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Inverse effects of the PPARγ2 Pro12Ala polymorphism on measures of adiposity over 15 years in African Americans and whites The CARDIA study

Myriam Fornage[a,*], David R. Jacobs Jr[c,d], Michael W. Steffes[e], Myron D. Gross[e], Molly S. Bray[b], Pamela J. Schreiner[c]

[a]*Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX 77030, USA*
[b]*Human Genetics Center, University of Texas Health Science Center at Houston, Houston, TX 77030, USA*
[c]*Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, MN 55454, USA*
[d]*Department of Nutrition, University of Oslo, Oslo, Norway*
[e]*Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55454, USA*

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Abstract

Few studies have addressed the association of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor γ2 (PPARγ2) gene with longitudinal measures of adiposity and insulin sensitivity during young adulthood, or reported on its relationship with these outcomes in African Americans. These issues were examined in the biracial Coronary Artery Risk Development in Young Adults (CARDIA) cohort, a population-based sample of 5115 African Americans and whites followed prospectively over 15 years. Frequency of the Ala12 allele was 2.1% in African Americans and 12.8% in whites, consistent with previous reports. A generalized estimating equation method was used to simultaneously examine the cross-sectional and longitudinal relationships between the Pro12Ala polymorphism and the measures of adiposity and insulin sensitivity. The Pro12Ala polymorphism was significantly associated with mean 15-year levels of adiposity, but these associations were in opposite direction in the 2 racial groups. On average, African Americans carrying the Ala12 allele had a 1.1 kg/m^2 lower body mass index (BMI) (P = .02) and whites a 0.6 kg/m^2 higher BMI (P = .01), as compared to Pro12 homozygotes. The Ala12 allele was also significantly associated with a decreased risk of incident insulin resistance syndrome in each race (OR = 0.44, P = .04 in African Americans; OR = 0.61, P = .01 in whites) and lower mean 15-year levels of fasting insulin (P = .02), glucose (P = .02), and homeostasis model assessment (P = .01) in African Americans but not in whites. Important roles of BMI and ethnic background in influencing the complex relationships among PPARγ gene variation, adiposity, and insulin resistance are suggested.

1. Introduction

The peroxisome proliferator-activated receptor γ (PPARγ) is a transcription factor activated by fatty acids that regulates energy storage, adipocyte differentiation, lipoprotein metabolism, and insulin action [1]. Three messenger RNA (mRNA) isoforms have been identified, PPARγ1, PPARγ2, and PPARγ3, which are produced by alternate promoters of the PPARγ gene and alternative splicing of the PPARγ mRNA [1,2]. PPARγ2 is most abundantly and relatively specifically expressed in adipose tissue [1]. A common missense mutation in the PPARγ2-specific domain has been identified, which results in the substitution of an alanine for a proline at codon 12 [3]. In transfection assays, the Ala allele of the nuclear receptor had a decreased binding affinity for its promoter element, and thus, had a reduced ability to transactivate the promoter of its target genes [4].

Allele frequencies of the Pro12Ala polymorphism vary among races. Multiple studies have been conducted in human populations, mostly in whites, to investigate the association of this polymorphism with obesity and type 2 diabetes, yielding conflicting results. Although the Ala12 allele has been more consistently associated with higher body mass

* Corresponding author. Tel.: +1 713 500 2463; fax: +1 713 500 2424.
E-mail address: myriam.fornage@uth.tmc.edu (M. Fornage).

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index (BMI) and waist circumference [5-8], some reports have found either no relationship between this allele and obesity [9-11] or its association with lower BMI [4,12,13]. Likewise, the Ala12 allele has been associated with improved insulin sensitivity and decreased risk for type 2 diabetes in many but not all studies [4,14-18]. Few studies have addressed the association of the Pro12Ala polymorphism with longitudinal changes in measures of adiposity and insulin sensitivity during young adulthood. Fewer yet have reported on its relationship with these outcomes in African Americans. We investigated the association of the Pro12Ala polymorphism with longitudinal measures of adiposity and insulin sensitivity in the biracial Coronary Artery Risk Development in Young Adults (CARDIA) cohort. We also examined the role of dietary fat intake, physical activity, and BMI in possibly modulating these relationships.

