

Effects of Peroxisome Proliferator-Activated Receptor- γ 2 Pro12Ala Polymorphism on Body Fat Distribution in Female Korean Subjects

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The effects of peroxisome proliferator-activated receptor gamma2 (PPAR γ 2) Pro12Ala (P12A) polymorphism on body mass index (BMI) and type 2 diabetes are well documented; however, until now, only a few studies have evaluated the effects of this polymorphism on body fat distribution. This study was conducted to elucidate the effects of this polymorphism on computed tomography (CT)-measured body fat distribution and other obesity-related parameters in Korean female subjects. The frequencies of PPAR γ 2 genotypes were: PP type, 93.0%; PA type, 6.8%; and AA type, 0.2%. The frequency of the A allele was 0.035. Body weight ($P = .012$), BMI ($P = .012$), and waist-to-hip ratio (WHR) ($P = .001$) were significantly higher in subjects with PA/AA compared with subjects with PP. When body composition was analyzed by bioimpedance analysis, lean body mass and body water content were similar between the 2 groups. However, body fat mass ($P = .003$) and body fat percent ($P = .025$) were significantly higher in subjects with PA/AA compared with subjects with PP. Among overweight subjects with BMI of greater than 25, PA/AA was associated with significantly higher abdominal subcutaneous fat ($P = .000$), abdominal visceral fat ($P = .031$), and subcutaneous upper and lower thigh adipose tissue ($P = .010$ and $.013$). However, among lean subjects with BMI of less than 25, no significant differences associated with PPAR γ 2 genotype were found, suggesting that the fat-accumulating effects of the PA/AA genotype were evident only among overweight subjects, but not among lean subjects. When serum lipid profiles, glucose, and liver function indicators were compared among overweight subjects, no significant difference associated with PPAR γ 2 genotype was found. Changes in body weight, BMI, WHR, and body fat mass were measured among overweight subjects who finished a 1-month weight loss program of a hypocaloric diet and exercise; no significant differences associated with PPAR γ 2 genotype were found. The results of this study suggest that the PPAR γ 2 PA/AA genotype is associated with increased subcutaneous and visceral fat areas in overweight Korean female subjects, but does not significantly affect serum biochemical parameters and outcomes of weight loss programs.

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THE NUCLEAR RECEPTOR, peroxisome proliferator-activated receptor-gamma (PPAR γ), is a transcription factor, which plays a major role in adipocyte differentiation.¹ Alternative use of promoters and differential splicing of the human PPAR γ gene result in 2 isoforms: PPAR γ 1 and PPAR γ 2. PPAR γ 2 contains 28 additional amino acids at its amino terminus.² PPAR γ 1 and PPAR γ 2 are both expressed in adipose tissue, but PPAR γ 2 is much more sensitive to insulin-mediated transcriptional activation and adipocyte differentiation, suggesting a distinct role for PPAR γ 2 in obesity and insulin resistance.³ Several rare dominant negative mutations in PPAR γ 2 have been detected in families with severe insulin resistance and diabetes,⁴ while a rare gain of function mutation has been detected in individuals with extreme obesity.⁵

A common variant of the human PPAR γ 2 gene that predicts substitution at amino acid 12 of alanine for proline (Pro12Ala) was found by Yen et al.⁶ Many studies were conducted to elucidate the relationship between this polymorphism and metabolic syndrome, because this amino acid position is within the domain of PPAR γ 2, which is involved in insulin-mediated transcriptional activation, and the substitution of alanine for proline could cause a significant change in protein structure. Although these studies were controversial and irreproducible, a

meta-analysis based on data from over 3,000 individuals demonstrated that the Pro12Ala polymorphism influences susceptibility to type 2 diabetes. The Pro allele was reported to be associated with a 1.25-fold increase in diabetes risk compared with the Ala allele.⁷ Recently, Masud and Ye⁸ performed a meta-analysis using data from 30 independent studies with a total number of 19,136 subjects and reported that body mass index (BMI) was significantly higher in Ala allele carriers compared with Pro allele homozygotes ($P = .019$).

However, the effects of Pro12Ala polymorphism on body fat distribution have been less well studied. This study was conducted to elucidate the effects of this polymorphism on computed tomography (CT)-measured abdominal and distal fat distribution along with other obesity-related phenotypes in female Korean subjects.

