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PPARG and *ADIPOQ* gene polymorphisms increase type 2 diabetes mellitus risk in Asian Indian Sikhs: Pro12Ala still remains as the strongest predictor

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Abstract

We have examined the association of 14 tagging single nucleotide polymorphisms (tagSNPs) in peroxisome proliferator activated receptor–γ transcripts 1 and 2 (*PPARG1* and *2*) and 5 tagSNPs in adiponectin (*ADIPOQ*) genes for their effect on type 2 diabetes mellitus (T2D) risk in Asian Indian Sikhs. A total of 554 T2D cases and 527 normoglycemic controls were examined for association with T2D and other subphenotypes of T2D. With the exception of a strong association of *PPARG2*/Pro12Ala with T2D (odds ratio, 0.13; 95% confidence interval, 0.03-0.56; $P = .0007$), no other tagSNP in the *PPARG* locus revealed any significant association with T2D in this population. Similarly, none of the tagSNPs in the *ADIPOQ* gene was associated with T2D susceptibility in single-site analysis. However, haplotype analysis provided strong evidence of association of these loci with T2D. Three-site haplotype analysis in the *PPARG* locus using the 2 marginally associated SNPs (P/rs11715073 and P/rs3892175) in combination with Pro12 Ala (P/rs1801282) revealed a strong association of 1 "risk" (CGC) ($P = .003$, permutation $P = .015$) and 1 "protective" (CAC) ($P = .001$, permutation $P = .005$) haplotype associated with T2D. However, the major effect still appears to be driven by Pro12Ala, as the association of these haplotypes did not remain significant when analyzed conditional upon Pro12Ala ($P = .262$). In addition, 2-site haplotype analysis in the *ADIPOQ* locus using only 2 marginally associated SNPs (AD/rs182052 and AD/rs7649121) revealed a significant protective association of the GA haplotype with T2D ($P = .009$, permutation $P = .026$). Multiple linear regression analysis also revealed significant association of an *ADIPOQ* variant (AD/rs12495941) with total body weight ($P = .010$), waist ($P = .024$), and hip ($P = .021$), although these associations were not significant after adjusting for multiple testing. Our new findings strongly suggest that the genetic variation in *PPARG* and *ADIPOQ* loci could contribute to the risk for the development of T2D in Indian Sikhs. Identification of causal SNPs in these important biological and positional candidate genes would help determine the true physiologic significance of these loci in T2D and obesity.

1. Introduction

Asian Indians, 25% of the global population, make up the greatest proportion of diabetic persons in the world [1]. The underlying reasons of the high prevalence of type 2 diabetes mellitus (T2D) and cardiovascular disease in Asian Indians are not well understood given the absence of conventional risk factors, like high smoking, diets rich in meat, or high body mass index (BMI). People from India, indeed the entire Indian subcontinent, have a high prevalence of a characteristic metabolic syndrome, including elevated plasma triglycerides (TG), low levels of high-density lipoprotein cholesterol, high prevalence of insulin resistance, and a

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tendency toward central obesity and premature atherosclerosis [2-4]. Family and migrant studies point to strong genetic and ethnic predisposition in response to certain environmental factors [5,6].

In this investigation, we have examined the association of 2 positional and biological candidate genes—peroxisome proliferator activated receptor–γ (*PPARG*) and adiponectin (*ADIPOQ*, also known as *ACRP30* or *APM*)—with T2D and related phenotypes. *PPARG* maps to chromosome 3p24 and has been implicated in several genomewide linkage scans for T2D [7], and is widely studied for its role in insulin resistance, central obesity, T2D, and other related phenotypes in different populations [8]. The most widely reproduced association between genetic variation and population risk in diabetes is the Pro12Ala (rs1801282) polymorphism in the *PPARG2* that has been confirmed in several recent genomewide association studies in white persons [9-11], except in the French genomewide association study [12]. Their latter study of obese and nonobese Europeans from France and Switzerland suggests a positive association of *PPARG2* (Pro12Ala) on T2D in obese individuals (BMI $\geq$30 kg/m^2) [13]. We previously replicated significant association of the *PPARG2*/Pro12Ala (rs1801282) polymorphism with T2D in Asian Indian Sikhs [14]. We have also observed a significant linkage peak for TG logarithm of odds (LOD 3.0) near the *PPARG* locus in our genomewide linkage scan performed in Khatri Sikh pedigrees (unpublished findings). Association of *PPARG2*/Pro12Ala (rs1801282) with T2D in this investigation was further confirmed in this study that included 14 additional T2D cases and 144 controls compared with the previously published association [14]. To further define the role of the *PPARG* and *PPARG*-target gene *ADIPOQ* in T2D pathophysiology in Indians, we performed a comprehensive screening of tagging single nucleotide polymorphisms (tagSNPs) in these loci in a case-control cohort of Khatri Sikhs from India. The tagSNP approach was applied to reduce project cost by avoiding genotyping redundant SNPs and increasing informativeness while maximizing target gene coverage.

