

Evaluation of single nucleotide polymorphisms of Pro12Ala in peroxisome proliferator-activated receptor- γ and Gly308Ala in tumor necrosis factor- α genes in obese Asian Indians: a population-based study

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Background: A population-based case control study was performed to determine the associations of Pro12Ala polymorphism in peroxisome proliferator-activated receptor- γ (PPARG) and Gly308Ala polymorphism in tumor necrosis factor- α (TNFA) genes in obese subjects.

Patients and methods: Of 1,400 eligible subjects, ≥ 20 years, we recruited only 1,127. For extreme phenotype case-control design, we evaluated 201 subjects with body mass index (BMI) ≥ 30 kg/m² (Group 1) and 143 with BMI < 20 kg/m² (Group 2). Clinical, anthropometric, biochemical, and nutritional details and polymorphisms were estimated.

Results: In Group 1, the dietary intake of calories and fats was higher, physical activity was lower, and prevalence of truncal obesity, hypertension, high total cholesterol, low high-density lipoprotein cholesterol, and diabetes was greater than in Group 2. There were no homozygous polymorphisms of either gene. Heterozygous Pro12Ala polymorphism in PPARG was found in 15 (7.5%) subjects in Group 1 and 3 (2.1%) subjects in Group 2 ($P = 0.028$), and heterozygous Gly308Ala polymorphism in TNFA was found in 19 (9.5%) in Group 1 and 7 (4.9%) in Group 2 ($P = 0.115$). Presence of heterozygous polymorphism in PPARG and TNFA-predicted obesity with univariate odds ratio ([OR], 95% confidence intervals) of 2.25 (1.32–3.84, $P = 0.003$) and 1.48 (1.10–1.99, $P = 0.009$) and with multivariate OR 1.74 (1.03–2.93, $P = 0.038$) and 1.46 (1.05–2.03, $P = 0.024$), respectively. The addition of dietary and physical activity variables did not result in significant change.

Conclusion: Obese Asian Indians have greater prevalence of heterozygous polymorphisms of Pro12Ala in PPARG and Gly308Ala in TNFA genes.

Keywords: obesity, Asian Indian, Pro12Ala polymorphism, diet, urban

Introduction

Obesity is emerging as a major public health problem in low income countries including India.^{1,2} Early in the 20th century, most populations in which obesity became a public health problem were in North America and Europe. Recent data show that the largest increases in obesity are in developing countries, such as Mexico, China, and Thailand.^{3,4} Global estimates using both longitudinal and cross-sectional data indicate that the prevalence of obesity in countries in intermediate development has increased by 30%–100% over the past decade.³ Serial studies in India have shown a significant increase in overweight and obesity in urban populations, and some studies have shown

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that the prevalence has almost doubled in the last 20 years.⁴⁻⁶ Significant prevalence of obesity has also been reported from industrial populations,⁷ urban slums,⁸ and among rural populations⁹ in India.

There are multiple factors associated with the increase in obesity in developing countries, perhaps the most important being urbanization and globalization of food production and marketing that result in an obesogenic environment.^{1,3,10} Obesity has a strong genetic component as well.¹¹ These genetic differences account for 30%–50% of the variation in fatness in a population.³ Multiple chromosomal locations, genes, and genetic polymorphisms have been implicated.¹¹ Although whole-genome analysis techniques have provided important information about chromosomal locations, linkages, and polymorphisms (eg, fat mass and obesity-associated [FTO] gene),¹² the study of single-nucleotide associations (SNPs) are important for identification of pathophysiological pathways.¹³ Limited research has been done on associations of SNPs with obesity in Asian Indians and none has been conclusive.¹⁴⁻¹⁸

We performed a population-based epidemiological study in an urban location in India to identify the prevalence of obesity.^{19,20} The study used an extreme phenotype case-control design and compared the prevalence of Pro12Ala polymorphism in peroxisome proliferator-activated receptor- γ (PPARG) and Gly308Ala polymorphism in tumor necrosis factor- α (TNFA) genes in obese (body mass index [BMI] ≥ 30 kg/m²) and thin (BMI ≤ 20 kg/m²) persons. These two SNPs were chosen because outside the relationship of FTO gene, these two have the most robust evidence for obesity.¹¹ Secondly, in India, obesity (especially truncal obesity) is significantly associated with impaired glucose tolerance and diabetes, and both of these genes have been identified as important in pathobiological pathways that lead to insulin resistance and inflammation in obese subjects. Significant association of these SNPs has previously been reported with type 2 diabetes, insulin resistance, and central obesity.^{11,14-18,21,22} The extreme phenotypic approach used in this study has been previously evaluated in genetic epidemiological studies and is an important method when the absolute number of subjects is small.^{23,24}