2. Materials and methods

2.1. Sample and laboratory methods

Participants were from the CARDIA study. The initial examination included 5115 African Americans and whites 18 to 30 years of age. Participants were recruited without regard to health status to represent proportionate racial, sex, age, and education groups from 4 communities: Birmingham, Ala; Chicago, Ill; Minneapolis, Minn; and Oakland, Calif. Details of the study design have been published [19]. Five sequential examinations have been conducted from the time of initiation of the study in 1985-1986 through year 15 (2000-2001).

Each participant's age, race, and sex were self-reported during the recruitment phase and verified during the baseline clinic visit. Body weight, height, and waist circumference were assessed at each examination. Body weight was measured to the nearest 0.1 kg, using a calibrated scale, with the participant in light clothing without shoes. Height was measured to the nearest 0.5 cm with a vertical ruler. Body mass index was computed as body weight per height squared (kg/m^2). Waist circumference was measured in duplicate midway between the iliac crest and the lowest lateral portion of the rib cage. Hip circumference was measured in duplicate at the maximal protrusion of the gluteal muscles at the level of the symphysis pubis. Triceps, subscapular, and suprailiac skinfold thicknesses were measured in duplicate using a Harpenden caliper. The sum of all 3 skinfold thicknesses was used in this report. Skinfold thicknesses and hip circumference were measured at the baseline, years 2, 7, and 10.

Sociodemographic risk factors such as education and smoking were measured using standardized questionnaires at each examination. Physical activity was measured in all 6 examinations, as a summary score computed on the basis of the frequency and metabolic cost of self-reported typical moderate to vigorous physical activities [20]. Dietary factors were assessed at baseline and year 7 by a food frequency questionnaire [21]. The means of baseline and year 7 energy intake and total fat intake as a percent of energy intake were used in this report.

Blood samples were drawn after an overnight fast. Total plasma cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured according to standardized methods [22]. Plasma glucose was measured by the hexokinase method and serum insulin was measured by standard radioimmunoassay. Homeostasis model assessment (HOMA) index was computed as fasting plasma glucose (mmol/L) times fasting serum insulin (μU/I) divided by 22.5 [23]. Low HOMA values indicate high insulin sensitivity. Metabolic syndrome was defined on the basis of recommended criteria [24] as having 3 or more of the following: abdominal obesity (waist circumference >102 cm for men, >88 cm for women), high normal blood pressure (systolic ≥ 130 mm Hg, and/or diastolic ≥ 85 mm Hg, and/or taking antihypertensive medication), hypertriglyceridemia (fasting triglycerides ≥ 150 mg/dL), low HDL levels (fasting HDL-C <40 mg/dL for men, <50 mg/dL for women), and hyperglycemia (fasting glucose ≥ 110 mg/dL, or taking diabetes medication). Incident metabolic syndrome was coded as a categorical variable with presence defined as occurrence of the syndrome at any examination over 15 years and absence defined as no occurrence of the syndrome at all examinations.

Genotyping of the Pro12Ala polymorphism was performed by TaqMan assay (Applied Biosystems, Foster City, Calif) using forward and reverse primers (AAACCCCTATTCCATGCGTTTATG and GCAGACAGTGTATCAGTGAAGGAATC) and allele-specific probes (FAM-CTCCTATTGACCC- AGAAA and VIC-TCCTATTGACGCAGAAA). Genotype data were obtained on 3798 individuals who participated in the year 10 examination. Those without genotype information were more likely to be African Americans, smokers, and men, and have a lower education and a higher total fat intake, but were otherwise similar to those with genotype data (not shown).

2.2. Statistical methods

Genotype frequencies were estimated by direct counting. Agreement of the Pro12Ala genotype frequencies with Hardy-Weinberg equilibrium expectations was tested using a χ^2 goodness-of-fit test. In all subsequent analyses, Pro12/Ala12 and Ala12/Ala12 genotypes were combined in a single category because of the low frequency of the Ala12 allele. Within each race, means and proportions of risk factor variables were compared between individuals carrying at least one copy of the Ala12 allele and those not carrying the Ala12 allele by t tests and χ^2 tests, respectively.

In cross-sectional analyses, general linear models were used to assess the association of the Pro12Ala polymorphism with each outcome measure of obesity and insulin sensitivity, including BMI, waist and hip circumferences, skinfolds, fasting plasma glucose, HDL-C, LDL-C, and the natural logarithm transformations of fasting insulin, triglycerides, and HOMA index.