Differential splicing of human messenger RNA generates 2 main isoforms—*pparg1* and *pparg2*—encoded by transcripts *PPARG1* and *PPARG2* that differ at their 5′ ends [15,16]. *Pparg1* is expressed in diverse tissues including adipose, skeletal muscle, heart, liver, and large intestine, whereas *pparg2* is exclusively expressed in adipose tissues [17]. The *ppargs* are also targets of the thiazolidinediones, a class of antidiabetic drugs widely used for improving insulin sensitivity through their strong binding affinity with *pparg* receptor [18,19]. These drugs also have been shown to stimulate adipocyte differentiation.

ADIPOQ maps to chromosome 3q27 and is implicated in T2D [20] and obesity [21,22] in multiple studies in different populations including 3 separate genomewide linkage scans on T2D conducted in French [23], US white [23], and Pima Indian [24] subjects. Patients with coronary heart disease, T2D, and obesity have reduced plasma levels of adiponectin, the protein product of *ADIPOQ* [25]. However, its role in T2D, obesity, insulin resistance, and cardiovascular diseases in Northern Indians is currently unknown.

Here we report the results of analysis of association of 14 tagSNPs in *PPARG* and 5 tagSNPs in *ADIPOQ* with T2D in a case-control cohort of Khatri Sikhs from India.

2. Subjects and methods

2.1. Human subjects

The study subjects are part of the ongoing Sikh Diabetes Study (SDS) [26]. The focus of this study is on an endogamous community of Khatri Sikhs living in the Northern states of India, including Punjab, Haryana, Himachal Pradesh, Delhi, and Jammu and Kashmir. The DNA and serum samples of 554 unrelated T2D cases (309 male, 245 female) and 527 normoglycemic (NG) (258 male, 269 female) subjects were used in this investigation. The T2D cases were 25 years or older with a mean age (mean ± SD) of 55.5 ± 11.1 years at the time of recruitment. The details of diagnostic criteria and recruitment of T2D and NG subjects have been described elsewhere [14,26,27]. Type 2 diabetes mellitus was diagnosed based on the guidelines of the American Diabetes Association [28]. *Type 2 diabetes mellitus* was defined as (1) a fasting plasma glucose level of at least 7.0 mmol/L after a minimum 12-hour fast or (2) a 2-hour postglucose level (2-hour oral glucose tolerance test [OGTT]) of at least 11.1 mmol/L on more than one occasion with symptoms of diabetes. *Impaired glucose tolerance* (IGT) was defined as a fasting plasma glucose level of at least 5.6 mmol/L but not exceeding 7.0 mmol/L or a 2-hour OGTT of at least 7.8 mmol/L but less than 11.1 mmol/L. *Normoglycemia* was defined as a fasting glycemia not exceeding 5.6 mmol/L or a 2-hour glucose less than 7.8 mmol/L. In the absence of medical record information, we confirmed self-reported T2D cases by performing a 2-hour OGTT following the criteria of the World Health Organization (75-g oral load of glucose). Control status was based on self-reports, medical records, and fasting glucose levels. The 2-hour OGTT was also performed on selected controls who presented impaired fasting glucose (>5.6 mmol/L) at the time of recruitment. The 2-hour OGTT was used to detect the presence of IGT. Subjects with impaired fasting glucose and/or IGT were excluded from analysis. Body mass index was calculated as weight (in kilograms)/height (in meters)^2, and waist-hip ratio (WHR) was calculated as the ratio of abdomen or waist circumference to hip circumference.

The NG subjects were recruited from the same Khatri Sikh community and geographic location as the T2D patients [26]. The average age of NG subjects (mean ± SD) was 51.5 ± 14.0 years. Most of the subjects were recruited from the state of Punjab from North India. Individuals of South, East, and Central Indian origin were excluded, as were individuals with type 1 diabetes mellitus (T1D) or a family

member with T1D, rare forms of T2D called *maturity-onset diabetes of young*, or secondary diabetes (eg, hemochromatosis, pancreatitis). Of the 527 NG controls, 204 were spouses of diabetic patients; and the remaining 323 were unrelated controls. These individuals had no first-degree relative with T2D or T1D, but did include individuals with other chronic illnesses such as hypertension, coronary heart disease, or arthritis.

In general, Sikhs do not smoke for religious and cultural reasons; and about 50% of participants were lifelong vegetarians. All blood samples were obtained at the baseline visit, and all participants provided a written informed consent for investigations. All SDS protocols and consent documents were reviewed and approved by the University of Oklahoma and the University of Pittsburgh Institutional Review Boards as well as the Human Subject Protection Committees at the participating hospitals and institutes in India.