Methods

A population-based genetic epidemiological study was performed in an ethnically homogenous group.¹⁹ Subjects belonging to a community that hailed from Punjab region in north India were studied. House-to-house survey was conducted to identify obese subjects in this population group and for this the whole population of Punjabi subjects living in that

location was screened. In a previous study, among a similar community at Jaipur, we reported the prevalence of obesity (BMI ≥ 30 kg/m²) of about 25%.²⁰ We, therefore, targeted a sample size of 1,400 subjects, expecting a response rate of 70%, so that more than 200 obese subjects could be recruited for the genetic epidemiological study.²⁰ The study was approved by the institutional ethics committee, and informed consent was obtained from all the participants.

Data collection

A detailed proforma was utilized for data collection. Briefly, we collected information regarding demographic data, past history of major illnesses such as coronary heart disease, hypertension, diabetes or high cholesterol levels, and smoking or tobacco intake and alcohol intake.²⁵ As the focus of this case-control genetic epidemiological study was subjects with high BMI (Group 1, ≥ 30 kg/m²) and low BMI (Group 2, < 20 kg/m²) in this subgroup of subjects with high and low BMI we performed a detailed dietary evaluation using a validated food frequency questionnaire and a single 24-hour recall.²⁶ Data from the food frequency questionnaire were used to identify calorie intake and intake of various macronutrients. Physical activity was inquired using a previously validated instrument.²⁷ Physical examination was performed to assess height, weight, waist and hip circumference, and blood pressure (BP) using previously reported methodology.²⁵ Body fat percent and visceral fat were analyzed using a Karada Fat-Scan machine (Omron Model No. HBF-362; Omron Healthcare Singapore Pte Ltd, Alexandra Technopark, Singapore) using bioelectric impedance method with this hand-held device. The inputs in the formula are electric resistance, height, weight, age, and gender. Fasting blood sample for glucose and lipid estimation (after overnight fast) was obtained from all the subjects with very low and high BMI. Fasting glucose was determined at a central laboratory using glucose-peroxidase method and external quality control. Quality control measures were also followed for estimation of total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides.²⁸ Low-density lipoprotein (LDL) cholesterol was estimated using the Friedewald formula.

Diagnostic criteria

The risk factors were classified using standard guidelines.^{25,29,30} Truncal obesity was defined by waist-to-hip ratio (WHR) of > 0.95 for men and > 0.85 for women.^{20,25} Smokers included subjects with present or past smoking or any tobacco use. Hypertension was diagnosed when the systolic

or diastolic BP was $\geq 140/\geq 90$ mm Hg on a repeated single-day measurements or when the individual was a known hypertensive. Dyslipidemia was defined by the presence of high total cholesterol (≥ 200 mg/dL), high LDL cholesterol (≥ 130 mg/dL), low HDL cholesterol (< 40 mg/dL), or high triglycerides (≥ 150 mg/dL) according to National Cholesterol Education Program Adult Treatment Panel-III (ATP-III) guidelines.³⁰ Diabetes was diagnosed when a subject provided history of previously diagnosed diabetes or the fasting blood glucose was ≥ 126 mg/dL.

Genetic analyses

Blood sample was collected for genetic analyses in groups with high and low BMI.²⁴ EDTA-anticoagulated venous blood samples were collected from all study subjects, and the genomic DNA was isolated from whole blood by proteinase K digestion followed by ethanol precipitation. DNA was isolated locally and stored at -70°C . Detection of the polymorphisms was carried out using amplification and restriction enzyme digestion technique. SNPs of possible

importance in obesity were tabulated (Table 1) and because of financial reasons, we evaluated polymorphisms only in PPARG gene important in adipogenesis and TNFA gene important in inflammatory pathways. In the PPARG gene, we studied the Pro12Ala polymorphisms, and in TNFA gene, the Gly318Ala polymorphism was studied. All the SNP analyses were performed at a national laboratory using previously described methodologies.³¹ The Pro12Ala polymorphism in PPARG gene was genotyped using the upstream primer 5'-GCC AAT TCA AGC CCA GTC-3' and the downstream primer 5'-GAT ATG TTT GCA GAC AGT GTA TCA GTG AAG GAA TCG CTT TCC G-3'. The polymerase chain reaction (PCR) product was digested overnight using the enzyme BStU1. The Gly318Ala polymorphism in TNFA gene was genotyped using upstream primer 5'-AGG CAA TAG GTT TTG AGG GCC AT-3' and downstream primer 5'-GAG CGT CTG CTG GCT GGG TG-3'. The amplified product was digested overnight using the restriction enzyme Nco1. The digested PCR products were resolved on 2%-3% agarose gel stained with ethidium bromide. Details including

