ORIGINAL ARTICLE

***Ala/Ala* Genotype of *Pro12Ala* Polymorphism in the *Peroxisome Proliferator-Activated Receptor- γ 2* Gene Is Associated with Obesity and Insulin Resistance in Asian Indians**

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Abstract

Aim: We determined the association of the *Pro12Ala* polymorphism of the *peroxisome proliferator activated receptor (PPAR)- γ 2* gene with obesity, insulin resistance (IR), and lipids in Asian Indians without diabetes in north India.

Subjects and Methods: In this cross-sectional study ($n=495$; 279 males and 216 females, 18–60 years of age), anthropometric (body mass index, waist and hip circumferences, and skinfold thickness) and biochemical (fasting glucose, lipid profile, fasting insulin, leptin, and adiponectin) parameters were assessed. Polymerase chain reaction–restriction fragment length polymorphism analysis was used for identification of individual genotypes.

Results: Frequencies of the *Pro* and *Ala* alleles were 0.89 and 0.11, respectively. The genotype frequencies (%) of *Pro/Pro*, *Pro/Ala*, and *Ala/Ala* were 82.6, 14.7, and 2.7, respectively, without any gender differences. The frequency of the *Ala/Ala* genotype was higher in obese than in nonobese subjects (4.9% vs. 1.5%, $P=0.06$). The *Ala/Ala* genotype was associated with higher values of hip circumference, subscapular skinfold thickness, and sum of four skinfold thickness than the *Pro/Pro* and *Pro/Ala* genotypes ($P<0.05$). Using a multivariate logistic regression model after adjusting for age, sex, and insulin, subjects with the *Ala/Ala* genotype showed a high risk of obesity (odds ratio [OR], 3.2, 95% confidence interval [CI] 1.2–12.9) and IR (OR, 3.6, 95% CI: 1.04–12.4).

Conclusion: The *Ala/Ala* genotype of the *PPAR- γ 2* gene is associated with obesity and IR in Asian Indians without diabetes living in north India.

Introduction

OBESITY IS ASSOCIATED with insulin resistance (IR) and consequent metabolic derangements that increase the likelihood of type 2 diabetes mellitus (T2DM) and coronary heart disease. Determinants of obesity and IR include lifestyle and genetic causes. Variants in the genes that regulate adipocyte metabolism may predispose individuals to develop obesity.^{1,2} One of the candidate genes, *peroxisome proliferator-activated receptor (PPAR)- γ* , is a member of the nuclear hormone receptor superfamily and activates adipocyte differentiation and mediates the expression of fat cell-specific genes.³ The gene is located on chromosome 3p25, and its protein product has three isoforms, γ 1, γ 2, and γ 3, produced by alternative splicing of the *PPAR- γ* mRNA.⁴ PPAR- γ 1 ex-

hibits widespread expression, albeit at low levels, and γ 2 and γ 3 are highly expressed in adipose tissues.⁵

PPAR- γ 2 is a lipid-activated transcription factor that has a key role in the expression of genes involved in adipocyte differentiation and function, as well as regulation of genes in several other tissues.^{5,6} PPAR- γ is activated by certain fatty acids, prostanoids, and thiazolidinediones, a class of insulin-sensitizing antihyperglycemic agents.⁷ It heterodimerizes with the retinoid X receptor and binds to specific PPAR-responsive elements of DNA to promote transcription of numerous target genes implicated in obesity, lipid metabolism, carcinogenesis, and inflammation.⁸

Polymorphism in the *PPAR- γ 2* gene (*Pro12Ala*) has been shown to be associated with IR⁹ and obesity.^{10,11} In addition, it has been suggested that the *Pro12Ala* polymorphism has an

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effect on body mass index (BMI) in individuals with marked obesity and that this effect is not apparent in lean individuals.^{11,12}