The longitudinal analyses used the generalized estimating equation method developed by Zeger and Liang [25]. For each racial group, the following typical model was estimated:

$$Y_{it} = \beta_0 + \beta_1 t + \beta_2 G_i + \beta_3 U_i + \beta_4 X_{it} + \beta_5 Z_{i0} + \beta_6 \Delta Z_{it} + \beta_7 (G_i \times t) + e_{it}$$

where for t = 0, 2, 5, 7, 10, and 15, Y_{it} is the measure of adiposity or insulin sensitivity of the ith person at year t; G_i is the Pro12Ala genotype category of the ith person; U_i is a time-independent covariate (eg, baseline age, baseline education); X_{it} is a time-dependent covariate for the ith person at time t (eg, X_{it} = 1 if the ith person is a current smoker and X_{it} = 0 otherwise); Z_{i0} is the baseline value of a time-dependent covariate (eg, baseline physical activity); ΔZ_{it} is the change in the value of that covariate from baseline (eg, change in physical activity between year t and baseline). Models were estimated that adjusted for age and sex (model 1); age, sex, smoking, education, and physical activity (model 2); variables in model 2 and BMI (model 3, where appropriate). For each model, the relevant time-dependent and time-independent variables were added according to the format illustrated in the typical model above. Potential genotype-by-environment interactions were assessed by adding the appropriate interaction terms. Interactions between genotype and total fat intake, physical activity, or BMI category were examined.

Multiple logistic regression models were used to assess the relationship between the metabolic syndrome and the Pro12Ala polymorphism. In each race, maximum likelihood estimates and 95% confidence intervals of the odds of having the metabolic syndrome in relation to Pro12Ala genotype category were obtained from models containing the following variables: age, sex, and Pro12Ala genotype category (model 1); variables in model 1, and BMI (model 2). Additional analyses were performed stratifying by obesity category (BMI $\geq$ 30 kg/m^2 and BMI < 30 kg/m^2).

3. Results

Frequency of the Ala12 allele was 2.1% in African Americans and 12.8% in whites, consistent with previous reports. In each racial group, genotype frequency distributions were in accordance with Hardy-Weinberg equilibrium expectations (P = .27 for African Americans, P = .64 for whites), and were significantly different between the 2 racial groups (Fisher exact test, P < .0001). African Americans and whites significantly differed with regards to most baseline characteristics, including age, BMI, HDL-C, triglycerides, fasting glucose and fasting insulin levels, physical activity, education level, dietary fat intake, sex ratio, and proportion of smokers (not shown). Table 1 shows the means of baseline measures of adiposity, insulin sensitivity, and lipids adjusted for age and sex, by genotype category in each race. In whites, there were significant associations between the Pro12Ala polymorphism and baseline measures of adiposity. White individuals carrying at least one copy of the Ala12 allele had significantly higher BMI (P = .001), greater circumferences (P = .01), and greater skinfold thickness (P = .0004) at baseline than Pro12 homozygotes. Pro12Ala genotype effects on measures of adiposity were not significantly different between Ala/Ala homozygotes and Ala/Pro heterozygotes, suggesting a dominant effect of the Ala12 allele in this race group (not shown). In African Americans, the Ala12 allele was significantly associated with lower baseline waist-to-hip ratio (P = .04) and lower subscapular-to-triceps skinfold ratio (P = .04). There was no significant association between the Pro12Ala polymorphism and the baseline measures of fasting glucose, insulin, triglycerides, LDL-C, HDL-C, and HOMA index in either race.

Table 1
Age- and sex-adjusted means (standard error) for selected baseline measures of adiposity, insulin sensitivity, and lipids by Pro12Ala genotype in African Americans and whites