MATERIALS AND METHODS

Subjects

The 1,051 female Korean subjects were recruited from Kirin Oriental Medical Hospital (Seoul, Korea). General characteristics of the subjects are listed in Table 1. Male subjects were also recruited, but the number of cases was not large enough for statistical analysis. Genomic DNA was obtained with informed consent. Body compositions were measured by bioimpedance analysis using a commercial device (Inbody 2.0; Biospace, Korea). The areas of abdominal subcutaneous fat, abdominal visceral fat, and subcutaneous fat at the upper and lower thigh of 471 subjects were measured using CT (Hispeed CT/e; GE). The 198 overweight subjects finished a 1-month weight loss program of an 800 kcal/d hypocaloric diet and aerobic exercise; changes in body weight, BMI, waist-to-hip ratio (WHR), and body fat mass during the program were measured.

Determination of the PPAR γ 2 Genotype

Genomic DNA was extracted from whole blood using a Qiagen kit. Polymerase chain reaction (PCR) was conducted to amplify the

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Submitted March 22, 2004; accepted June 19, 2004.

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0026-0495/04/5312-0003\$30.00/0

doi:10.1016/j.metabol.2004.06.019

Table 1. General Characteristics of Study Subjects

	Total Subjects (n = 1051)
Age (yr)	27.06 ± 0.20
Weight (kg)	65.98 ± 0.33
BMI (kg/m ²)	25.59 ± 0.12
WHR	0.872 ± 0.002
SBP (mm Hg)	115.72 ± 0.39
DBP (mm Hg)	71.65 ± 0.32

NOTE. Data are mean ± SE.

Abbreviations: SBP, Systolic blood pressure, DBP, diastolic blood pressure.

genomic DNA fragment containing the Pro12Ala position of the PPAR γ 2 gene. Upstream primer (5'TCT GGG AGA TTC TCC TAT TGG3'), downstream primer (5'GTG GAA GAC AAC TAC AAG AG3'), 3 μ L dNTP mix (1 mmol/L), 0.2 μ L Taq DNA polymerase (1 U), and 3 μ L PCR buffer (10 $\times$) were added and adjusted to a total volume of 30 μ L with distilled water. The amplification protocol consisted of 35 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 30 seconds. The amplified PCR products were checked for correct size of 154 bp by electrophoresis in a 3% agarose gel. The PCR products were subsequently digested with the restriction enzyme *Hha*I for 2 hours at 37°C and subjected to electrophoresis in a 3% agarose gel. The resulting band patterns were the PP type (a single band of 154 bp), the PA type (3 bands of 154, 132, and 22 bp), and the AA type (2 bands of 132 and 22 bp).

Biochemical Analysis

Blood samples were obtained after fasting overnight for more than 12 hours and centrifuged at 2,000 rpm for 30 minutes. Serum was collected and concentrations of fasting glucose, total cholesterol (TC) and high-density lipoprotein (HDL) cholesterol, triglyceride (TG), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and total bilirubin were measured by auto-biochemical analyzer (SP-4410, ARKRAY, Japan). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation [LDL cholesterol = TC–HDL cholesterol – TG/5].

Statistical Analysis

All values are presented as mean ± SE. Age-adjusted univariate analysis of variance was performed by the General Linear Model procedure to examine the independent effect of the PPAR γ 2 genotype on dependent variables. The χ^2 test was used to compare PPAR γ 2 genotype frequencies among the high-fat content group and the normal group. Multivariate analyses were conducted using the General Linear Model procedures, in which the effects of the PPAR γ 2 genotype, age, lean body mass, and serum TG were included. The source of variation in subcutaneous and visceral fat area was computed using the type III sum of squares, which can quantify the effects of an independent variable after adjustment for all other variables included in the model, as described in the report by Robitaille et al.⁹ Statistical significance was established at the level of $P < .05$. All analyses were performed using SPSS version 10.0 (Chicago, IL).