Table 1 Candidate genes and single nucleotide polymorphisms associated with obesity according to biological pathways

Central neuronal signaling pathway	Adipogenesis	Energy metabolism and thermogenesis	Leptin-insulin signaling pathway	Inflammatory cytokines	Hormone signaling pathway	Renin-angiotensin pathway	Undetermined pathways
AGRP	ACDC	ACPI	ABCC8	IL6	AR	ACE	FTO
CART	ADPN	DA	BTC	IL6R	CCKAR	AGT	—
DRD2	APM1	ADRA2B	GCGR	IL10	CRHR1	HSD11B1	—
DRD4	APOA1	ADRB2	IDE	LTA	CYP11B2	—	—
GHRL	APOA2	ADRB3	IGF2	SERPINE1	CYP19A1	—	—
GPR24	APOA4	ATPIA2	INS	TNF	ESR1	—	—
HTR1B	APOB	CAPN10	IRS1	—	ESR2	—	—
HTR2A	APOD	ENPP1	IRS2	—	GHRHR	—	—
HTR2C	APOE	FABP1	LEP	—	MAOA	—	—
IDE	CBFA2T1	FABP2	LEPR	—	MAOB	—	—
MC3R	FOXC2	FABP4	PTPRF	—	MED12	—	—
MC4R	GNB3	FASN	RETN	—	NROB2	—	—
MC5R	INSIG2	GAD2	TBC1D1	—	NCOA3	—	—
NPR3	LDLR	GYS1	TCF1	—	PGR	—	—
NPY	LIPC	HSPA1B	—	—	SGK	—	—
NPY2R	LIPE	PPRG1A	—	—	SLC6A3	—	—
NR3C1	LMNA	PTPNI	—	—	SLC6A14	—	—
POMC	LPL	TUB	—	—	VDR	—	—
PYY	MACS2	UCP1	—	—	—	—	—
TH	PLIN	UCP2	—	—	—	—	—
UBL5	PON1	UCP3	—	—	—	—	—
Y2R	PPARA	—	—	—	—	—	—
—	PPARD	—	—	—	—	—	—
—	PPARG	—	—	—	—	—	—
—	SAH	—	—	—	—	—	—
—	SCARB1	—	—	—	—	—	—
—	SORBS1	—	—	—	—	—	—
—	SREBF1	—	—	—	—	—	—

location of SNPs in the respective genes, primer sequences, PCR conditions, and restriction enzymes with product sizes have been reported earlier.³¹

Statistical analyses

The numerical variables are reported as mean $\pm$ 1 SD, and ordinal variables are reported in percent. Significance of inter-group differences was determined using unpaired *t*-test for numerical variables and χ^2 test for ordinal variables. Hardy–Weinberg equilibrium was tested for each of the SNPs based on the genotyping of 440 chromosomes from normal healthy individuals (average age 35.1 $\pm$ 9 years). These were recruited on a random basis from different locations, including public meeting places, offices, colleges, markets, and hospitals, and represented population-based controls as reported earlier.³² Pearson's χ^2 test (3 $\times$ 2 contingency table) was used to assess association of SNPs with obesity using the cases (Group 1) and controls (Group 2). Allelic and genotypic associations of SNPs found significant by the χ^2 test were evaluated by computing odds ratio (OR) and 95% confidence interval (CI). χ^2 values were derived from a series of 2 $\times$ 2 contingency tables based on the frequency of each haplotype vs all others between the two groups. Logistic regression analysis was carried out to correlate various clinical parameters with genotypes and to study pair wise interactions between SNPs of different genes. *P* values were subject to Bonferroni's correction and considered significant when <0.05 .