Variable	African Americans			Whites		
	Pro/Ala + Ala/Ala	Pro/Pro	P	Pro/Ala + Ala/Ala	Pro/Pro	P
N	79	1765		473	1481	
Age	24.1 (4.0)	24.4 (3.8)	.50	25.7 (3.4)	25.5 (3.4)	.48
% Males	48.8	46.2	.34	39.2	42.1	.70
BMI (kg/m^2)	24.3 (0.6)	25.3 (0.1)	.16	24.2 (0.2)	23.5 (0.1)	.001
Waist (cm)	76.8 (1.3)	78.6 (0.3)	.15	78.3 (0.4)	77.0 (0.2)	.01
Triceps skinfold (mm)	15.8 (1.0)	16.4 (0.2)	.54	16.9 (0.3)	15.9 (0.2)	.01
Subscapular skinfold (mm)	15.9 (1.0)	16.9 (0.2)	.32	14.9 (0.3)	14.2 (0.2)	.06
Suprailiac skinfold (mm)	17.3 (1.2)	18.5 (0.3)	.34	18.9 (0.5)	18.4 (0.3)	.34
Hip (cm)	100.4 (1.3)	100.8 (0.3)	.75	100.0 (0.4)	98.3 (0.2)	.0004
Waist-to-hip ratio	0.76 (0.01)	0.78 (0.01)	.04	0.78 (0.01)	0.78 (0.01)	.43
Subscapular/triceps ratio	1.08 (0.03)	1.16 (0.01)	.04	0.95 (0.01)	0.98 (0.01)	.12
Glucose (mg/dL)	80.6 (1.8)	82.2 (0.4)	.37	82.9 (0.5)	82.9 (0.3)	.94
Log insulin (mU/L)	2.36 (0.05)	2.44 (0.01)	.14	2.28 (0.02)	2.27 (0.01)	.60
Log HOMA (mU mmol/L^2)	0.75 (0.06)	0.83 (0.01)	.14	0.69 (0.02)	0.68 (0.01)	.62
Log triglycerides (mg/dL)	4.04 (0.05)	4.09 (0.01)	.30	4.22 (0.02)	4.21 (0.01)	.92
HDL-C (mg/dL)	55.2 (1.5)	54.3 (0.3)	.54	51.6 (0.6)	51.8 (0.3)	.79
LDL-C (mg/dL)	109.7 (3.5)	109.6 (0.8)	.96	107.8 (1.4)	109.1 (0.8)	.41

Table 2
Adjusted differences in measures of adiposity and insulin sensitivity averaged over all examinations (mean) and in the average annual change in these measures (change per year) between genotype categories (Ala/X[a] minus Pro/Pro) by race

	Adjusted difference: Ala/X[a] minus Pro/Pro (95% CI)			
	African Americans		Whites	
	Mean	Change per year	Mean	Change per year
A				
BMI (kg/m^2)				
Model 1	−1.15 (−2.12; −0.18)*	0.006 (−0.056; 0.069)	0.56 (0.11; 1.02)*	0.02 (−0.01; 0.04)
Model 2	−1.05 (−2.05; −0.06)*	0.006 (−0.056; 0.068)	0.66 (0.20; 1.11)**	0.01 (−0.01; 0.04)
Waist (cm)				
Model 1	−2.32 (−4.44; −0.20)*	0.02 (−0.12; 0.16)	1.03 (0.03; 2.03)*	0.01 (−0.05; 0.07)
Model 2	−2.03 (−4.17; 0.12)	0.02 (−0.11; 0.16)	1.28 (0.29; 2.27)**	0.003 (−0.06; 0.06)
Hip (cm)				
Model 1	−1.48 (−3.82; 0.85)	−0.03 (−0.02; 0.15)	1.60 (0.65; 2.56)**	0.01 (−0.08; 0.06)
Model 2	−1.57 (−3.96; 0.81)	−0.02 (−0.21; 0.16)	1.75 (0.80; 2.70)***	−0.02 (−0.09; 0.05)
Skinfolds[b] (mm)				
Model 1	−2.75 (−8.45; 2.94)	0.23 (−0.22; 0.69)	1.99 (−0.28; 4.26)	0.00 (−0.19; 0.20)
Model 2	−2.97 (−8.70; 2.77)	0.27 (−0.19; 0.73)	2.48 (0.24; 4.72)*	−0.01 (−0.21; 0.19)
Subscapular/triceps ratio (unit)				
Model 1	−0.09 (−0.15; −0.03)**	0.002 (−0.01; 0.01)	−0.03 (−0.06; −0.01)*	−0.003 (−0.007; 0.001)
Model 2	−0.07 (−0.14; −0.01)*	−0.00 (−0.01; 0.01)	−0.03 (−0.06; −0.004)*	−0.002 (−0.005; 0.001)
Model 3	−0.08 (−0.14; −0.01)*	0.00 (−0.01; 0.01)	−0.04 (−0.07; −0.01)***	−0.003 (−0.006; 0.002)
B				
Log (HOMA) (unit)				
Model 1	−0.12 (−0.21; −0.03)**	0.001 (−0.01; 0.01)	0.01 (−0.04; 0.05)	−0.002 (−0.006; 0.002)
Model 2	−0.12 (−0.21; −0.03)**	0.002 (−0.01; 0.01)	0.01 (−0.03; 0.06)	−0.002 (−0.006; 0.001)
Model 3	−0.07 (−0.14; 0.01)	0.003 (−0.005; 0.011)	−0.02 (−0.06; 0.07)	−0.003 (−0.006; 0.001)
Log (insulin) (μU/mL)				
Model 1	−0.10 (−0.19; −0.02)*	0.00 (−0.01; 0.01)	0.01 (−0.03; 0.04)	−0.002 (−0.005; 0.002)
Model 2	−0.10 (−0.19; −0.01)*	0.00 (−0.01; 0.01)	0.01 (−0.02; 0.05)	−0.002 (−0.006; 0.001)
Model 3	−0.06 (−0.13; 0.01)	0.002 (−0.005; 0.009)	−0.01 (−0.05; 0.02)	−0.003 (−0.006; 0.001)
Glucose (mg/dL)				
Model 1	−2.10 (−3.72; −0.47)*	0.12 (−0.32; 0.57)	−0.23 (−1.18; 0.71)	0.03 (−0.09; 0.15)
Model 2	−1.79 (−3.49; −0.08)*	0.11 (−0.33; 0.57)	−0.13 (−1.07; 0.81)	0.03 (−0.09; 0.16)
Model 3	−1.30 (−3.05; 0.44)	0.17 (−0.30; 0.64)	−0.77 (−1.82; 0.28)	0.03 (−0.10; 0.15)
Log triglycerides (mg/dL)				
Model 1	−0.07 (−0.17; 0.02)	−0.003 (−0.01; 0.004)	−0.05 (−0.10; 0.005)	−0.001 (−0.005; 0.002)
Model 2	−0.06 (−0.15; 0.04)	−0.002 (−0.01; 0.004)	−0.04 (−0.09; 0.09)	−0.001 (−0.005; 0.002)
Model 3	−0.04 (−0.14; 0.05)	−0.003 (−0.01; 0.004)	−0.06 (−0.11; −0.02)**	−0.001 (−0.005; 0.002)