2.2. Metabolic estimations

Blood glucose levels were measured by portable Life Scan glucometer (Johnson & Johnson, Langhorne, PA). Calibration of the glucometer was routinely verified using test strips provided by the manufacturer. Blood pressure was measured twice by an Omron (Warminster, PA) blood pressure machine (HEM-705 CP) [29] in sitting position with the left arm resting on a table, legs uncrossed, and feet flat. Quantitation of lipids (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, and TG) and measurement of insulin levels in serum were performed. Lipids were quantified using standard enzymatic methods (Roche, Basel, Switzerland). Insulin was measured by radioimmunoassay (Diagnostic Products, Cypress, CA). All quantitative parameters were determined per manufacturer's instructions using a Hitachi 902 autoanalyzer (Roche). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting glucose − fasting insulin/22.5. Pancreatic β-cell function (HOMA-B) was calculated as (fasting insulin − 20)/(fasting glucose − 3.5), as described by Matthews et al [30].

2.3. SNP genotyping

DNA was extracted from buffy coats using QiaAmp blood kits (Qiagen, Chatsworth, CA) or by the salting out procedure [31]. The tagSNPs were selected using HapMap database (http://hapmap.org). The HapMap data from white persons were chosen to provide the closest possible SNP coverage because allelic distributions of the Asian Indian population from North India are similar to populations of European ancestries [32]; HapMap's tagSNP browser generates a list of maximally informative SNP markers. TagSNPs with minor allele frequency of at least 10% and that passed the Hardy-Weinberg equilibrium (HWE) test ($P < .05$) were chosen using a pairwise r^2 cutoff of 0.8. This is more conservative and thus selects more SNPs than the haplotype R^2 method [33]. We included SNPs in the 5′ untranslated regions and the 2 to 3 kilobases upstream of the transcription site. All selected SNPs in these genes are validated SNPs.

The 14 tagSNPs spanned the entire region of the *PPARG* locus (NM_138711, NM_015869) and included 2 exonic SNPs: a common nonsynonymous coding SNP Pro12Ala (rs1801282) from exon B and a nonsynonymous His447His (rs3856806) from exon 6 in *PPARG2* (online Figure 1s). All 5 tagSNPs from *ADIPOQ* locus (NM_004797) were intronic SNPs (online Figure 2s). Genotyping of all investigated SNPs was performed using TaqMan Pre-Designed or TaqMan Made-to-Order SNP genotyping assays from Applied Biosystems (ABI, Foster City, CA). TaqMan genotyping reactions and florescence detection were performed on an ABI prism 7900HT sequence detection system using 20 ng of genomic DNA following the manufacturer's instructions. Genotypes were scored by analyzing data on both real-time as well as allele discrimination assay platforms using sequence detection system software provided by ABI. For quality control, 30 replicate positive controls and 8 negative controls were included in each run to match the concordance; and the discrepancy rate in the concordance was less than 0.2%. Genotyping success rate ranged between 93% and 99% for all the investigated SNPs.

2.4. Statistical analysis

Departure from HWE in cases and controls was tested using Pearson χ^2 goodness-of-fit test. The genotype and allele frequencies in T2D cases were compared with those in control subjects using χ^2 test or Fisher exact probability test when expected cell values were less than 3. Pairwise linkage disequilibrium (LD) analysis in this sample was performed using HAPLOVIEW version 4.0 (http://www.broad.mit.edu/mpg/haploview/) [34]. Association analyses were performed assuming additive, dominant, and recessive models. Statistical evaluations for testing genetic effects were performed using multivariate logistic regression analysis with adjustments for age, sex, and other covariates. We also performed multiple linear regression analysis to examine the impact of these variants on quantitative risk variables of T2D, including BMI, waist circumference, WHR, fasting insulin, glucose, and lipid levels. Skewed variables detected by Shapiro-Wilk test for the continuous traits (eg, fasting insulin, fasting glucose, HOMA-IR, HOMA-B, TG, cholesterol) were log-transformed before statistical comparisons, and all P values were derived from analyses of transformed data. Significant covariates for each dependent trait were identified by using Spearman correlation and stepwise multiple linear regression with an overall 5% level of significance. Analyses were adjusted for the confounding effects of age, BMI, sex, medication, and also disease status in the combined analysis where appropriate. For lipid traits, the individuals on lipid-

lowering medications were excluded. These analyses were performed using SPSS (Chicago, IL) version 15.0. Haplotype-based association (with disease status) and permutation tests were performed using HAPLOVIEW (version 4.0) that uses an accelerated expectation maximization algorithm to estimate the haplotype frequencies [34]. Haplotype analysis was mainly performed to explore the combined effects of significant SNPs, and empirical significance was derived using permutation tests. Conditional haplotype analysis was performed using PLINK (version 1.06, Boston, MA). We applied Bonferroni adjustments of significance levels to correct for multiple testing for both single-site and haplotype analysis.

3. Results

Table 1 shows the demographic and clinical characteristics of the study subjects separated by sex. The genomic positions and genotypic frequencies of the investigated SNPs within *PPARG* and *ADIPOQ* are shown in Tables 2 and 3, respectively. None of the SNP genotype distributions deviated significantly from HWE in the controls.