Several rare, loss-of-function mutations in the *PPAR- γ 2* gene have been detected in three families with severe IR, T2DM, and hypertension,¹³ and a rare (*Pro12Ala*) gain-of-function mutation has been detected in four unrelated individuals with extreme obesity. *PPAR- γ Pro12*, which had greater in vitro activity than *Ala12*, was found to be associated with metabolically deleterious phenotypes, such as decreased insulin sensitivity, obesity, and T2DM in the Finnish population.¹² The *Pro/Ala* genotype was not associated with the metabolic syndrome, T2DM, and obesity in Asian Indians in South India.¹⁴

Obesity and the metabolic syndrome are increasing in Asian Indians.¹⁵ Although these disorders in Asian Indians are mostly governed by imbalanced diets and physical inactivity, the genetic basis of these diseases has been less investigated. In this study, we aim to investigate association of *PPAR- γ 2* gene polymorphism with obesity, IR, and lipids in Asian Indians residing in a metropolitan city of North India.

Subjects and Methods

This cross-sectional population-based study involved 495 adult subjects without diabetes (279 males, 216 females) and was conducted at the All India Institute of Medical Sciences, New Delhi, India, from April 2006 to July 2011. The study was approved by the institutional ethics committee, and informed consent was obtained. Subjects were randomly selected from residential colonies to have approximate representation from each income group (high income group, approximately 10%; middle income group, approximately 65–70%; and low income group, approximately 15–20%) according to the proportion living in a metropolitan city. First, a list of total number of houses in each locality with the number of adult subjects in each household was obtained. Subsequently a random number list was generated to select the household that was approached for the participation in the study. Only one individual from one household was selected. Subjects with known diabetes or detected to have fasting blood glucose in the diabetes range, any severe acute or chronic illness, or known human immunodeficiency virus seropositivity and pregnant and lactating women were excluded from the study.

Clinical and anthropometric measurements

Height, weight, waist circumference (WC), hip circumference (HC), waist-to-hip ratio, and skinfold thickness at four sites (triceps, biceps, suprailiac, and subscapular) were measured according to standard protocols.¹⁶ BMI was calculated by dividing the weight (in kg) by the height (in m) squared, and total skinfold (TSF) thickness was calculated as the sum of the four skinfold thicknesses.

Biochemical assays

Venous blood samples were obtained after an overnight fast for 8–10 h. Fasting plasma glucose (FPG), total cholesterol, triglycerides (TGs), and high-density lipoprotein cholesterol (HDL-C) were estimated as described previously.¹⁷ The value of low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's equation. Fasting insulin levels were

measured using commercially available radioimmunoassay insulin kits (Immunotech, Marseille, France) as described previously.¹⁸ The intra- and inter-assay variations were less than 5%. Leptin and adiponectin levels were measured by enzyme-linked immunosorbent assay-based kits (LINCO Research, St. Charles, MO) as described previously.^{19,20} The intra- and inter-assay percentage variations were 2.1% and 3.3% for leptin and 1.8% and 2.9% for adiponectin, respectively.

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes by the high salt precipitation method.²¹ The *Pro12Ala* single nucleotide polymorphism in the *PPAR- γ 2* gene was analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism. The primers (New England Biolab, Ipswich, MA) used for the *PPAR- γ 2* gene were as follows: forward, 5'-GCCAATTCAAGCCCAGTC-3'; reverse, 5'-GATATGTTTGCAGACAGTGTATCAGTGAAGGAATCGC TTTCCG-3'. Each PCR procedure was carried out in a total volume of 25 μ L. Two hundred nanograms of genomic DNA was added to 2.5 μ L of each primer, 2.5 μ L of 10 $\times$ PCR buffer, 0.25 mM deoxynucleotide triphosphates, and 0.5 unit of *Taq* DNA polymerase in a total PCR mixture. In the thermal reactor, amplification of the *PPAR- γ 2* gene was achieved under the following conditions: denaturation at 94°C for 10 min followed by 35 amplification cycles of (denaturation at 94°C for 5 min, annealing at 52°C for 1 min, extension at 72°C for 1 min) and a final extension step of 10 min at 72°C. Ten microliters of the amplified product was then digested with 0.1 μ L of the restriction enzyme *Bst*UI and 1.0 μ L of enzyme buffer for 12 h (overnight) at 60°C. The digested products were resolved on 2.5% agarose gels, and the bands were visualized by ethidium bromide staining.

