

# Association of the Pro12Ala Variant in the Peroxisome Proliferator-Activated Receptor- $\gamma$ 2 Gene With Obesity in Two Caucasian Populations

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**T**he nuclear receptor, peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ), is an important regulator of adipocyte differentiation and a modulator of intracellular insulin-signaling events (1). PPAR- $\gamma$  mRNA expression in both skeletal muscle and adipose tissue *in vitro* is induced by insulin (2,3). In skeletal muscle of obese subjects, PPAR- $\gamma$  mRNA is elevated in direct relation to BMI and fasting insulinemia (2); reports are mixed as to whether expression is increased in adipose tissue of obese subjects (3–5). In one report, activators of PPAR- $\gamma$  were shown to increase adiposity in a rodent model (6), while in another (7) they were found to increase the number of adipocytes, but not the mass of adipose tissue. Clinically, most studies have not found that PPAR- $\gamma$ -activating thiazolidinediones cause weight gain when administered to humans for treatment of diabetes (1).

Alternate use of promoters and differential splicing of the human PPAR- $\gamma$  gene results in two isoforms: PPAR- $\gamma$ 1 and PPAR- $\gamma$ 2. PPAR- $\gamma$ 2 contains 28 additional amino acids at its NH<sub>2</sub> terminus (1,8). PPAR- $\gamma$ 1 and PPAR- $\gamma$ 2 both are expressed in adipose tissue (1,3–5), and there appears to be no difference in the abilities of the two isoforms to participate in ligand-induced initiation of transcription of target genes or in ligand-induced adipocyte differentiation (9). Interestingly, however, it was demonstrated recently that PPAR- $\gamma$  could activate transcription in a ligand-independent fashion and that insulin potentiated this activity (9).

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AA, Ala<sup>12</sup> homozygotes; ANCOVA, analysis of covariance; BLSA, Baltimore Longitudinal Study on Aging; JHU-WMC, Johns Hopkins University Weight Management Center; PA, Pro12Ala heterozygotes; PCR, polymerase chain reaction; PP, Pro<sup>12</sup> homozygotes; PPAR- $\gamma$ , peroxisome proliferator-activated receptor- $\gamma$ ; RFLP, restriction fragment length polymorphism.

Furthermore, the  $\gamma$ 2 isoform is much more potent at doing so than is the  $\gamma$ 1 isoform (9), suggesting a possible distinct role for PPAR- $\gamma$ 2 in obesity, insulin resistance, and diabetes.

Recently, we reported a naturally occurring variant in the human PPAR- $\gamma$ 2 gene that predicts substitution at amino acid 12 of alanine for the proline that is present in both normal human and mouse PPAR- $\gamma$ 2 sequences (10). This amino acid position is within the domain of PPAR- $\gamma$ 2 that enhances ligand-independent activation, as described by Werman et al. (9). Because the substitution of alanine for proline is non-conservative and could cause a significant change in protein structure, we hypothesize that it may alter the function of PPAR- $\gamma$ 2 such that individuals with this variant may be at an increased genetic risk for obesity and/or insulin resistance.

To test this hypothesis, we genotyped and performed association studies of the Pro12Ala PPAR- $\gamma$ 2 variant in two independently recruited cohorts of unrelated, nondiabetic, adult Caucasian subjects from the Baltimore metropolitan area. One cohort of 517 subjects, with a distribution of being lean-to-moderately obese (mean BMI 26.5 kg/m<sup>2</sup>, range 18.6–43.2 kg/m<sup>2</sup>), is from the Baltimore Longitudinal Study on Aging (BLSA) and has been recruited continuously since 1958. The other cohort is of 169 very obese subjects (mean BMI 36.5 kg/m<sup>2</sup>, range 24.2–76.8 kg/m<sup>2</sup>), recruited prospectively from August 1994 through June 1996 from the Johns Hopkins University Weight Management Center (JHU-WMC). Subjects with diabetes by history or with fasting glucose  $\geq 7.1$  mmol/l (126 mg/dl) were excluded to avoid the well-known confounding effects of diabetes and its treatment on the obesity-related traits that were studied. All protocols were approved by the Institutional Review Board of the Johns Hopkins University, and informed written consent was obtained from all subjects.

Genomic DNA was obtained from peripheral blood using standard methods, and the Pro12Ala PPAR- $\gamma$ 2 variant was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. This PCR-RFLP analysis, previously described (10), uses a mutagenic PCR primer to introduce a *Bst*UI site only when a C  $\rightarrow$  G substitution at nucleotide 34 of the PPAR- $\gamma$ 2 gene is present. Genotyping was repeated for all Ala<sup>12</sup> homozygotes, several Pro12Ala heterozygotes chosen randomly, and several Pro<sup>12</sup> homozygotes chosen randomly; reproducibility was 100%. Because the number of Ala<sup>12</sup> homozygotes (AA) was small (two in JHU-WMC, three in BLSA), these were collapsed

TABLE 1  
JHU-WMC and BLSA subject characteristics by Pro12Ala PPAR- $\gamma$ 2 genotype adjusted for age and/or BMI