RESULTS

The frequencies of the PPAR γ 2 Pro12Ala (P12A) polymorphic genotypes were measured in 1,051 female subjects. The PP type was 93.0% (n = 978), the PA type was 6.8% (n = 71), and the AA type was 0.2% (n = 2); these frequencies are in

agreement with Hardy-Weinberg equilibrium. The frequency of the A allele of 0.035 was similar to the frequencies reported in other east Asian populations of 0.039 for Chinese,¹⁰ 0.040 for Taiwanese,¹¹ and 0.041 for Japanese.¹² However, the A allele frequency is much smaller than the frequencies reported in the Caucasian population of 0.11,¹³ 0.12,¹⁴ and 0.13.¹⁵

Table 2 shows the comparison of physical characteristics and body compositions of the subjects along with PPAR γ 2 genotypes. The only 2 AA type cases were combined with the PA type into the PA/AA type for comparison with the PP type. Weight ($P = .012$), BMI ($P = .012$), and WHR ($P = .001$) were significantly higher in the PA/AA type compared with the PP type. Blood pressures did not differ significantly with PPAR γ 2 genotype ($P > .05$). When body composition was measured by bio-impedance analysis, lean body mass and water content were similar between the 2 groups. However, body fat mass ($P = .003$) and body fat percent ($P = .025$) were significantly higher in the PA/AA type compared with the PP type, indicating that the increased body weight of PA/AA type carriers is the result of a selective increase in body fat rather than lean body mass. Body weight and BMI were 5.0% and 4.6% higher, respectively, in PA/AA type carriers than in PP type carriers; however, body fat mass was 12.3% higher, indicating that the PPAR γ 2 genotype has a more powerful effect on body fat content than on body weight and BMI.

To estimate the effect of the PPAR γ 2 genotype on the risk of body fat overaccumulation, the subjects were divided into 2 groups of normal fat range (<32%) and unhealthy fat range (>32%) by the criteria suggested for female subjects,¹⁶ and the distribution of PPAR γ 2 genotypes was compared among the 2 groups (Table 3). Subjects with unhealthy fat overaccumulation accounted for 59.6% of the PP type carriers and 71.6% of the PA/AA type carriers ($P = .048$). The odd ratio was 1.713 with a 95% confidence interval of 1.017 ~ 2.885, demonstrating that subjects with the PA/AA genotype have a 1.7-fold higher risk of unhealthy body fat overaccumulation than PP type carriers.

To more accurately evaluate the effects of PPAR γ 2 genotype on body fat accumulation, 471 subjects were tested using CT to

Table 2. Comparisons of Physical Characteristics and Body Compositions by Genotypes of PPAR γ 2

Genotype	PP Type (n = 977)	PA/AA Type (n = 74)	P Value
Physical characteristics			
Weight (kg)	65.65 ± 0.35	68.92 ± 1.62	.012*
BMI (kg/m ²)	25.46 ± 0.13	26.64 ± 0.57	.012
WHR	0.871 ± 0.002	0.893 ± 0.009	.001
SBP (mm Hg)	115.51 ± 0.42	117.94 ± 1.62	.093
DBP (mm Hg)	71.72 ± 0.35	73.56 ± 1.38	.109
Body composition			
Water (kg)	30.06 ± 0.27	30.22 ± 0.45	.872
Fat mass (kg)	22.49 ± 0.24	25.26 ± 1.13	.003
Lean body mass (kg)	43.28 ± 0.16	43.88 ± 0.66	.280
Body fat (%)	33.50 ± 0.19	35.12 ± 0.88	.025

NOTE. Data are mean ± SE.

*P values were obtained by general linear model (covariance) analysis adjusted for age.

Table 3. Distribution of PPAR γ 2 Genotypes in Subjects With Normal and Unhealthy Body Fat Levels

	Normal Range (fat < 32%)	Unhealthy Range (fat > 32%)	Total	<i>P</i> Value*	Odds Ratio (95% CI)
PP type	395 (40.4)†	582 (59.6)	977 (100.0)		
PA/AA type	21 (28.4)	53 (71.6)	74 (100.0)	.048	1.713 (1.017~2.885)
Total	416 (39.6)	565 (60.4)	1051 (100.0)		

**P* value and odd ratio were obtained by χ^2 test.

†Number of subjects (%).

measure the cross-sectional fat areas at the abdominal and distal parts of the body (Table 4). Abdominal subcutaneous fat area was 28% greater in the PA/AA type compared with the PP type ($P = .000$). Abdominal visceral fat area was increased by 16% in the PA/AA type, although the difference was not statistically significant ($P = .117$). Total abdominal fat area (combined subcutaneous fat and visceral fat) was significantly greater in PA/AA type carriers ($P = .000$), but the visceral fat to subcutaneous fat ratio was not significantly different ($P = .376$). Subcutaneous fat areas in the distal part of the body, measured at the upper and lower thigh, were 11% greater at the upper thigh ($P = .009$) and 18% greater at the lower thigh ($P = .002$) in the PA/AA type compared with the PP type.