3.1. Single-site analyses of PPARG and ADIPOQ SNPs

A significant association with T2D was seen in 3 of the 14 tagSNPs examined in the *PPARG* locus (Table 2). Exonic SNP rs1801282 (Pro12Ala) from *PPARG2* revealed significant association with T2D under a recessive model with an age-, sex- and BMI-adjusted odds ratio (OR) of 0.13 (95% confidence interval [CI], 0.03-0.56; P = .0007). The remaining 2 significant SNPs (P/rs11715073 and P/rs3892175) were from *PPARG1*. The age-, sex-, and BMI-adjusted ORs were 0.52 (95% CI, 0.31-0.86; P = .010) for P/rs11715073 under a recessive model and 0.69 (95% CI, 0.52-0.92; P = .010) for P/rs3892175 under a log-additive model (Table 2). Of the 5 tagSNPs analyzed in the *ADIPOQ* gene, 2 (AD/rs182052 and AD/rs7649121) variants revealed marginally significant associations with T2D when tested individually (Table 3). Age-, sex-, and BMI-adjusted ORs were 1.23 (95% CI, 1.02-1.48; P = .027) for rs182052 and 1.36 (95% CI, 1.03-1.79; P = .029) for rs7649121 under log-additive and dominant models, respectively. However, after applying Bonferroni correction for multiple testing for a total of 14 SNPs and 3 genetic models, only Pro12Ala (P/rs1801282) remained significantly associated with T2D at P = .0012 in the *PPARG* locus. None of the SNPs in the *ADIPOQ* locus remained significant with a significance threshold of .0033 after adjusting for multiple testing.

Table 1
Clinical characteristics of study population stratified by disease status and sex (mean ± SD)

	Sex	NG	T2D	P value[a] (2-tailed)
		n = 527 (258 M/269 F)	n = 554 (309 M/245 F)[a]	
Age (y)	M	51.8 ± 15.6	56.1 ± 11.1	<.0001
	F	51.5 ± 13.3	55.2 ± 11.1	.001
Age at diagnosis (y)	M	–	47.3 ± 11.0	–
	F	–	48.2 ± 10.6	–
Duration of diabetes (y)	M	–	8.9 ± 7.4	–
	F	–	7.4 ± 6.0	–
BMI	M	26.7 ± 4.2	26.6 ± 4.4	.817
	F	27.5 ± 4.8	29.0 ± 5.4	.001
WHR	M	0.97 ± 0.08	0.99 ± 0.08	1.44×10^{-3}
	F	0.91 ± 0.07	0.93 ± 0.07	1.32×10^{-5}
Fasting glucose (mmol/L)	M	5.4 ± 0.6	9.4 ± 3.3	4.57×10^{-44}
	F	5.5 ± 0.6	9.6 ± 3.1	4.09×10^{-49}
Fasting insulin (pmol/mL)	M	93.0 ± 95.6	72.5 ± 82.9	.008
	F	93.6 ± 96.0	70.5 ± 74.4	.003
Total cholesterol (mg/dL)	M	178.7 ± 50.6	181.1 ± 56.9	.612
	F	179.8 ± 42.9	192.6 ± 54.3	.004
LDL cholesterol (mg/dL)	M	103.0 ± 35.4	100.7 ± 39.0	.474
	F	105.3 ± 37.5	111.9 ± 40.6	.065
TG (mg/dL)	M	173.6 ± 91.8	184.2 ± 109.4	.237
	F	154.6 ± 77.0	174.7 ± 94.0	.011

M indicates male; F, female; LDL, low-density lipoprotein.
[a] Difference between T2D cases and NG controls.

3.2. Haplotype analyses of PPARG and ADIPOQ SNPs

To further evaluate the role of these SNPs with T2D, we sought to determine whether these SNPs demonstrated any additional evidence of association when examined together by performing haplotype analysis. For *PPARG*, neither a 14-site haplotype analysis nor a 12-site haplotype analysis excluding P/rs10510419 and P/rs709154 revealed any significant association of a haplotype with T2D in case-control analysis (online Table 1s). P/rs10510419 and P/rs709154 were excluded because these SNPs were in strong LD with P/rs2938395 (D' >0.90) (online Figure 1s). However, a 3-site haplotype analysis using 2 SNPs from *PPARG1* that showed marginal trends (P/rs11715073 and P/rs3892175) together with the strongly significant SNP (Pro12Ala/rs1801282) in *PPARG2* revealed a significant difference in the frequencies of the common (CGC) and rare (CAC) haplotypes between cases and controls (Table 4). The frequency of the most common haplotype CGC was significantly higher in T2D cases compared with NG controls (77% vs 71%; P = .003, permutation test P = .015; Bonferroni P = .008), whereas a protective association was seen with the rare (CAC) haplotype showing significantly lower frequency in cases compared with controls (0.4% vs 2%; P = .001, permutation test P = .005; Bonferroni P = .008) (Table 4). Interestingly, both haplotypes carry the same "C" (Pro) allele at the rs1801282 site in *PPARG2*. The significant association of 3-SNP haplotypes including P/rs 11715073, P/rs3892175, and P/rs1801282 did not remain significant when we analyzed haplotype association conditional upon Pro12Ala (P/rs 1801282) (P = .262) by applying conditional haplotype analysis using PLINK.