	JHU-WMC						BLSA					
	Men			Women			Men			Women		
	PP	PA/AA	<i>P</i> value	PP	PA/AA	<i>P</i> value	PP	PA/AA	<i>P</i> value	PP	PA/AA	<i>P</i> value
<i>n</i>	47	10	—	94	18	—	246	70	—	162	39	—
Age (years)	44.3 $\pm$ 1.7	42.9 $\pm$ 4.8	0.75	43.2 $\pm$ 1.1	41.7 $\pm$ 2.0	0.57	65.7 $\pm$ 1.0	62.3 $\pm$ 1.8	0.92	63.3 $\pm$ 1.4	64.8 $\pm$ 2.6	0.76
BMI (kg/m <sup>2</sup> )*	37.5 $\pm$ 0.9	42.5 $\pm$ 2.0	0.03	34.3 $\pm$ 0.9	41.3 $\pm$ 2.2	0.004	26.7 $\pm$ 0.2	27.6 $\pm$ 0.5	0.08	25.5 $\pm$ 0.4	26.9 $\pm$ 0.7	0.09
Maximum BMI (kg/m <sup>2</sup> )* $\ddagger$	NA	NA		NA	NA		27.7 $\pm$ 0.3	28.7 $\pm$ 0.5	0.08	26.2 $\pm$ 0.4	27.6 $\pm$ 0.8	0.09
Weight (kg)*	118.5 $\pm$ 3.2	136.2 $\pm$ 7.1	0.03	92.7 $\pm$ 2.6	114.5 $\pm$ 6.0	0.001	82.5 $\pm$ 0.9	86.1 $\pm$ 1.6	0.05	66.6 $\pm$ 1.0	68.2 $\pm$ 2.1	0.47
Waist circumference (cm)*	124.2 $\pm$ 2.4	134.5 $\pm$ 5.2	0.08	98.0 $\pm$ 2.0	114.4 $\pm$ 4.5	0.001	95.6 $\pm$ 0.7	98.2 $\pm$ 1.3	0.08	83.6 $\pm$ 0.9	84.5 $\pm$ 1.8	0.66
Waist-to-hip ratio*	0.98 $\pm$ 0.01	1.01 $\pm$ 0.02	0.23	0.80 $\pm$ 0.01	0.85 $\pm$ 0.02	0.02	0.95 $\pm$ <0.01	0.95 $\pm$ <0.01	0.50	0.82 $\pm$ <0.01	0.83 $\pm$ 0.01	0.72
Fasting glucose (mmol/l) $\dagger$	5.2 $\pm$ 0.1	4.9 $\pm$ 0.2	0.25	5.0 $\pm$ 0.1	5.1 $\pm$ 0.1	0.78	5.4 $\pm$ <0.1	5.4 $\pm$ <0.1	0.96	5.1 $\pm$ <0.1	5.0 $\pm$ <0.1	0.16
2-h glucose (mmol/l) $\dagger$ $\S$	NA	NA		NA	NA		7.3 $\pm$ 0.1	7.1 $\pm$ 0.2	0.38	6.0 $\pm$ 0.1	6.6 $\pm$ 0.3	0.2
Fasting insulin (pmol/l) $\dagger$	120.4 $\pm$ 14	156.0 $\pm$ 37	0.38	95.5 $\pm$ 7	89.9 $\pm$ 16	0.75	70 $\pm$ 2	74 $\pm$ 4	0.45	67 $\pm$ 3	61 $\pm$ 6	0.33
2-h insulin (pmol/l) $\dagger$ $\S$	NA	NA		NA	NA		369 $\pm$ 15	364 $\pm$ 27	0.88	314 $\pm$ 19	242 $\pm$ 39	0.1
HDL cholesterol (mmol/l) $\dagger$	1.08 $\pm$ 0.06	0.73 $\pm$ 0.16	0.04	1.29 $\pm$ 0.04	1.38 $\pm$ 0.08	0.33	1.12 $\pm$ 0.02	1.07 $\pm$ 0.04	0.26	1.40 $\pm$ 0.03	1.37 $\pm$ 0.05	0.69
LDL cholesterol (mmol/l) $\dagger$	3.74 $\pm$ 0.16	3.89 $\pm$ 0.42	0.75	3.78 $\pm$ 0.11	3.44 $\pm$ 0.25	0.23	2.88 $\pm$ 0.05	2.84 $\pm$ 0.10	0.71	2.73 $\pm$ 0.07	2.71 $\pm$ 0.14	0.89
Total cholesterol (mmol/l) $\dagger$	5.74 $\pm$ 0.19	5.86 $\pm$ 0.42	0.81	5.75 $\pm$ 0.11	5.53 $\pm$ 0.25	0.43	4.61 $\pm$ 0.06	4.58 $\pm$ 0.11	0.79	4.74 $\pm$ 0.07	4.63 $\pm$ 0.15	0.49
Log triglyceride $\dagger$ $\parallel$	0.229 $\pm$ 0.031	0.471 $\pm$ 0.080	0.007	0.160 $\pm$ 0.025	0.109 $\pm$ 0.056	0.41	0.023 $\pm$ 0.01	0.054 $\pm$ 0.03	0.32	0.022 $\pm$ 0.019	-0.046 $\pm$ 0.039	0.13
Systolic blood pressure (mmHg) $\dagger$	137 $\pm$ 2	143 $\pm$ 5	0.29	129 $\pm$ 2	129 $\pm$ 4	0.98	136 $\pm$ 1	140 $\pm$ 2	0.19	133 $\pm$ 2	131 $\pm$ 3	0.56
Diastolic blood pressure (mmHg) $\dagger$	90 $\pm$ 2	96 $\pm$ 4	0.16	84 $\pm$ 1	84 $\pm$ 2	0.98	83 $\pm$ 1	84 $\pm$ 1	0.42	78 $\pm$ 1	77 $\pm$ 2	0.71