Definitions

Obesity was defined as BMI ≥ 25 kg/m².²² High WC was defined as >90 cm in males and >80 cm in females. Impaired fasting glucose was defined as an FPG value of ≥ 6.1 mmol/L and ≤ 7.0 mmol/L.^{22,23} The modified criteria of the National Cholesterol Education Program, Adult Treatment Panel III were used to define the metabolic syndrome as presence of any three or more of the following: WC >90 cm in males and >80 cm in females, serum TGs ≥ 1.695 mmol/L mg/dL, HDL-C <1.036 mmol/L in males and <1.295 mmol/L in females, fasting plasma glucose ≥ 6.1 mmol/L, and blood pressure $\geq 130/85$ mm Hg.²³ Truncal subcutaneous obesity was defined as subscapular skinfold thickness values in the highest quartile (>35 mm). The value of homeostasis model assessment (HOMA) denoting IR was termed as HOMA-IR and was calculated as equal to (fasting insulin [in μ U/mL] $\times$ fasting glucose [in mmol/L]/22.5).²⁴ IR was defined based on fasting insulin values. Subjects having HOMA-IR values in the highest quartile, ≥ 2.29 (corresponding value of fasting insulin levels ≥ 10.4 μ U/mL), were defined as insulin resistant.²⁵

Statistical analysis

The allelic and genotypic frequencies were determined by manual counting. Statistical analysis was performed using

STATA version 9 (StataCorp LP, College Station, TX). After the normality aspect of quantitative variables was confirmed, descriptive statistics were computed using mean $\pm$ SD values and Student's *t* test. The difference between proportions was tested using the χ^2 test. The influence of the genotype on the clinical parameters was estimated by the analysis of variance test with multiple comparisons. Age, sex, insulin, TG, and WC were included in multivariate logistic regression to identify the independent predictors of obesity and IR and to estimate the odds ratio (OR) and 95% confidence interval (CI). Allelic and genotypic frequencies were evaluated for Hardy–Weinberg equilibrium. A value of $P < 0.05$ was considered as significant.

Results

The clinical, anthropometric, and biochemical characteristics are summarized in Table 1. Generalized obesity and IR were present in 55.1% and 24.1% of subjects, respectively. Values of FPG were comparable among subjects with and without obesity and IR. Levels of lipid (except HDL-C and LDL-C) parameters were significantly higher in the obese and IR subjects compared with nonobese and non-IR subjects ($P < 0.0001$ for all). Mean values of BMI, WC, HC, skinfolds (biceps, triceps, subscapular, and suprailiac), and TSF were significantly higher in obese and IR subjects ($P < 0.001$ for all). Fasting insulin, HOMA-IR, and leptin levels were also significantly higher in obese and IR subjects ($P < 0.001$). Mean values of serum adiponectin were significantly higher in non-obese and non-IR subjects compared with obese and IR subjects ($P < 0.05$).

Analysis of PPAR- γ 2 Pro12Ala genotype

The observed allelic frequency of the *Pro* allele was 0.89, and that of the *Ala* allele was 0.11. Furthermore, overall, 82.6% of subjects were *Pro/Pro* homozygous, 14.7% were *Pro/Ala* heterozygous, and 2.7% were *Ala/Ala* homozygous (Table 2). The frequency of the *Ala/Ala* genotype was higher in obese compared with nonobese subjects (4.9% vs. 1.5%, $P = 0.06$). The *PPAR- γ 2* genotype frequencies did not follow the Hardy–Weinberg equilibrium ($\chi^2 = 16.2$, $P = 0.001$). The reproducibility of the genotyping data was checked by replicating the genotyping in 75 randomly selected samples. The frequency distribution of the genotypes of the *PPAR- γ 2* gene and their association with clinical, anthropometric, and biochemical profiles are given in Table 2. HC ($P = 0.03$), subscapular skinfold ($P = 0.0003$), and TSF ($P = 0.0008$) levels were highest in subjects with the *Ala/Ala* genotype. Prevalence of hyperinsulinemia was higher with *Ala/Ala* than *Pro/Pro* and *Pro/Ala* (57.1%, 21.7%, and 21.2, respectively; $P = 0.004$). Metabolic syndrome was present in 44.1% of subjects (men, 47.7%, women, 39.2%) (data not shown) and did not show any association with *PPAR- γ 2* gene polymorphism.