Model 1, adjusted for sex and age; model 2, adjusted for sex, age, education, smoking status, and exercise; model 3, adjusted for variables in model 2 and BMI.
[a] X = Ala or Pro.
[b] Sum of all three skinfold thicknesses.
* $P < .05$.
** $P < .01$.
*** $P < .001$.

Effects of the Pro12Ala polymorphism on longitudinal measures of adiposity and insulin sensitivity are shown in Table 2. There were significant interactions between race and genotype on measures of adiposity, including BMI ($P = .003$), waist circumference ($P = .007$), and hip circumference ($P = .006$), and on measures of insulin sensitivity, including HOMA ($P = .01$) and fasting insulin ($P = .02$). Thus, results are presented separately by race. In the generalized estimating equation analyses, the estimated coefficient for the genotype effects reflects the difference in measures of adiposity (or insulin sensitivity), averaged over all years, between Ala12 carriers and Pro12 homozygotes adjusting for other covariates. The estimated coefficient for the Genotype × Time effects reflects the average difference in the annual change in measures of adiposity (or measures related to insulin sensitivity) between genotype categories adjusting for other covariates. On average, over 15 years, African American carriers of the Ala12 allele had a lower BMI and smaller waist and hip circumferences as compared to Pro12 homozygotes after controlling for the effects of age and sex (Table 2A). However, in whites, Ala12 allele carriers had a higher BMI and greater waist and hip circumferences as compared to Pro12 homozygotes. Further controlling for the effects of smoking, education, and physical activity minimally affected these associations. The Ala12 allele was significantly associated with a lower subscapular-to-triceps skinfold ratio in both races, and this association was independent from