For *ADIPOQ*, a 5-site haplotype analysis showed that the frequency of the most common haplotype (GTACG) was

Table 2
PPARG (*1* and *2*) polymorphisms and T2D risk in Asian Indian Sikhs

TagSNP	Position	Genotype	NG (%)	T2D (%)	Model	ORs[a] (95% CI)	*P* value
P/rs 2972164	12309416	CC	273 (54.9)	263 (49.8)	Dominant	1.30 (1.00-1.68)	.048
		CT	179 (36.0)	219 (41.5)	CC vs CT + TT		
		TT	45 (9.1)	46 (8.7)			
		C/T[b]	0.73/0.27	0.71/0.29			
P/rs 11715073	12327971	CC	297 (55.5)	330 (61.1)	Recessive	0.52 (0.31-0.86)	.010
		CG	188 (36.7)	184 (34.1)	CC + CG vs GG		
		GG	42 (7.8)	26 (4.8)			
		C/G[c]	0.74/0.26	0.78/0.22			
P/rs 3892175	12343038	GG	376 (76.4)	421 (82.7)	Log additive	0.69 (0.52-0.92)	.010
		AG	105 (21.3)	82 (16.1)	GG, AG, AA		
		AA	11 (2.2)	6 (1.2)			
		G/A[c]	0.87/0.13	0.91/0.09			
P/rs 12490265	12359542	GG	329 (67.0)	339 (65.2)	Dominant	1.15 (0.88-1.52)	.311
		AG	139 (28.3)	160 (30.8)	GG vs AG + AA		
		AA	23 (4.7)	21 (4.0)			
		G/A[b]	0.81/0.19	0.81/0.19			
P/rs 12497191	12365135	AA	391 (75.2)	402 (77.9)	Log additive	0.82 (0.64-1.06)	.106
		AG	115 (22.1)	105 (20.3)	AA, AG, GG		
		GG	14 (2.7)	9 (1.7)			
		A/G[c]	0.86/0.14	0.88/0.12			
P/rs 1801282	12368125	CC	396 (76.3)	422 (80.1)	Recessive	0.13 (0.03-0.56)	.0007
		CG	108 (20.9)	103 (19.5)	CC + CG vs GG		
		GG	14 (2.8)	2 (0.4)			
		C/G[c]	0.87/0.13	0.90/0.10			
P/rs 4135247	12371588	GG	152 (31.0)	166 (32.0)	Recessive	0.80 (0.58-1.09)	.157
		AG	230 (46.9)	252 (48.6)	GG + AG vs AA		
		AA	108 (22.0)	100 (19.3)			
		G/A[c]	0.54/0.46	0.56/0.44			
P/rs 2972162	12399793	CC	194 (38.5)	201 (38.4)	Recessive	0.88 (0.63-1.23)	.448
		CT	223 (44.2)	241 (46.0)	CC + CT vs TT		
		TT	87 (17.3)	82 (15.6)			
		C/T[b]	0.61/0.39	0.61/0.39			
P/rs 10510419	12401936	GG	352 (70.0)	357 (70.7)	Recessive	0.71 (0.35-1.42)	.329
		GT	131 (26.0)	133 (26.3)	GG + GT vs TT		
		TT	20 (4.0)	15 (3.0)			
		G/T[c]	0.83/0.17	0.84/0.16			
P/rs 2938395	12404468	AA	316 (63.1)	316 (58.7)	Dominant	1.21 (0.93-1.58)	.148
		AG	159 (31.7)	194 (36.1)	AA vs AG + GG		
		GG	26 (5.2)	28 (5.2)			
		A/G[b]	0.79/0.21	0.77/0.23			
P/rs 709154	12431834	AA	362 (71.5)	365 (69.8)	Log additive	1.10 (0.87-1.39)	.433
		AT	130 (25.7)	142 (27.2)	AA, AT, TT		
		TT	14 (2.8)	16 (3.1)			
		A/T[b]	0.84/0.16	0.83/0.17			
P/rs 1797912	12445239	AA	346 (69.9)	345 (66.3)	Dominant	1.20 (0.90-1.58)	.209
		AC	131 (26.5)	156 (30.0)	AA vs AC + CC		
		CC	18 (3.6)	19 (3.7)			
		A/C[b]	0.83/0.17	0.81/0.19			
P/rs 7650895	12450162	AA	284 (58.1)	314 (61.3)	Dominant	0.82 (0.63-1.07)	.141
		AC	179 (36.6)	167 (32.6)	AA vs AC + CC		
		CC	26 (5.3)	31 (6.1)			
		A/C[c]	0.76/0.24	0.78/0.22			
P/rs 3856806	12450557	CC	406 (82.0)	414 (78.0)	Log additive	1.26 (0.95-1.66)	.103
		CT	82 (16.6)	105 (19.8)	CC, CT, TT		
		TT	7 (1.4)	12 (2.3)			
		C/T[b]	0.90/0.10	0.88/0.12			

[a] Odds ratios were adjusted for age, sex, and BMI. Position of SNPs on chromosome was taken from the National Center for Biotechnology Information (Genome Build 36.3).