Logistic regression analysis

On univariate logistic regression analysis, subjects with the *PPAR- γ 2 Ala/Ala* genotype had 4.4 times higher odds of developing obesity (OR [95% CI], 1.2–16.4; $P = 0.02$) and 3.8 times higher odds of developing IR (OR [95% CI], 1.24–11.5; $P = 0.01$). Using a multivariate logistic regression model after adjusting for age, sex, fasting insulin, TG, and WC, subjects with the *Ala/Ala* genotype showed high risk of obesity

TABLE 1. CLINICAL, ANTHROPOMETRIC, AND BIOCHEMICAL PROFILES

Variable	Obese (n=273)	Nonobese (n=222)	P	IR (n=117)	Non-IR (n=368)	P
Age (years)	38.4 $\pm$ 9.1	40.8 $\pm$ 8.3	0.003 ^a	39.6 $\pm$ 8.9	39.2 $\pm$ 8.5	0.6
Body mass index (kg/m ²)	28.6 $\pm$ 2.8	21.5 $\pm$ 2.3	0.01 ^a	27.3 $\pm$ 4.2	24 $\pm$ 4.1	0.001 ^a
Waist circumference (cm)	93.7 $\pm$ 9.8	81.0 $\pm$ 10.5	0.001 ^a	91.6 $\pm$ 10.5	85.0 $\pm$ 12.2	0.001 ^a
Hip circumference (cm)	101.5 $\pm$ 7.9	90.2 $\pm$ 7.8	0.001 ^a	99.5 $\pm$ 7.8	93.7 $\pm$ 9.7	0.001 ^a
Waist-hip ratio	0.91 $\pm$ 0.06	0.89 $\pm$ 0.09	0.004 ^a	0.90 $\pm$ 0.1	0.90 $\pm$ 0.2	0.5
Skinfold thickness (mm)						
Biceps	20.7 $\pm$ 8.5	14.5 $\pm$ 7.7	0.001 ^a	21.3 $\pm$ 8.1	16.0 $\pm$ 5.2	0.001 ^a
Triceps	28.2 $\pm$ 9.6	20.5 $\pm$ 7.9	0.001 ^a	27.5 $\pm$ 8.5	22.7 $\pm$ 9.2	0.001 ^a
Subscapular	35.8 $\pm$ 7.7	27.1 $\pm$ 7.9	0.001 ^a	35.2 $\pm$ 7.9	29.5 $\pm$ 8.8	0.001 ^a
Suprailiac	39.7 $\pm$ 7.7	27.8 $\pm$ 9.2	0.001 ^a	41.6 $\pm$ 7.2	30.4 $\pm$ 10.6	0.001 ^a
Total	119.7 $\pm$ 26.9	90 $\pm$ 27.1	0.001 ^a	120.4 $\pm$ 24.3	97.2 $\pm$ 21.2	0.001 ^a
FPG (mmol/L)	5.2 $\pm$ 1.3	5.0 $\pm$ 0.7	0.2	5.1 $\pm$ 1.0	5.0 $\pm$ 0.9	0.4
Triglycerides (mmol/L)	9.2 $\pm$ 2.4	7.0 $\pm$ 2.9	0.001 ^a	9.1 $\pm$ 2.6	7.1 $\pm$ 2.9	0.001 ^a
Total cholesterol (mmol/L)	10.6 $\pm$ 2.1	9.9 $\pm$ 2.7	0.001 ^a	11.1 $\pm$ 2.2	10 $\pm$ 2.3	0.008 ^a
LDL-C (mmol/L)	6.4 $\pm$ 1.9	6.1 $\pm$ 2.0	0.1	6.5 $\pm$ 1.6	5.6 $\pm$ 1.2	0.2
HDL-C (mmol/L)	2.3 $\pm$ 0.6	2.3 $\pm$ 0.6	0.5	2.3 $\pm$ 0.5	2.6 $\pm$ 0.8	0.5
VLDL (mmol/L)	1.8 $\pm$ 0.6	1.43 $\pm$ 0.5	0.04 ^a	1.8 $\pm$ 0.9	1.5 $\pm$ 0.8	0.01 ^a
Serum leptin (μ g/L)	28.1 (0.4–98.3) ^b	10.8 (0.3–61.2) ^b	0.001 ^a	24 $\pm$ 7.2	17.2 $\pm$ 6.2	0.002 ^a
Serum adiponectin (ng/L)	26.8 $\pm$ 9.5	34.8 $\pm$ 9.2	0.02 ^a	26.2 $\pm$ 8.9	32.1 $\pm$ 9.2	0.005 ^a
Fasting insulin (μ U/L) ^b	9.9 (0.3–41.2)	6.5 (0.2–52.3)	0.001 ^a	15.5 (1.2–63.4)	5.7 (0.5–41.6)	0.001 ^a
HOMA-IR ^b	3.2 (0.1–9.7)	1.3 (0–12.1)	0.001 ^a	3.5 (0.1–11.7)	1.2 (0.1–9.9)	0.003 ^a