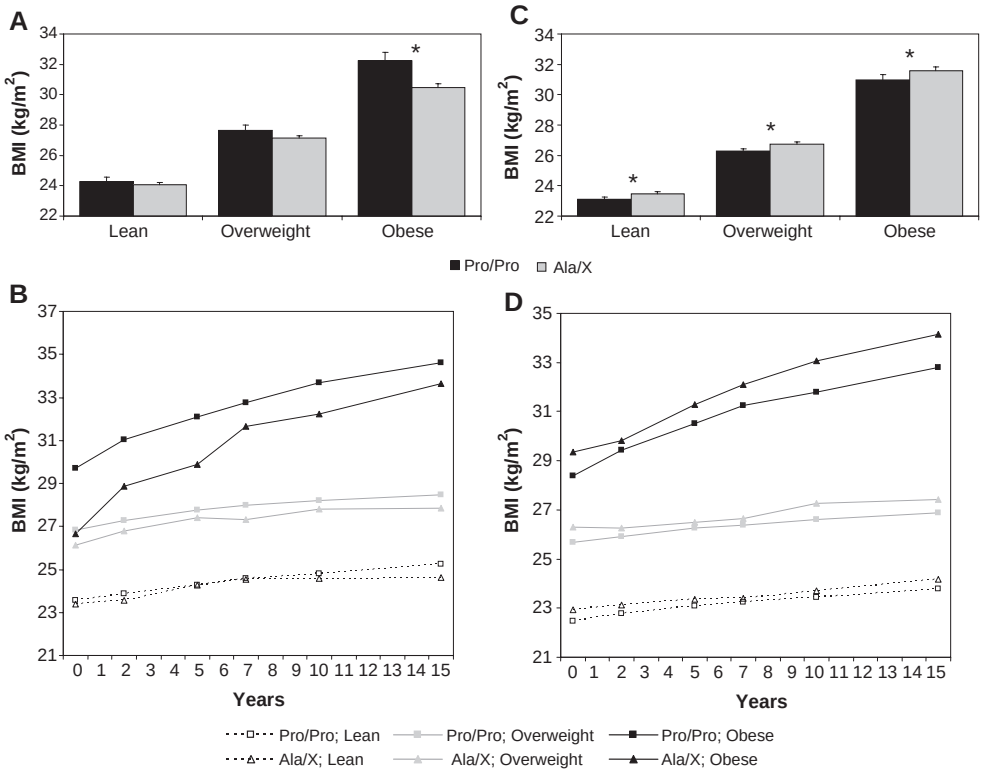

Fig. 1. Adjusted BMI (adjusted for age and sex) (averaged over 15 years) by genotype and BMI category (lean, BMI < 25; overweight, 25 ≤ BMI < 30; obese, BMI ≥ 30 kg/m^2) in African Americans (A) and in whites (C). Longitudinal profiles of adjusted BMI (adjusted for age and sex) by genotype and BMI category in African Americans (B) and in whites (D). Asterisk indicates $P < .05$.

effects on BMI. There was no significant effect of the Pro12Ala polymorphism on the annual change in measures of adiposity in either race. In other words, the difference in measures of adiposity that existed at baseline between the genotype categories remained the same over time (Table 2A).

There was a significant association of the Pro12Ala polymorphism with measures of insulin sensitivity in African Americans but not in whites (Table 2B). On average, over all examinations, Ala12 carriers had a lower HOMA index, lower fasting plasma insulin, and glucose levels as compared to Pro12 homozygotes. However, these associations did not remain statistically significant after controlling for the effects of BMI. There was no significant association between the Pro12Ala polymorphism and the fasting plasma lipid levels (not shown), with the exception of triglycerides. In whites, the Ala12 allele was significantly associated with lower triglyceride levels, independent of BMI. There was no significant effect of the Pro12Ala polymorphism on the rate of change in measures of insulin sensitivity and lipid levels in either race (Table 2B).

Body mass index may have modulating effects on the relationship between the Pro12Ala polymorphism and the measures of adiposity or insulin sensitivity. Therefore, we examined the interaction of BMI category (defined as lean, overweight, and obese) with Pro12Ala genotypes on changes in measures of adiposity and insulin sensitivity. Body mass index category significantly influenced the association of Pro12Ala genotypes with BMI in African Americans (P = .01) but not in whites (P = .90). The difference in mean 15-year BMI between Ala/Pro and Pro/Pro genotypes was greater among obese African Americans (Fig. 1A), whereas it remained constant and significant across BMI categories in whites (Fig. 1C). There was no significant effect of BMI category on the relationship of genotype with change in BMI over time in either race (P = .32 in African Americans; 0.22 in whites). In other words, within a BMI category, the difference in BMI between Ala-carrying and Pro/Pro genotypes remained the same over time (Fig. 1B and D).