Results

The overall response rate in the population study was 80.5%, and 1,127 (men 556, women 571) of 1,400-invited subjects participated in the study. There was a significant prevalence of cardiovascular risk factors in the overall study subjects.¹⁹ In men and women, respectively, smoking or tobacco use was in 347 (62.4%) and 12 (2.2%), obesity (BMI $\geq$ 25 kg/m²) in 303 (54.5%) and 350 (61.3%), truncal obesity with high WHR in 339 (61.0%) and 310 (54.30%), and hypertension in 322 (57.9%) and 279 (48.9%). Blood biochemistry results for fasting glucose and lipids were available for 644 subjects (57.1%). High total cholesterol was in 111 (32.6%) men and 120 (39.5%) women, low HDL cholesterol in 103 (30.3%) and 83 (27.3%), high triglycerides in 146 (42.9%) and 132 (43.4%), metabolic syndrome in 166 (48.8%) and 137 (45.1%), and diabetes in 88 (25.9%) and 64 (21.1%), respectively. For the present study, subjects were divided into two groups, respectively. Subjects with BMI $\geq$ 30 kg/m² were categorized into Group 1 (*n* = 201) and those with BMI $\leq$ 20 kg/m² as Group 2 (*n* = 143). There were more women in Group 1

(119, 59.2%) compared with Group 2 (62, 43.4%; *P* = 0.004; Table 2). In Groups 1 vs Group 2, the dietary intake of calories, fat energy percent (en%), saturated fat en%, and proteins was significantly greater. Physical activity measured using physical activity level score (PALS) was greater in Group 2 than in Group 1. In Group 1 vs Group 2, the prevalence (%) of truncal obesity (49.8 vs 19.6, *P* < 0.001), hypertension (71.1 vs 46.8, *P* < 0.001), high total cholesterol $\geq$ 200 mg/dL (29.9 vs 18.2, *P* = 0.006), low HDL cholesterol < 40 mg/dL (28.9 vs 23.1, *P* = 0.031), and diabetes (29.9 vs 12.6, *P* = 0.001) was significantly greater.

There was no patient with homozygosity in the PPAR γ or TNF α SNPs in the study Groups (Table 3). The alleles were in Hardy–Weinberg equilibrium. Heterozygous Pro12Ala polymorphism (AB allele) in PPAR γ was in 15 (7.5%) subjects in Group 1 and 3 (2.1%) in Group 2 (*P* = 0.028), and heterozygous Gly308Ala polymorphism (AB allele) in TNF α was in

Table 2 Demographic details, lifestyle variables, and cardiovascular risk factors in the study subjects

	Group 1 BMI $\geq$ 30 kg/m² N = 201	Group 2 BMI $<$ 20 kg/m² N = 143
Men:women	82:119 (40.8%) ^a	81:62 (56.6%)
Age (y)	48.1 $\pm$ 12.9 ^a	39.5 $\pm$ 17.7
Dietary variables		
Calories (per d)	2157 $\pm$ 383 ^a	1637 $\pm$ 204
Fat (g/d)	93.4 $\pm$ 18.8 ^a	51.0 $\pm$ 8.3
Fat energy (%)	39.0 $\pm$ 4.0 ^a	28.4 $\pm$ 5.4
SFA energy (%)	23.6 $\pm$ 8.1 ^a	13.7 $\pm$ 5.8
MUFA energy (%)	9.4 $\pm$ 3.1	9.4 $\pm$ 2.2
PUFA energy (%)	5.9 $\pm$ 3.3 ^b	5.2 $\pm$ 1.4
Proteins (g/d)	64.9 $\pm$ 18.1 ^a	53.1 $\pm$ 10.1
Physical activity variables		
Energy expenditure (kcal)	1870 $\pm$ 142 ^a	2127 $\pm$ 417
PALS	1.41 $\pm$ 0.12 ^a	1.50 $\pm$ 0.13
Risk factors		
BMI (kg/m ²)	34.1 $\pm$ 3.8 ^a	18.6 $\pm$ 1.2
Waist (cm)	102.9 $\pm$ 8.1 ^a	65.0 $\pm$ 7.0
WHR	0.92 $\pm$ 0.07 ^a	0.80 $\pm$ 0.08
Hypertension (%)	143 (71.1) ^a	67 (46.8)
Systolic BP (mm Hg)	132.6 $\pm$ 15.8 ^a	124.5 $\pm$ 14.6
Diabetes (%)	60 (29.8) ^a	18 (12.6)
Glucose fasting (mg/dL)	116.9 $\pm$ 5.3 ^a	95.6 $\pm$ 40.8
Cholesterol (mg/dL)	204.7 $\pm$ 52.0	194.6 $\pm$ 39.3
HDL cholesterol (mg/dL)	42.6 $\pm$ 6.0	43.1 $\pm$ 6.3
Triglycerides (mg/dL)	172.8 $\pm$ 70.6	158.9 $\pm$ 70.5

Significance level: ^a*P* < 0.01; ^b*P* < 0.05.