Data are mean $\pm$ SD values for the indicated number of subjects (*n*) unless indicated otherwise.

^aSignificant difference.

^bMedian (minimum – maximum).

FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment for insulin resistance; IR, insulin resistance; LDL-C, low-density lipoprotein cholesterol; VLDL, very low-density lipoprotein.

TABLE 2. ASSOCIATION OF THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ 2 *Pro12Ala* GENOTYPE WITH CLINICAL, ANTHROPOMETRIC, AND BIOCHEMICAL PARAMETERS

Variable	<i>Pro/Pro</i> (n=409)	<i>Pro/Ala</i> (n=73)	<i>Ala/Ala</i> (n=13)	P
Age (years)	39.5 $\pm$ 8.7	38.5 $\pm$ 9.9	40.5 $\pm$ 6.8	0.6
Sex				0.4
Males [n (%)]	233 (57)	36 (49.3)	7 (53.8)	
Females [n (%)]	176 (43)	37 (50.7)	6 (46.2)	
Body mass index [n (%)]				0.02 ^a
Obese	175 (42.8)	37 (50.6)	10 (76.9)	
Nonobese	234 (47.2)	36 (49.4)	3 (23.1)	
Waist circumference (cm)	86.3 $\pm$ 12.0	85.5 $\pm$ 11.5	94.4 $\pm$ 10.8	0.06
Hip circumference (cm)	94.8 $\pm$ 9.7	95.0 $\pm$ 8.7	101.7 $\pm$ 7.9	0.03 ^a
Waist-to-hip ratio	0.90 $\pm$ 0.09	0.89 $\pm$ 0.08	0.92 $\pm$ 0.05	0.7
Skinfold thickness (mm)				
Biceps	17.0 $\pm$ 5.1	17.6 $\pm$ 6.4	19.4 $\pm$ 4.5	0.2
Triceps	23.4 $\pm$ 9.3	24.4 $\pm$ 9.1	27.9 $\pm$ 6.4	0.2
Subscapular	30.1 $\pm$ 8.6	31.4 $\pm$ 8.9	40.5 $\pm$ 10.2	0.0003 ^a
Suprailiac	32.8 $\pm$ 13.9	31.7 $\pm$ 10.3	39.4 $\pm$ 11.5	0.5
Total	101.1 $\pm$ 20.6	104.1 $\pm$ 26.9	126.7 $\pm$ 24.9	0.008 ^a
FPG (mmol/L)	5.01 $\pm$ 0.70	4.99 $\pm$ 0.78	5.21 $\pm$ 0.79	0.6
Triglycerides (mmol/L)	1.63 $\pm$ 0.92	1.59 $\pm$ 0.83	1.54 $\pm$ 0.54	0.9
Total cholesterol (mmol/L)	4.78 $\pm$ 1.04	4.61 $\pm$ 1.06	4.93 $\pm$ 0.82	0.3
LDL-C (mmol/L)	2.96 $\pm$ 0.93	2.81 $\pm$ 0.91	3.15 $\pm$ 0.71	0.2
HDL-C (mmol/L)	1.09 $\pm$ 0.28	1.09 $\pm$ 0.33	1.07 $\pm$ 0.15	0.9
VLDL (mmol/L)	0.75 $\pm$ 0.50	0.72 $\pm$ 0.37	0.70 $\pm$ 0.24	0.2
Insulin [n (%)]				0.03 ^a
IR	94 (23.5)	16 (22.2)	7 (53.9)	
Non-IR	306 (76.5)	56 (77.8)	6 (46.1)	
Serum leptin (μ g/L) ^b	19.3 (0.2–88.9)	16.6 (0.5–92.1)	15.6 (0.1–93.4)	0.8
Serum adiponectin (ng/L)	31.1 $\pm$ 8.8	25.3 $\pm$ 8.2	37.3 $\pm$ 4.7	0.3