To evaluate the effects of the PA/AA genotype on body fat distribution more closely, the subjects were divided into lean and overweight groups by BMI criteria. The PA/AA type carriers were more common in the overweight group than in the lean group, but a statistically significant difference was not found by the χ^2 test ($P = .145$) (data not shown). Among the lean group with BMI of less than 25, none of the subcutaneous fat and visceral fat areas differed significantly according to PPAR γ 2 genotypes ($P > .05$) (Table 5). However, among the overweight group with BMI of greater than 25, PA/AA type

carriers had significantly higher fat content in the abdominal subcutaneous ($P = .000$), abdominal visceral ($P = .031$), and thigh subcutaneous adipose tissue ($P = .010, 0.013$) (Table 6). In particular, the abdominal visceral fat area was significantly greater in the PPAR γ 2 overweight subjects ($P = .031$), even though no statistical significance was found among total subjects ($P = .117$) (Table 4). These results suggest that the fat-accumulating effects of PA/AA genotype are evident only among overweight subjects and not among lean subjects.

The effects of PPAR γ 2 genotype on subcutaneous and visceral fat areas were also verified using multivariate analyses (Table 7). Age, lean body mass, and serum TG level were also included in the model. PPAR γ 2 genotype explained 6.2% ($P = .000$) of the variation in subcutaneous fat area and 1.8% ($P = .045$) of the variation in visceral fat area. These results clearly demonstrate that PPAR γ 2 genotype has a greater effect on subcutaneous fat than on visceral fat. Age and serum TG level could explain the variation in visceral fat areas, and subcutaneous fat area variation was partly explained by lean body mass.

Serum lipid profiles, glucose, and liver function indicators did not differ significantly among the 456 overweight subjects with regard to PPAR γ 2 genotype (Table 8). Similarly, serum

Table 4. Comparison of CT-Measured Fat Areas by PPAR γ 2 Genotype

Genotype	PP Type (n = 434)	PA/AA Type (n = 37)	<i>P</i> Value
Abdominal subcutaneous fat (mm ²)	25,478 $\pm$ 490	32,641 $\pm$ 2,687	.000*
Abdominal visceral fat (mm ²)	5,574 $\pm$ 135	6,477 $\pm$ 509	.117
Total abdominal fatt† (mm ²)	30,510 $\pm$ 555	39,118 $\pm$ 3,083	.000
V/S ratio‡ (mm ²)	0.234 $\pm$ 0.007	0.206 $\pm$ 0.011	.376
Upper thigh subcutaneous fat (mm ²)	14,715 $\pm$ 166	16,325 $\pm$ 898	.009
Lower thigh subcutaneous fat (mm ²)	9,807 $\pm$ 151	11,593 $\pm$ 863	.002

NOTE. Data are mean $\pm$ SE.

**P* values were obtained by general linear model (covariance) analysis adjusted for age.

†Total abdominal fat is the sum of abdominal subcutaneous fat and abdominal visceral fat.

‡V/S ratio is the ratio of abdominal visceral fat to abdominal subcutaneous fat.

Table 5. Comparison of CT-Measured Fat Areas by PPAR γ 2 Genotype in Lean Subjects With BMI of less than 25

Genotype	PP Type (n = 217)	PA/AA Type (n = 12)	<i>P</i> Value
Abdominal subcutaneous fat (mm ²)	19,375 $\pm$ 458	17,162 $\pm$ 1,571	.262*
Abdominal visceral fat (mm ²)	4,054 $\pm$ 117	3,380 $\pm$ 411	.209
Total abdominal fatt† (mm ²)	23,069 $\pm$ 461	20,542 $\pm$ 1,876	.220
V/S ratio‡ (mm ²)	0.217 $\pm$ 0.005	0.199 $\pm$ 0.017	.480
Upper thigh subcutaneous fat (mm ²)	12,879 $\pm$ 147	11,672 $\pm$ 846	.054
Lower thigh subcutaneous fat (mm ²)	8,080 $\pm$ 130	7,546 $\pm$ 545	.314

NOTE. Data are mean $\pm$ SE.