[b] Least frequent allele to be associated with risk.

[c] Least frequent allele to be associated with protection.

Table 3
ADIPOQ polymorphisms and T2D risk in Asian Indian Sikhs

TagSNP	Position	Genotype	NG (%)	T2D (%)	Model	ORs[a] (95% CI)	*P* value
AD/rs 182052	188043476	GG	240 (48.6)	233 (42.1)	Log additive	1.23 (1.02-1.48)	.027
		GA	204 (41.3)	253 (45.7)	GG, GA, AA		
		AA	50 (10.1)	68 (12.3)			
		G/A[b]	0.69/0.31	0.65/0.35			
AD/rs 12495941	188050874	GG	153 (30.6)	158 (30.9)	Recessive	0.83 (0.61-1.12)	.222
		GT	228 (45.6)	245(47.9)	GG + GT vs TT		
		TT	119 (23.8)	108 (21.1)			
		G/T[c]	0.54/0.46	0.55/0.45			
AD/rs 7649121	188051479	AA	381 (74.3)	376 (68.7)	Dominant	1.36 (1.03-1.79)	.029
		AT	116 (22.6)	148 (27.1)	AA vs AT + TT		
		TT	16 (3.1)	23 (4.2)			
		A/T[b]	0.86/0.14	0.82/0.18			
AD/rs 3821799	188054180	CC	174 (35.2)	174 (32.0)	Dominant	1.17 (0.92-1.50)	.207
		CT	231 (46.8)	278 (51.1)	CC vs CT + TT		
		TT	89 (18.0)	92 (16.9)			
		C/T[b]	0.59/0.41	0.58/0.42			
AD/rs 6773957	188056399	GG	227 (45.7)	229 (42.6)	Dominant	1.25 (0.97-1.60)	.085
		GA	205 (41.2)	247 (46.0)	GG vs GA + AA		
		AA	65 (13.1)	61 (11.4)			
		G/A[b]	0.66/0.34	0.66/0.34			

[a] ORs were adjusted for age, sex, and BMI. Position of SNPs on chromosome was taken from the National Center for Biotechnology Information (Genome Build 36.3).
[b] Least frequent allele to be associated with risk.
[c] Least frequent allele to be associated with protection.

significantly lower in T2D cases (17%) compared with NG controls (20%) ($P = .039$), however, this was not significant with permutation testing (1000 permutations, $P = .135$) (online Table 2s). A 2-site haplotype analysis using only the 2 marginally associated SNPs (AD/rs182052 and AD/rs7649121) revealed a protective association for the most common (GA) haplotype with T2D (Table 5). The frequency of the GA haplotype was significantly lower in T2D cases compared with controls (62% vs 67%; $P = .009$, permutation $P = .026$; Bonferroni $P = .0125$).

3.3. Association of PPARG and ADIPOQ variants with quantitative subphenotypes of T2D

Indians generally have a strong tendency toward upper body adiposity. As we reported earlier, despite normal BMI, this Khatri Sikh sample presented with an uneven distribution of fat, with a strong tendency toward central adiposity [26]. We investigated the effect of each SNP separately on the obesity-related quantitative traits: BMI, WHR, and waist circumference. No significant association was observed with any of the *PPARG* genotypes (data not shown). There was also no evidence of association of *PPARG* variants with T2D-associated complications such as hypertension, coronary heart disease, quantitative levels of serum lipids, fasting glucose, or insulin. However, multiple linear regression analysis revealed a marginal association of an intronic variant (AD/rs12495941) from LD block 1 (Figure 2s) with hip circumference ($P = .04$) in controls and total weight ($P = .01$) in cases; and a consistent association of obesity-related variables like weight ($R^2 = 0.010$, $P = .010$), waist circumference ($R^2 = 0.005$, $P = .024$), and hip circumference ($R^2 = 0.006$, $P = .021$) but not BMI ($R^2 = 0.001$, $P = .104$)

Table 4
Three-site haplotype analysis (P/rs11715073, P/rs3892175, and P/rs1801282) using selected significant SNPs from the *PPARG* locus

Haplotype sequence	Haplotype frequency		χ^2	*P* value	Permutation *P* value
	NG	T2D			
CGC	0.710	0.765	8.75	.003	.015
GGG	0.109	0.089	2.52	.112	.443
GAC	0.106	0.090	1.59	.207	.667
GGC	0.036	0.040	0.26	.609	.990
CGG	0.015	0.011	0.812	.368	.890
CAC	0.018	0.004	10.75	.001	.005

Underlined fonts in haplotypes indicate the combination of 3 risk alleles with more frequent presence in T2D cases than NG controls; Bonferroni $P = .008$.

Table 5
Two-site haplotype analysis (AD/rs182052 and AD/rs7649121) using only those SNPs from the *ADIPOQ* locus showing an evidence of association

Haplotype	Haplotype frequency		χ^2	*P* value	Permutation *P* value
	NG	T2D			
GA	0.67	0.62	6.77	.009	.026
AA	0.19	0.21	1.39	.238	.546
AT	0.12	0.14	2.77	.096	.240
GT	0.02	0.03	1.61	.205	.486

Underlined fonts indicate the haplotype combination of protective alleles showing more frequent presence in NG controls than T2D cases; Bonferroni $P = .0125$.