Data are mean $\pm$ SD values for the indicated number of subjects (n) except as indicated.

^aSignificant difference.

^bMedian (minimum – maximum).

FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; IR, insulin resistance; LDL-C, low-density lipoprotein cholesterol; VLDL, very low-density lipoprotein.

(OR, 3.2; 95% CI, 1.2–12.9) and IR (OR, 3.6; 95% CI, 1.04–12.4) (Table 3).

Discussion

In this cross-sectional study, we have observed a low frequency of the *Ala* allele (0.11), similar to that observed in the Danish population (0.142).²⁶ It is important that, for the first

time, we report a significant association of obesity and IR with the *Ala/Ala* genotype of the *PPAR*- γ 2 gene in Asian Indians without diabetes.

The associations of *PPAR*- γ 2 gene polymorphisms with IR and obesity have been reported to be varied in different populations and ethnic groups. Tonjes et al.²⁷ performed a classical meta-analysis of data from approximately 32,000 German subjects without diabetes and reported that fasting glucose and IR were significantly greater in the *Pro/Pro* genotype compared with the *Pro/Ala* and *Ala/Ala* genotypes. In addition, the BMI of individuals with the *Pro/Pro* genotype did not significantly differ from individuals with the *Pro/Ala* genotype in the global analysis. It is interesting that, in the Caucasian subgroup, the *Pro/Ala* genotype was associated with significantly increased BMI. Another meta-analysis using data from 30 independent studies (n=19,136) from white British patients with coronary artery disease found a greater BMI in *Ala* allele carriers than those who did not carry that allele (P=0.0006). These data also indicated that the *Pro12Ala* polymorphism is a genetic modifier of obesity and are consistent with the *Ala* allele.²⁸

Association of the *Ala* allele with low fasting plasma insulin concentrations and improved insulin sensitivity has been reported in the Finnish and Swedish populations, respectively.¹³ Nelson et al.²⁹ observed that the *Ala* allele is not associated with BMI and WC in the Hispanic population, but

TABLE 3. ASSOCIATION OF THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ 2 *Pro12Ala* GENOTYPE WITH OBESITY AND INSULIN RESISTANCE

Parameter	<i>Pro/Pro</i>	<i>Pro/Ala</i>	<i>Ala/Ala</i>	P
BMI odds ratio (95% CI)				
Unadjusted	1.0	1.4 (0.8–2.3)	4.4 (1.2–16.4)	0.02 ^a
Adjusted	1.0	1.3 (0.8–2.1)	3.2 (1.2–12.9)	
IR odds ratio (95% CI)				
Unadjusted	1.0	0.93 (0.5–1.7)	3.8 (1.24–11.5)	0.01 ^a
Adjusted	1.0	0.82 (0.4–1.63)	3.6 (1.04–12.4)	0.04 ^a

Pro/Pro, *Pro/Ala*, and *Ala/Ala* genotypes are expressed as the representative baseline with 95% class interval (CI). Body mass index (BMI) was adjusted for age, gender, and fasting insulin; insulin resistance (IR) was adjusted for age, gender, triglycerides, and waist circumference).