**P* values were obtained by general linear model (covariance) analysis adjusted for age.

†Total abdominal fat is the sum of abdominal subcutaneous fat and abdominal visceral fat.

‡V/S ratio is the ratio of abdominal visceral fat to abdominal subcutaneous fat.

Table 6. Comparison of CT-Measured Fat Areas by PPAR γ 2 Genotype in Overweight Subjects With BMI of Greater Than 25

Genotype	PP Type	PA/AA Type	P Value
Abdominal subcutaneous fat (mm ²)	31,363 $\pm$ 645	40,071 $\pm$ 2,898	.000*
Abdominal visceral fat (mm ²)	7,064 $\pm$ 193	7,963 $\pm$ 503	.031
Total abdominal fat† (mm ²)	37,816 $\pm$ 717	48,034 $\pm$ 3,179	.000
V/S ratio‡ (mm ²)	0.250 $\pm$ 0.014	0.210 $\pm$ 0.014	.426
Upper thigh subcutaneous fat (mm ²)	16,518 $\pm$ 241	18,558 $\pm$ 996	.013
Lower thigh subcutaneous fat (mm ²)	11,517 $\pm$ 216	13,534 $\pm$ 1,050	.010

NOTE. Data are mean $\pm$ SE.

*P values were obtained by general linear model (covariance) analysis adjusted for age.

†Total abdominal fat is the sum of abdominal subcutaneous fat and abdominal visceral fat.

‡V/S ratio is the ratio of abdominal visceral fat to abdominal subcutaneous fat.

biochemistry of total (lean and overweight) subjects did not differ significantly (data not shown). Among the overweight subjects, 198 subjects finished a 1-month weight loss program of a hypocaloric diet and exercise, and changes in body weight, BMI, WHR, and body fat mass during the program were compared by PPAR γ 2 genotypes (Table 9). The results showed no significant differences. Overall, the results of this study suggest that PPAR γ 2 PA/AA genotype has fat-accumulating effects at abdominal and distal adipose tissues among overweight subjects; the PPAR γ 2 PA/AA genotype has a greater influence on accumulation of subcutaneous adipose tissue than visceral adipose tissue.

DISCUSSION

Recently, Masud and Ye⁸ performed a meta-analysis using data from 30 independent studies with a total number of 19,136 subjects and reported that BMI was significantly higher in Ala allele carriers compared with Pro allele homozygotes ($P = .019$). The results in Table 2 show that weight, BMI, and WHR are significantly higher in Ala allele carriers than in noncarriers, which is consistent with the meta-analysis results. This consistency of the

Table 7. Source of Variation in Subcutaneous and Visceral Fat Areas in Overweight Subjects With BMI of Greater Than 25

	Subcutaneous Fat Area		Visceral Fat Area	
	% of Variance	P Value	% of Variance	P Value
PPAR γ 2 P12A	6.2	.000	1.8	.045
Age	—	NS	16.7	.000
Lean body mass	15.9	.000	4.7	.001
Serum triglyceride	—	NS	5.1	.001

Abbreviation: NS, not significant.

Table 8. Comparison of Serum Biochemical Parameters by PPAR γ 2 Genotype in Overweight Subjects With BMI of Greater Than 25

Genotype	PP Type (n = 423)	PA/AA Type (n = 33)	P Value
Lipid profiles			
Total cholesterol (mg/dL)	182.11 $\pm$ 1.49	182.67 $\pm$ 6.53	.904†
LDL cholesterol (mg/dL)	112.73 $\pm$ 1.38	116.61 $\pm$ 4.78	.425
HDL cholesterol (mg/dL)	47.67 $\pm$ 0.58	47.77 $\pm$ 1.94	.952
Triglyceride (mg/dL)	109.90 $\pm$ 2.11	108.83 $\pm$ 8.65	.929
Atherogenic index*	3.05 $\pm$ 0.05	3.10 $\pm$ 0.19	.750
LDL/HDL†	2.53 $\pm$ 0.05	2.59 $\pm$ 0.16	.688
Fasting blood glucose			
Glucose (mg/dL)	103.92 $\pm$ 1.06	105.38 $\pm$ 3.12	.678
Liver function indicators			
Total bilirubin (mg/dL)	0.694 $\pm$ 0.037	0.572 $\pm$ 0.032	.333
GOT (IU/L)	22.06 $\pm$ 0.78	19.61 $\pm$ 2.19	.371
GPT (IU/L)	28.31 $\pm$ 1.25	27.09 $\pm$ 6.43	.782
Albumin (g/dL)	4.36 $\pm$ 0.02	4.38 $\pm$ 0.04	.824
Protein (g/dL)	7.59 $\pm$ 0.02	7.66 $\pm$ 0.07	.435

NOTE. Data are mean $\pm$ SE.