We next examined the possible interactions of total fat intake with Pro12Ala genotypes on longitudinal measures of adiposity and insulin sensitivity, as suggested by previous reports [26-28]. With the exception of BMI in African Americans (P = .19), total fat intake significantly influenced longitudinal change in measures of adiposity and insulin sensitivity in both races ($P < .05$). However, in either race, there was no significant impact of total fat intake on the relationship between the Pro12Ala polymorphism and the measures of adiposity or insulin sensitivity or longitudinal changes in these measures (not shown).

Associations between Pro12Ala polymorphism and incident metabolic syndrome are shown in Table 3. There was a significant interaction between race and genotypes (P = .002), thus, results are presented separately by race. The association between the Ala12 allele and the decreased risk

Table 3
Logistic regression results evaluating the relationship between PPARγ2 Pro12Ala genotype category and incident metabolic syndrome (over 15 years) by race, with and without stratification by obesity category

	OR	95% CI
All African Americans		
Pro12Ala genotype[a] (model 1)	0.44*	0.20-0.98
Pro12Ala genotype[a] (model 2)	0.51	0.21-1.22
Obese African Americans*		
Pro12Ala genotype[a] (model 1)	0.50	0.20-1.28
Pro12Ala genotype[a] (model 2)	0.53	0.20-1.41
Nonobese African Americans		
Pro12Ala genotype[a] (model 1)	0.47	0.06-3.56
Pro12Ala genotype[a] (model 2)	0.44	0.06-3.34
All whites		
Pro12Ala genotype[a] (model 1)	0.93	0.69-1.25
Pro12Ala genotype[a] (model 2)	0.61**	0.42-0.89
Obese[b] whites		
Pro12Ala genotype[a] (model 1)	0.61*	0.39-0.95
Pro12Ala genotype[a] (model 2)	0.50**	0.31-0.80
Nonobese whites		
Pro12Ala genotype[a] (model 1)	1.30	0.75-2.24
Pro12Ala genotype[a] (model 2)	1.10	0.63-1.93

Model 1, adjusted for age and sex; model 2, adjusted for age, sex, and BMI.
[a] Ala/Ala + Ala/Pro vs Pro/Pro (reference).
[b] BMI ≥ 30 kg/m^2.
* $P < .05$.
** $P < .01$.

of incident metabolic syndrome was significant in both races. African Americans carrying the Ala12 allele had a ~50% reduction in risk of metabolic syndrome compared to Pro12 homozygotes ($P = .04$). This estimate did not significantly change after further controlling for BMI effects, or among obese and nonobese subgroups, although it was no longer statistically significant. In whites, BMI significantly influenced the relationship between the Pro12Ala polymorphism and the risk of metabolic syndrome (P for BMI × Genotype interaction = .004). Stratified analyses by BMI category showed that obese whites carrying the Ala12 allele had a 40% to 50% reduced risk of metabolic syndrome as compared to obese whites who did not carry this allele ($P < .05$). There was no significant association between the Pro12Ala polymorphism and the risk of metabolic syndrome in nonobese whites.

4. Discussion

We report significant associations between the Pro12Ala polymorphism and the longitudinal measures of adiposity, including BMI, waist and hip circumferences, and skinfolds thickness, but these associations were in opposite direction in African Americans and whites. The Ala12 allele was consistently associated with higher mean 15-year measures of adiposity in whites and lower mean 15-year measures of adiposity in African Americans. Several factors may contribute to these results. Although the functional differences between the Pro- and the Ala-containing protein products and the lack of other common missense mutations have strengthened the case for etiological relevance of the Pro12Ala polymorphism [4], it is possible that another neighboring causative locus (loci) exists (elsewhere in the PPARγ gene or in another nearby gene), which influences the effects of the Pro12Ala polymorphism on adiposity. Population-specific linkage disequilibrium between the Pro12Ala polymorphism and this variant(s) may, thus, explain the differing associations in the 2 populations. This hypothesis is supported by the recent data revealing differences in the pattern of variation in the PPARγ2 gene between African Americans and whites [29]. Moreover, a study by Doney et al [13], showing inversed effects on BMI of the Pro12Ala polymorphism and a C to T polymorphism at nucleotide 1431 of the PPARγ gene, further bolsters the notion that multiple variants may be at play in the relationship between the PPARγ2 gene and the adiposity. The effects of the Pro12Ala polymorphism may also be modified by environmental factors, which may differ between populations. Although an interaction between dietary fat and the Pro12Ala polymorphism on BMI was previously reported [26-28], and total dietary fat intake was significantly different between African Americans and whites, we did not find evidence that the effects of the Pro-12Ala polymorphism on adiposity were significantly influenced by dietary fat intake in our study. These results may reflect, in part, the difficulties in accurately assessing dietary fat intake and/or the necessity of larger samples to adequately detect such interactions. We also cannot exclude that other factors, not examined in this study, may influence the magnitude and/or direction of the associations described here.