Note: Numbers in parentheses are percent. Statistical *t*-test for continuous variables and chi-square test for ordinal variables.

Abbreviations: BMI, body mass index; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; PALS, physical activity level score; WHR, waist-to-hip ratio; BP, blood pressure; HDL, high-density lipoprotein.

Table 3 Distribution of PPARG and TNFA allele polymorphisms in obese and thin subjects

	PPARG Pro12Ala polymorphisms				TNFA Gly308Ala polymorphisms			
	AA allele	BB allele	AB allele	P value (χ^2 test)	AA allele	BB allele	AB allele	P value (χ^2 test)
Obese (BMI ≥ 30 kg/m ²); n = 201	0	0	15	0.028	0	0	19	0.115
Thin (BMI < 20 kg/m ²); n = 143	0	0	3		0	0	7	

Abbreviations: PPARG, peroxisome proliferator-activated receptor- γ ; TNFA, tumor necrosis factor- α .

19 (9.5%) in Group 1 and 7 (4.9%) in Group 2 ($P = 0.115$). Presence of heterozygous polymorphism of PPARG and TNFA genes significantly predicted obesity with univariate OR (95% CIs) of 2.25 (1.32–3.84, $P = 0.003$) and 1.48 (1.10–1.99, $P = 0.009$), respectively. These ORs remained significant after multivariate adjustments for age, gender, and comorbidities at 1.74 (1.03–2.93, $P = 0.038$) for AB allele in PPARG and 1.46 (1.05–2.03, $P = 0.024$) for AB allele in TNFA. Addition of dietary and physical activity variables did not result in significant change, suggesting negligible gene-diet or gene-physical activity interactions.

To study the gene-environment interactions, we classified lifestyle variables according to the genetic heterozygosity (Table 4). It was observed that subjects with PPARG AB allele were less physically active and had greater intake of calories and fats. These subjects also had significantly greater BMI, waist size, and WHR. The mean systolic BP and the prevalence of hypertension were not significantly different among all the three allelic groups. Fasting blood glucose level was

significantly greater in those with PPARG heterozygosity, and the prevalence of diabetes was significantly greater in these subjects. Cholesterol and triglyceride levels also were significantly greater in these subjects. Subjects with TNFA heterozygosity with AB allele or having heterozygous alleles in both the SNPs were also less physically active, consumed more calories and fats, and although BMI and waist size were greater, there was no difference in WHR. The prevalence of hypertension, diabetes and mean lipid levels was not significantly different in groups with either TNFA or both polymorphisms.

Discussion

This is the first study to show that obese Asian Indians residing in north India have significant polymorphisms of heterozygous AB alleles in Pro12Ala in PPARG and in Gly308Ala in TNFA genes. This significance remains after adjustment for comorbidities and dietary and lifestyle variables, suggesting direct pathophysiological influence of these genes. Study of gene-environment interactions shows

Table 4 Lifestyle and phenotypic characteristics of study subjects (n = 344) with and without the presence of PPARG and TNFA polymorphisms