*Atherogenic index (AI) = (total cholesterol – HDL cholesterol)/HDL cholesterol.

†LDL cholesterol to HDL cholesterol ratio.

‡P values were obtained by general linear model (covariance) analysis adjusted for age.

results from this study with the meta-analysis involving an extremely large number of subjects may suggest that the subjects involved in this study are representative of the general population, and other data in this study may be equally reliable.

Until now, the effect of PPAR γ 2 polymorphism on CT-measured body fat distribution had not been studied extensively. Mori et al¹⁷ reported no difference in BMI, subcutaneous fat area, and visceral fat area with respect to PPAR γ 2 genotype among 215 nondiabetic male Japanese subjects. In this report, however, the number of subjects was small (203 Pro homozygotes and 12 Ala allele carriers), and the effect on BMI was not consistent with the meta-analysis results. Robitaille et

Table 9. Changes in Physical Characteristics and Body Fat Mass During a 1-Month Weight Loss Program Among Overweight Subjects With BMI of Greater Than 25

Genotype	PP Type (n = 181)	PA/AA Type (n = 17)	P Value
Weight (kg)	–7.06 $\pm$ 0.19	–7.23 $\pm$ 0.79	.799*
BMI (kg/m ²)	–2.92 $\pm$ 0.12	–2.81 $\pm$ 0.33	.755
WHR	–0.035 $\pm$ 0.005	–0.054 $\pm$ 0.010	.243
Fat mass (kg)	–5.05 $\pm$ 0.23	–5.98 $\pm$ 0.91	.249

NOTE. Data are mean $\pm$ SE.

*P values were obtained by general linear model (covariance) analysis adjusted for age.

al⁹ reported that carriers of the Ala allele had greater BMI, waist circumference, fat mass, as well as subcutaneous fat and visceral fat areas than Pro homozygotes among 720 French Canadians participating in a Quebec Family Study. They reported that fat areas of visceral adipose tissue and subcutaneous adipose tissue were 14% and 27% higher in Ala allele carriers compared with noncarriers. The effects of the Ala allele in female Korean subjects were almost identical to the effects in French Canadian subjects, with fat areas of visceral adipose tissue and subcutaneous adipose tissue 16% and 28% higher in Ala allele carriers (Table 4). The results in Table 2 and Table 4 show that the effects of the Ala allele on BMI, fat mass, and CT-measured abdominal fat areas among the Korean population and the Caucasian population in the Quebec Family Study are similar, even though genetic backgrounds and dietary patterns could be expected to differ significantly between the 2 populations. In this study, fat areas at distal parts of the body (the upper and lower thigh) were also measured; the Ala allele had a similar effect on the central (abdominal) and distal (thigh) adipose tissues (Table 4).

Data shown in Table 5 and Table 6 show that the effect of the Ala allele on body fat accumulation varies according to obesity status of the subjects. Among lean subjects, the fat-accumulating effect of the Ala allele was not evident, while a statistically significant effect was found among overweight subjects. This result is also consistent with the meta-analysis of Masud and Ye,⁸ in which the Ala 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 disparate effects of the Ala allele on body fat accumulation in lean and overweight subjects suggest that the impact of PPAR γ 2 genotype can be modified by other factors. The expression level of PPAR γ 2 mRNA in adipose tissue of obese subjects was higher than in lean subjects, and the level was decreased by a low calorie diet.¹⁸ The increased expression level of PPAR γ 2 mRNA among obese subjects could amplify the small difference in PPAR γ 2 activity caused by the Pro12Ala substitution, while the amplification might not be enough to affect a difference in lean subjects. In addition, it was reported that low dietary polyunsaturated fat to saturated fat ratios are correlated with greater BMI in Ala allele carriers than in noncarriers, while high dietary ratios are correlated with the opposite.¹⁹ Gene-nutrient interactions may also cause the disparate effects of the Ala allele on fat accumulation.