^aSignificant difference.

in other studies the *Ala* allele has been associated with higher BMI and increased weight gain.^{30,31} The findings of increased risk of obesity and IR in subjects with the *Ala/Ala* genotype in our study population differs from that observed in several other populations.

Our study population is representative of the general urban population in Northern India. Previous reports on *PPAR-γ2* gene polymorphism in Asian Indians have been reported in mixed populations from South India, consisting of patients with T2DM or metabolic syndrome and subjects without diabetes.^{14,32} The study from South India showed no difference in fasting and 2-h insulin levels between Asian Indians with T2DM with the *Pro12Ala* and wild-type genotype, compared with lower insulin levels in white Caucasians with the *Pro12Ala* genotype.³² Metabolic syndrome consists of a cluster of clinical and biochemical variables, which may not only be influenced by multiple lifestyle factors but also by myriad genetic influences. We did not observe any association of *PPAR-γ2* gene polymorphism with the metabolic syndrome in the present study, which is similar to observations of a study conducted on Chinese subjects.³³ Haseeb et al.¹⁴ reported no association of *Pro12Ala* with the metabolic syndrome, T2DM, and obesity, suggesting lack of protectiveness of this polymorphism for T2DM in Asian Indians in South India.

Studies of association between *PPAR-γ2* gene polymorphism and lipids are fewer. González Sánchez et al.³⁴ reported an association of *Ala12* carriers with lower levels of TGs. A population study of 973 elderly Finnish subjects revealed that individuals with the *Ala/Ala* genotype had considerably higher HDL-C and lower TG levels.¹² In the present study, we did not observe any association of *PPAR-γ2* gene polymorphism with the lipid parameters. Some of the earlier studies in different populations have reported similar observations in Japanese subjects³⁵ and the French WHO-MONICA population.³⁶ The reasons for the lack of association of the *Ala/Ala* genotype with lipids in our study may be the following: First, the low frequency of the *Ala/Ala* genotype (2.7%) observed in our study may make it difficult to draw any firm conclusions about the association with lipids. Second, other factors like ethnicity and nutrition profile may have affected the results. The present study population is relatively small; thus the result could have low statistical power. Further studies of this polymorphism are warranted in a larger number of subjects.

We have previously shown that Asian Indians have relatively higher truncal and abdominal fat mass compared with white Caucasians and black populations³⁷ and that high subcutaneous adiposity is an independent risk factor for hyperinsulinemia.³⁸ In the present study we report for the first time that truncal subcutaneous adiposity is associated with the *Ala/Ala* genotype. In general, *PPAR-γ2* gene expression is increased in the adipose tissue of obese subjects. However, pathogenesis of obesity probably involves a large number of genetic and environmental factors. The disparate effects of the *PPAR-γ2 Pro12Ala* polymorphism on BMI in obese and lean individuals suggest that the impact of this genetic variant can be modified by other environmental and/or genetic factors. The gene–nutrient interaction^{39,40} may, in part, explain the disparate effects of the *Ala12* variant on BMI in obese and lean subjects.

A limitation of our study may be that we did not use a more accurate method of measurement of insulin sensitivity like the hyperinsulinemic euglycemic clamp technique. This was

primarily due to the infrastructural constraints and also because all the sampling was done in a large number of subjects in the community setting.

To conclude, the risk imparted by the *Ala/Ala* genotype for generalized obesity and IR in northern Asian Indians is an important observation. However, this observation needs to be evaluated in a larger population for robust results.

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Author Disclosure Statement

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