Data are adjusted means  $\pm$  SE. \*Adjusted for age by ANCOVA;  $\dagger$ adjusted for age and BMI by ANCOVA;  $\ddagger$ maximal BMI recorded to date during BLSA subjects' enrollment;  $\S$ glucose and insulin at 120 min during oral glucose tolerance test;  $\parallel$ triglycerides measured in millimoles per liter, transformed to log base 10 to give more normal distribution. NA, these data are not available for the JHU-WMC cohort.

with Pro12Ala heterozygotes (PA), and compared with Pro<sup>12</sup> homozygotes (PP) for all analyses. Analysis of covariance (ANCOVA) was used to compare quantitative phenotypic traits, with results presented as adjusted means  $\pm$  SE.

Allele frequencies of the Pro12Ala PPAR- $\gamma$ 2 were 0.11 in both Caucasian cohorts. Genotype frequencies were also similar and conformed to expectations of the Hardy-Weinberg rule. In both cohorts, the Pro12Ala PPAR- $\gamma$ 2 variant associated with higher age-adjusted means for BMI and weight. In the obese JHU-WMC cohort, the PA/AA subjects had a higher mean BMI than PP subjects (mean BMI 41.5  $\pm$  1.6 kg/m<sup>2</sup> vs. 35.3  $\pm$  0.7 kg/m<sup>2</sup>; *P* < 0.001), as well as greater weight (122.3  $\pm$  5.2 kg vs. 101.2  $\pm$  2.3 kg; *P* < 0.001). When stratified by sex, these associations were more evident in women (Table 1). In the BLSA cohort, there also were significant associations of the PA/AA genotype with higher BMI from the last visit (27.3  $\pm$  0.4 kg/m<sup>2</sup> vs. 26.1  $\pm$  0.2 kg/m<sup>2</sup>; *P* = 0.01), higher maximal BMI recorded during BLSA enrollment (28.1  $\pm$  0.4 kg/m<sup>2</sup> vs. 26.9  $\pm$  0.2 kg/m<sup>2</sup>; *P* = 0.01), and greater weight (77.6  $\pm$  1.3 kg vs. 74.4  $\pm$  0.7 kg; *P* = 0.03). However, when stratified by sex, the associations did not reach statistical significance in the BLSA cohort (Table 1).

Furthermore, the mean waist circumference and waist-to-hip ratio were significantly higher in the PA/AA women of the JHU-WMC, with a similar trend in men. Men of the JHU-

WMC cohort also had significant differences in fasting triglycerides and HDL cholesterol, with a trend toward higher fasting insulin and blood pressure. By contrast, in women of both groups there were tendencies toward lower insulin and triglyceride levels in those with the PA/AA genotype, with *P* values that did not reach statistical significance (Table 1). There were no significant associations with glucose levels in any subset.

Our findings that the Pro12Ala PPAR- $\gamma$ 2 missense mutation associates with higher BMI in two independent cohorts suggest that it (or another mutation in linkage disequilibrium) may contribute to the genetic susceptibility for the multifactorial disorder of obesity. Because associations with BMI, waist circumference, and weight are much less strong in the larger BLSA cohort of less obese subjects than in the smaller JHU-WMC cohort of very obese subjects, these comparisons need to be examined in other Caucasian populations, as well as in other ethnic groups. The association we found with altered lipid profile was in the small subset of very obese men only, but suggests this variant's role in cardiovascular disease deserves further investigation. Seemingly contrasting trends in lipid profile in women may suggest interaction between the Pro12Ala variant and sex. We found no significant associations with fasting insulin or glucose levels, though other studies will

be needed to examine the variant's role in overt diabetes. Functional evaluation of the ability of the Pro12Ala PPAR- $\gamma$ 2 variant protein to activate transcription in the typical ligand-dependent fashion, as well as in the insulin-sensitive ligand-independent fashion, is needed to demonstrate directly a functional role for this amino acid substitution.

In summary, we performed association studies in two independent Caucasian populations and found that the Pro12Ala PPAR- $\gamma$ 2 variant associates with higher BMI. These results suggest that genetic variation at the PPAR- $\gamma$  locus may influence susceptibility to obesity in humans. These results also may imply that the PPAR- $\gamma$ 2 isoform plays a role in adipocyte metabolism that is distinct from that of the PPAR- $\gamma$ 1 isoform.

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