Table 4 shows that subcutaneous fat, but not visceral fat, differed significantly according to PPAR γ 2 genotype among all the subjects. Among overweight subjects, the abdominal subcutaneous fat area and visceral fat area were 28% and 13% higher in Ala allele carriers, suggesting that PPAR γ 2 genotype has a greater effect on subcutaneous fat than on visceral fat (Table 6). Data in Table 7 more clearly show the greater effects

of PPAR γ 2 genotype on subcutaneous fat (6.2%) than on visceral fat (1.8%). Lefebvre et al²⁰ reported that PPAR γ is expressed in higher levels in human subcutaneous adipose tissue than in visceral adipose tissue. The higher expression level of PPAR γ in subcutaneous adipose tissue could lead to higher amplification of the subtle change in its activity caused by Pro12Ala substitution, while the amplification may be lower in visceral adipose tissue. It is well known that visceral fat accumulation is related to aberrant metabolic profiles.²¹ However, a relationship between subcutaneous fat and metabolic syndrome has not yet been identified. Troglitazone, a PPAR γ activator, was reported to increase subcutaneous fat area while improving metabolic profiles, providing evidence that subcutaneous fat accumulation is not related to metabolic syndrome.²² Matsuzawa et al²³ suggested that the visceral fat to subcutaneous fat ratio (V/S ratio) is a better indicator of metabolic syndrome. In this study, V/S ratio was not significantly different and was even slightly decreased in Ala allele carriers (Table 4 and Table 6), providing some explanation for the similar metabolic profiles among PPAR γ 2 genotypes shown in Table 8.

The molecular mechanism of the effects of PPAR γ 2 polymorphism on adipogenic activity was not completely elucidated until now. Deeb et al²⁴ reported that the substitution of Ala for Pro at the 12th codon resulted in a decrease in binding affinity of PPAR γ 2 to the cognate promoter element and reduced the ability to transactivate responsive promoters in *in vitro* experiments. On the contrary, however, the results in this study along with the report by Robitaille et al⁹ show that the Ala allele is associated with increased body fat in humans, suggesting that the Ala allele might be associated with increased adipogenic activity of PPAR γ 2. The effect of the Ala allele might differ in *in vivo* and *in vitro* conditions. Various kidney, hepatoma, and preadipocyte cells lines were studied experimentally *in vitro*. However, cell lines often lack some cellular pathways and may differ from whole body conditions. Soukas et al²⁵ found that the gene expression pattern in adipocytes *in vitro* and *in vivo* overlap, but also differ importantly in some respects, and suggested that one or more transcriptional programs are activated exclusively *in vivo* to generate the full adipocyte phenotype. In *in vitro* experiments, nonphysiologically high amounts of PPAR γ 2 proteins were expressed by the transfection of high-efficiency expression vectors, which may not occur under normal physiologic conditions. Furthermore, the *in vitro* experiments were conducted over a very short time period, while fat accumulation in humans is the integrative result of a lifetime process beginning in the prenatal stage. Long-term effects may differ from short-term effects. More study is needed to elucidate the mechanism underlying the *in vivo* effects of PPAR γ 2 polymorphism.

REFERENCES

1. Spiegelman BM: PPAR γ : Adipogenic regulator and thiazolidinedione receptor. *Diabetes Care* 47:507-514, 1998
2. Elbrecht A, Chen Y, Cullinan CA, et al: Molecular cloning, expression and characterization of human peroxisome proliferators activated receptors γ 1 and γ 2. *Biochem Biophys Res Commun* 224:431-437, 1996
3. Werman A, Hollenberg A, Solanes G, et al: Ligand-independent

activation domain in the N-terminus of peroxisome proliferators activated receptor γ : Differential activity of PPAR γ -1 and -2 isoforms and influence of insulin. *J Biol Chem* 272:20230-20235, 1997

4. Barroso I, Gurnell M, Growley VE, et al: Dominant negative mutations in human PPAR γ associated with severe insulin resistance, diabetes mellitus and hypertension. *Nature* 402:880-883, 1999