	PPARG		TNFA		Both PPARG and TNFA	
	Present (n = 18)	Absent (n = 326)	Present (n = 26)	Absent (n = 318)	Present (n = 7)	Absent (n = 337)
Men:women	9:9 (50.0)	154:172 (47.2)	11:15 (42.3)	152:166 (47.8)	3:4 (42.8)	160:177 (47.5)
PALS	1.48 $\pm$ 0.13 ^a	1.56 $\pm$ 1.15	1.50 $\pm$ 0.18	1.56 $\pm$ 1.17	1.47 $\pm$ 0.11	1.55 $\pm$ 1.10
Calorie intake/d	2472 $\pm$ 667 ^a	1911 $\pm$ 372	2232 $\pm$ 526 ^a	1917 $\pm$ 391	2581 $\pm$ 712 ^a	1927 $\pm$ 393
Fat en%/d	40.0 $\pm$ 7.5 ^a	34.3 $\pm$ 6.9	34.6 $\pm$ 8.7	34.5 $\pm$ 6.9	40.3 $\pm$ 6.4 ^b	34.5 $\pm$ 7.0
SFA en%/d	25.5 $\pm$ 9.7 ^a	19.2 $\pm$ 8.5	21.8 $\pm$ 10.4	19.3 $\pm$ 8.5	25.5 $\pm$ 8.5	19.4 $\pm$ 8.7
BMI (kg/m ²)	38.0 $\pm$ 10.5 ^a	27.1 $\pm$ 7.7	33.3 $\pm$ 11.1 ^a	27.2 $\pm$ 7.8	34.0 $\pm$ 10.4 ^a	26.9 $\pm$ 7.6
Waist (cm)	108.1 $\pm$ 20.6 ^a	86.0 $\pm$ 19.5	95.0 $\pm$ 24.6 ^b	86.5 $\pm$ 19.7	98.4 $\pm$ 22.9 ^a	85.8 $\pm$ 19.4
WHR	1.01 $\pm$ 0.25 ^a	0.88 $\pm$ 0.14	0.98 $\pm$ 0.23 ^a	0.88 $\pm$ 0.13	0.90 $\pm$ 0.10	0.87 $\pm$ 0.9
Hypertension	9 (50.0)	201 (61.6)	15 (57.7)	195 (61.3)	21 (47.7)	189 (63.0)
Systolic BP (mm Hg)	128.6 $\pm$ 15.9	129.3 $\pm$ 15.8	127.9 $\pm$ 13.4	129.4 $\pm$ 16.0	128.8 $\pm$ 13.8	129.3 $\pm$ 16.0
Diabetes	18 (100.0) ^a	60 (18.4)	11 (42.3) ^b	67 (21.1)	22 (50.0) ^a	56 (18.7)
Glucose fasting (mg/dL)	235.0 $\pm$ 65.4 ^a	99.9 $\pm$ 35.9	142.8 $\pm$ 72.9 ^a	105.5 $\pm$ 47.1	155.8 $\pm$ 97.9 ^a	82.7 $\pm$ 50.1
Cholesterol (mg/dL)	220.4 $\pm$ 40.9	199.6 $\pm$ 47.9	209.5 $\pm$ 41.4	200.0 $\pm$ 48.2	188.3 $\pm$ 66.3	157.4 $\pm$ 77.2
HDL cholesterol (mg/dL)	40.8 $\pm$ 5.8	42.9 $\pm$ 6.1	42.3 $\pm$ 5.2	42.8 $\pm$ 6.2	38.4 $\pm$ 13.1	35.3 $\pm$ 17.5
Triglycerides (mg/dL)	223.1 $\pm$ 69.5 ^a	164.0 $\pm$ 69.5	187.4 $\pm$ 72.8	165.7 $\pm$ 70.4	168.5 $\pm$ 78.5 ^a	125.5 $\pm$ 75.9

Significance level: ^a $P < 0.01$; ^b $P < 0.05$.

Note: Numbers in parentheses are percent. Statistical t-test for continuous variables and chi-square test for ordinal variables.

Abbreviations: PPARG, peroxisome proliferator-activated receptor- γ ; TNFA, tumor necrosis factor- α ; PALS, physical activity level score; SFA, SFA, saturated fatty acid; BMI, body mass index; WHR, waist-to-hip ratio; BP, blood pressure; HDL, high-density lipoprotein.

that those with PPARG or TNFA polymorphisms consumed more calories and fats, and subjects with PPARG had greater prevalence of diabetes and lipid levels were more. But larger studies are required to confirm these observations.