Table 6
Association of *ADIPOQ* (rs12495941) with obesity related traits

	Controls (n = 500)				Cases (n = 511)			
	GG (153)	GT (228)	TT (119)	*P* corrected[a]	GG (158)	GT (245)	TT (108)	*P* corrected[a]
BMI	26.7 (25.9-27.4)	27.3 (26.7-27.9)	27.4 (26.5-28.2)	.205	27.3 (26.5-28.1)	27.6 (27.0-28.2)	28.0 (27.0-29.0)	.350
Weight (kg)	69.6 (67.4-71.8)	70.6 (68.9-72.4)	72.1 (69.3-74.9)	.113	70.4 (68.4-72.4)	71.7 (70.0-73.3)	73.4 (70.5-76.4)	.010
Waist (cm)	88.7 (87.0-90.4)	90.5 (89.0-92.0)	90.6 (88.3-93.0)	.158	93.2 (91.5-94.8)	93.8 (92.5-95.1)	95.5 (93.2-97.7)	.117
Hip (cm)	95.0 (93.5-96.5)	96.0 (94.8-97.2)	97.3 (95.5-99.1)	.040	97.0 (95.5-98.5)	97.6 (96.4-98.8)	98.7 (96.5-100.8)	.521
Fasting glucose (mg/dL)	98.3 (96.1-100.5)	98.3 (96.4-100.1)	99.1 (96.7-101.5)	.888	183.8 (172.5-195.1)	176.9 (168.4-185.4)	180.6 (165.4-195.9)	.641
Fasting insulin (pmol/L)[b]	56.8 (48.1-67.2)	64.3 (56.1-73.6)	54.7 (45.2-66.2)	.558	42.8 (36.8-49.7)	47.4 (41.8-53.8)	40.3 (33.1-48.9)	.764
HOMA-IR[b]	1.5 (1.2-1.8)	1.6 (1.3-1.8)	1.5 (1.2-1.9)	.874	2.2 (1.9-2.6)	2.4 (2.1-2.8)	1.8 (1.5-2.3)	.242
HOMA-B[b]	64.9 (53.7-78.4)	70.2 (58.7-84.0)	64.2 (51.2-80.4)	.970	17.8 (14.8-21.3)	20.1 (17.4-23.3)	17.0 (14.0-20.6)	.915
TG (mg/dL)[b]	144.2 (133.0-156.3)	155.9 (145.9-166.7)	137.5 (127.1-148.7)	.504	157.6 (146.3-169.8)	157.0 (146.4-168.4)	158.3 (143.4-174.7)	.964

	Combined (n = 1011)			
	GG (311)	GT (473)	TT (227)	*P* corrected[a]
BMI	27.0 (26.4-27.5)	27.4 (27.0-27.8)	27.7 (27.0-28.3)	.104
Weight (kg)	70.0 (68.5-71.5)	71.2 (70.0-72.4)	72.7 (70.7-74.7)	.010
Waist (cm)	91.0 (89.8-92.2)	92.2 (91.2-93.2)	93.0 (91.3-94.6)	.024
Hip (cm)	96.0 (95.0-97.1)	96.8 (96.0-97.6)	98.0 (96.6-99.3)	.021
Fasting glucose (mg/dL)	146.3 (137.8-154.7)	144.8 (138.2-151.4)	144.0 (133.5-154.5)	.697
Fasting insulin (pmol/L)[b]	49.1 (43.9-54.9)	54.9 (50.0-60.2)	47.0 (41.0-53.9)	.544
HOMA-IR[b]	1.9 (1.7-2.1)	2.0 (1.8-2.3)	1.7 (1.5-2.0)	.465
HOMA-B[b]	30.9 (26.4-36.1)	33.6 (29.4-38.5)	30.0 (25.0-36.1)	.917
TG (mg/dL)[b]	151.1 (143.0-159.6)	156.5 (149.1-164.3)	147.2 (138.2-156.8)	.710

Data represent mean and its 95% CI from analysis of variance.

[a] *P* values were derived from multiple linear regression analysis using the best genetic model after adjusting for age, sex, BMI, medication, and disease status where appropriate.

[b] Log-transformed traits; mean values of log-transformed variables are presented as geometric mean, and other untransformed variables are presented as arithmetic mean.

was seen in the combined cohort after adjusting for age, sex, disease stratus, and medication (Table 6). Applying a conservative Bonferroni adjustment for considering 9 independent variables requires a level of significance of .0056; no trait remained significantly associated with this *ADIPOQ* SNP at this level.