Although a few longitudinal studies in selected populations with relatively small sample sizes have suggested that the Ala12 allele was associated with greater weight gain [30,31], we did not detect any significant difference in the rate of change in measures of adiposity between genotype categories in either race.

Association of the Ala12 allele with reduced risk for type 2 diabetes and improved insulin sensitivity has been one of the most consistent results among studies addressing the role of the Pro12Ala polymorphism in susceptibility to diabetes and diabetes-related traits [9,14,15,32-34]. In accord with these data, we report a significant association between the Ala12 allele and the lower 15-year HOMA index, fasting insulin, and glucose levels in African Americans. Moreover, this allele was significantly associated with a reduced risk of metabolic syndrome in both African Americans and whites, and this, despite significant racial differences in the relationship between BMI and genotype.

The modulating effects of BMI on these associations further emphasize the complexity of the relationships between ethnicity, variation in the PPARγ gene, adiposity, and insulin sensitivity. Because PPARγ2 is abundantly expressed in adipocytes, alteration in adipose tissue is likely

to affect Pro12Ala polymorphism metabolic effects. In African Americans, the effect of the Pro12Ala polymorphism on HOMA, fasting insulin, and fasting glucose levels, and the risk for metabolic syndrome was exerted largely through its influence on adiposity, because adjusting for BMI attenuated the associations. In whites, the association of the Ala12 allele with reduced risk for the metabolic syndrome was restricted to obese subjects. These data are consistent with previous reports suggesting a more pronounced effect of the Ala12 allele on insulin sensitivity among obese subjects [9,12,14].

A unifying model to explain these results may propose that the Ala12 allele has pleiotropic beneficial effects on insulin sensitivity and adiposity in both populations, but that an additional variant(s) in or near the PPARγ gene, in linkage disequilibrium with the Pro12Ala polymorphism in whites, unfavorably influences body weight, modifying the net observed effect of the Pro12Ala polymorphism on BMI in this population. The finding that the ratio of mRNA encoding PPARγ2 to that encoding γ1 is positively correlated with BMI [35,36] underscores the complex interactions between the multiple PPARγ isoforms in relation to adiposity, hence, possibly the genetic variants that may influence their expression and/or function. To test the hypothesis proposed here, we are measuring additional polymorphisms in and around the PPARγ gene and analyzing the association of these PPARγ genotypes and haplotypes with adiposity and insulin sensitivity.

The molecular and cellular mechanisms underlying these relationships is unknown, but studies in animal models and humans have suggested that they may involve effects on free fatty acids release from fat tissue and/or modulation of expression and release of adipocytokines [37]. In this study, there was no association between the Pro12Ala polymorphism and the adiponectin levels in either race, suggesting that the observed Pro12Ala polymorphism effects are unlikely to be mediated through effects on adiponectin (DR Jacobs and the YALTA investigators, personal communication).

Strengths of our study include a long period of follow-up, which allowed us to examine the effect of the Pro12Ala polymorphism on longitudinal changes in measures of adiposity and insulin sensitivity; an enhanced power to detect gene effects due to repeated measures of outcomes; and an assessment of potential confounders such as dietary fat intake, which allowed us to evaluate possible gene-environment interactions. Limitations of our study include the young age of the participants, which presents a disadvantage for studying effects of the Pro12Ala polymorphism on overt disease, especially type 2 diabetes; possible false positive results due to the large number of statistical test performed; and possible confounding from other variants within the PPARγ gene or in another nearby gene, due to linkage disequilibrium.

In summary, we report race-specific associations between the Pro12Ala polymorphism and the indices of adiposity and insulin action in the biracial CARDIA cohort. We suggest that the combination of multiple variants in or around the PPARγ gene, with race-specific effects on these metabolic traits, may influence the complex relationships among PPARγ gene variation, ethnic background, adiposity, and insulin sensitivity. Studies are needed to further investigate the cellular and molecular mechanisms underlying the complex effects of PPARγ2 on adiposity and insulin sensitivity, to understand the biological basis of these associations.

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