Over the past two decades, serious efforts were made to unravel genes and genetic markers that predispose to common obesity.¹¹ The initial epidemiological approaches have been limited to candidate gene and linkage studies. These approaches have led to the identification of a large number of potential candidate genes and quantitative trait loci, but very few have been confirmed convincingly.¹¹ The candidate gene approach is hypothesis-driven and relies on current understanding of the biology and pathophysiology of obesity and related traits. The hypothesis is based on the data derived from animal models, cellular systems, and extreme or monogenic forms of obesity.³ Genetic variants at these loci are then tested in population level association studies. The number of genetic association studies has grown exponentially over the past 15 years. The latest update of human obesity gene map reports 127 candidate genes associated with obesity related traits.^{11,33} Of these, 12 genes (ADIPOQ, ADRB2, ADRB3, GNB3, HTR2C, NR3C1, LEP, LEPR, PPARG, UCP1, UCP2, and UCP3) have been replicated in 10 or more studies.²¹ However, the major problem that has plagued the candidate gene approach is that many of these studies are small (including less than 1,000 individuals) and, thus, often are underpowered.¹² With a small sample size, positive results do not prove and negative results do not disprove a true association.^{24,34} This is a limitation of the present study also because we studied only 343 subjects. However, this study used an extreme phenotypic case-control design (very high BMI vs very low BMI), and the significant presence of heterozygous polymorphisms suggests possible importance of these genes. However, as there were no subjects with homozygous polymorphisms and the absolute number of AB polymorphisms in cases and controls was small, we suggest studies with larger sample sizes to validate these results. These observations, however, are also in consonance with earlier studies.¹¹ Another issue in genetic epidemiological studies is genetic heterogeneity, eg, the FTO gene identified recently using genome-wide association studies³⁵ appears important mainly in Caucasians and among non-Caucasian populations variable results have been reported.^{36,37}

Several groups have reported loss-of-function mutations in the ligand-binding domain of human PPARG, specifically in the adipose-specific PPARG-2.^{21,23} Inherited lipodystrophic syndromes caused by PPARG mutations are characterized by altered distribution of subcutaneous fat, insulin resistance,

diabetes, elevated triglycerides, low HDL cholesterol, hypertension, and polycystic ovarian syndrome, but are rare. The most common PPARG genetic variant is a polymorphism replacing alanine with proline at codon 12 (Pro12Ala) in exon B, which encodes part of the PPARG transactivation domain.³⁸ This variant has an heterozygous allele frequency of 4% in Japanese and can reach up to 28% in white cohorts. Low overall frequency (5.2%) is observed in the present cohort that is consistent with other Asian studies. Multiple gene-gene interactions of PPARG have been reported and increased risk of obesity and diabetes reported for SNPs in APM1g11391A locus, beta-3 adrenergic receptor, interleukin-6, acyl coenzyme A synthetase, and adiponectin gene loci.³⁸ In the present study, no interaction was observed with TNFA polymorphism, but because of the small study size and as we did not study the other polymorphisms, we cannot comment on this issue. Gene-environment interactions have also been reported for PPARG with dietary factors and physical activity. The effect of Pro12Ala variant was more apparent in patients with low physical activity, and the effects of polyunsaturated:saturated fat ratio were additive.³⁸ In this smaller study, we observed that those with PPARG were less physically active and consumed more calories and fats. Whether PPARG polymorphisms influence physical activity or appetite through known or unknown mechanisms needs more studies. Greater prevalence of diabetes in those with the presence of homozygous Pro12Ala polymorphisms in PPARG gene and higher total cholesterol and triglyceride levels confirms the well known influence of PPARG in glucose and lipid metabolism³⁸ and is similar to earlier studies.^{11,18,22,38-40} In the present study, we found only heterozygous Pro12Ala polymorphisms and therefore the results are not comparable with other studies.

TNFA is expressed in adipocytes, and the elevated levels of this cytokine have been linked to obesity and insulin resistance.²¹ Several population-based studies among Caucasian subjects have reported association of Gly380Ala SNP in TNFA gene and obesity but some have reported no association.⁴¹⁻⁴⁴ On one hand, Brand et al⁴¹ studied 176 German Caucasian subjects for this polymorphism and reported a significant association of this polymorphism with high BMI levels ($P = 0.013$). These findings were replicated in some more studies.^{42,43} On the other hand, Corbolan et al⁴⁴ failed to report a significant association of this polymorphism with BMI in Spanish Caucasians subjects ($n = 313$). Similarly, some studies reported a positive association of this polymorphism with waist size and WHR, whereas others failed to confirm this association.¹¹ This study has greater sample sizes than most of above-mentioned studies, and there is a significant association.

Larger studies are needed to confirm these observations as there is a physiological role of TNFA system in obesity and related metabolic complications.⁴⁵

Obesity is an important emerging issue in developing countries.^{3,10} It is predicted that obesity-related syndromes can lead to decline in life expectancy.⁴⁶ Obesity is considered a heritable neurobehavioral disorder that is highly sensitive to environmental conditions.⁴⁷ Large-scale molecular approaches shall continue to identify genetic factors important in predisposition to obesity.¹¹ Clinical significance of such associations needs further studies.

Disclosure

The authors report no conflicts of interest in this work.

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