato KA, Rohde FC, Garrison R, Obarzanek E, Ernst ND, Horan M. 2000. Body mass index and the prevalence of hypertension and dyslipidemia. *Obes Res* 8: 605–619.

40) Luan J, Browne PO, Harding AH, Halsall DJ, O’Rahilly S, Chatterjee VKK, Wareham NJ. 2001. Evidence for genetic interaction PPARY locus. *Diabetes* 50: 686–689.

41) Nicklas BJ, van Rossum EIIC, Berman DM, Ryan AS, Dennis KE, Shuldiner AR. 2001. Geetic variation in the peroxisome proliferator-activated receptor-2 gene (Pro12Ala) affects metabolic responses to weight loss and subsequent weight regain. *Diabetes* 50: 2172–2176.

42) Lindi VL, Uusitupa MIJ, Lindstrom J, Louheranta A, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Kinnunen-Kiukaanniemi S, Laakso M, Tuomilehto J. 2002. Association of the Pro12Ala polymorphism in the PPARG-y2 gene with 3-year incidence of type 2 diabetes and body weight change in the Finnish diabetes prevention study. *Diabetes* 51: 2581–2586.