5. Ristow M, Muller-Wieland D, Pfeiffer A, et al: Obesity associated with a mutation in a genetic regulator of adipocyte differentiation. *N Engl J Med* 339:953-959, 1998
6. Yen C-J, Beamer BA, Negri C, et al: Molecular scanning of the human peroxisome proliferators activated receptor γ (hPPAR γ) gene in diabetic Caucasians: Identification of a Pro12Ala PPAR- γ 2 missense mutation. *Biochem Biophys Res Commun* 241:270-274, 1997
7. Altshuler D, Hirschhorn JN, Klannemark M, et al: The Common PPARGamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76-80, 2000
8. Masud S, Ye S: Effect of the peroxisome proliferators activated receptor- γ gene Pro12Ala variant on body mass index: A meta-analysis. *J Med Genet* 40:773-780, 2003
9. Robitaille J, Despres JP, Perusse L, et al: The PPAR-gamma P12A polymorphism modulates the relationship between dietary fat intake and components of the metabolic syndrome: Results from the Quebec Family Study. *Clin Genet* 63:109-116, 2003
10. Fu M, Chen H, Li X, et al: Association of Pro12Ala variant in peroxisome proliferator-activated receptor-gamma2 gene with type 2 diabetes mellitus. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 19:234-238, 2002
11. Lei HH, Chen MH, Yang WS, et al: Peroxisome proliferator-activated receptor gamma 2 Pro12Ala gene variant is strongly associated with larger body mass in the Taiwanese. *Metabolism* 49:1267-1270, 2000
12. Mori H, Ikegami H, Kawaguchi Y, et al: The Pro12Ala substitution in PPARGamma is associated with resistance to development of diabetes in the general population. *Diabetes* 50:891-894, 2001
13. Beamer BA, Yen CJ, Andersen RE, et al: Association of the Pro12Ala variant in the peroxisome proliferator-activated receptor-gamma2 gene with obesity in two Caucasian populations. *Diabetes* 47:1806-1808, 1998
14. Schaffler A, Barth N, Schmitz G, et al: Frequency and significance of Pro12Ala and Pro115Gln polymorphism in gene for peroxisome proliferation-activated receptor-gamma regarding metabolic parameters in a Caucasian cohort. *Endocrine* 14:369-373, 2001
15. Kolehmainen M, Uusitupa MI, Alhava E, et al: Effect of the Pro12Ala polymorphism in the peroxisome proliferator-activated receptor (PPAR) gamma2 gene on the expression of PPARGamma target genes in adipose tissue of massively obese subjects. *J Clin Endocrinol Metab* 88:1717-1722, 2003
16. Nieman DC: Exercise Testing and Prescription: A Health Related Approach (ed 4). Mayfield, CA, Mountain View, 1999
17. Mori Y, Kim-Motoyama H, Katakura T, et al: Effect of the Pro12Ala variant of the human peroxisome proliferators-activated receptor gamma2 gene on adiposity, fat distribution, and insulin sensitivity in Japanese men. *Biochem Biophys Res Commun* 251:195-198, 1998
18. Vidal-Puig AN, Considine RV, Jimenez-Linan M, et al: 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, 1997
19. Luan J, Browne PO, Harding AH, et al: Evidence for gene-nutrient interaction at the PPARGamma locus. *Diabetes* 50:686-689, 2001
20. Lefebvre A, Laville M, Vaga N, et al: Depot-specific differences in adipose tissue gene expression in lean and obese subjects. *Diabetes* 47:98-103, 1998
21. Bjorntorp P: Metabolic implication of body fat distribution. *Diabetes Care* 14:1132-1143, 1991
22. Akazawa S, Kawasaki E, Sun F, et al: Efficacy of troglitazone on body fat distribution in type 2 diabetes. *Diabetes Care* 23:1067-1071, 2000
23. Matsuzawa Y, Nakamura T, Shimomura I, et al: Visceral fat accumulation and cardiovascular disease. *Obes Res* 3:645S-647S, 1995 (suppl 5)
24. Deeb SS, Fajas L, Nemoto M, et al: A Pro12Ala substitution in PPARGamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 20:284-287, 1998
25. Soukas A, Socci ND, Saatkamp BD, et al: Distinct transcriptional profiles of adipogenesis in vivo and in vitro. *J Biol Chem* 36:34167-34174, 2001