4. Discussion

With the exception of a strong association of *PPARG2*/Pro12Ala with T2D (OR, 0.13; 95% CI, 0.03-0.56; P = .0007), no other tagSNP in the *PPARG* locus revealed any significant association with T2D in this population. Two SNPs (P/rs11715073 and P/rs3892175) in *PPARG1* revealed some moderate trend toward association with T2D in this population. However, our follow-up haplotype analysis using these 3 SNPs suggested a strong evidence of association with T2D susceptibility with the most common haplotype (CGC) (P = .003, permutation P = .015) due to the unique combination of 3 common risk alleles of these 3 SNPs: "C" of P/rs11715073, "G" of P/rs3892175, and "C" of P/rs1801282 (Pro12Ala). Whether this associated haplotype is actually causative has yet to be determined. The major affect of association still appears to be driven by Pro12Ala (P/rs1801282) because the association of these haplotypes with T2D no longer remained significant (P = .262) when we analyzed these haplotype combinations of 3 selected SNPs (P/rs 11715073, P/rs3892175, and P/rs1801282) conditional upon Pro12Ala (P/rs 1801282). These analyses further confirmed that the haplotype analysis do not add more information beyond the known association of Pro12Ala with T2D. In addition, the empirical P value for the CGC haplotype by permutation distribution (P = .015) under the null hypothesis of no association of CGC haplotype is dependent upon their observed association that was significant in these SNPs, whereas the permutation P value for these 3 variants carrying CGC combination in 14 SNP haplotype was not significant in 1000 permutations (P = .257). Therefore, the major contribution appears to be determined from Pro12Ala variant. It is possible that a yet to be discovered causative variant could be located within this locus and may be in strong LD with these SNPs. There is presence of strong LD between P/rs11715073 and P/rs1801282 (D' = 0.84, r^2 = 0.29) and between P/rs11715073 and P/rs3892175 (D' = 0.87, r^2 = 0.30) (online Figure 1s).

PPARG has been shown to increase or decrease the transcription of target genes [35,36]. The *PPARG* target genes including *TNFα*, leptin, resistin, *ADIPOQ*, *IL-6*, and *PAI-1* encode proteins or peptides that participate in the pathogenesis of insulin resistance [37-41]. Interestingly, adiponectin is one of those insulin-sensitizing target proteins whose expression is increased upon activation of *PPARG* by thiazolidinediones [37]; and increased expression of adiponectin improves the insulin sensitivity [42]. Therefore, the genetic variations in the *PPARG* are expected to modulate the secretion of plasma adiponectin levels. In fact, adiponectin levels were shown to increase significantly among *PPARG2*-Ala allele carriers in a Finnish study when weight loss was induced by heavy exercise [43]. Thus, it is possible that the *PPARG2* (Pro12Ala)-associated increase in T2D risk could be associated with lower adiponectin levels. However, because of the lack of data on serum adiponectin levels in this population, we were unable to test this hypothesis. The promoter region of *ADIPOQ* also harbors PPAR response elements, binding sites for *PPARG* [44]. Perhaps the marginally associated SNP from 5′ region *ADIPOQ* gene (AD/rs182052) could be in LD with a causal SNP in promoter region residing in PPAR response elements. Genotyping of additional SNPs in the *ADIPOQ* and *PPARG* genes along with the data on serum adiponectin levels may help detect causative variants. Interestingly, our preliminary results on gene-gene interaction using PIA version 2.0 (http://www3.cancer.gov/intra/lhc/PIA2-distribution.zip), MDR version 1.2.4 (http://mac.softpedia.com/developer/MDR-Team-2175.html), and logistic regression analysis tools have consistently identified the 3 SNPs—2 from *PPARG1* (P/rs2972164 and P/rs4135247) and 1 from *ADIPOQ* (AD/rs3821799)—showing significant interaction (P = .00009) in 3-SNP model. However, caution should be observed in interpreting these findings until confirmed on a large size sample. The general estimates of power revealed that our sample had 80% power to calculate the minimum detectable ORs for risk and maximum detectable OR for protective models at α = 0.05 in all *ADIPOQ* and *PPARG* variants except P/rs10510419 under dominant and recessive models (online Table 4s). Our sample also had 62% to 80% power to detect significant association with T2D in AD/rs182052 and P/rs3892175 using log-additive model; however, the power was less than 40% for the remaining 3 SNPs (P/12497191, P/rs709154, and P/3856806) using log-additive genetic model (online Table 5s). Nonetheless, with the exception of Pro12Ala (P/rs1801282), none of the other variants remained significant after applying Bonferroni correction (P = .0012).

Our study also observed a statistically significant association of one of the *ADIPOQ* variants (AD/rs12495941) with some obesity-related traits (Table 6). This result is in agreement with several earlier published reports where different variants in *ADIPOQ* were associated with obesity-related traits in different populations [21,45-48]. However, unlike other studies, the association of this variant (AD/rs12495941) with obesity-related traits was independent of BMI in Khatri Sikhs. Body mass index is not considered an accurate measure for obesity, especially in the populations from South East Asia where their muscle mass is typically low and visceral and subcutaneous fat is increased [49-51]. This association with obesity-related traits is not statistically significant after applying a Bonferroni adjustment. However, this association with obesity-related traits is supported by the observation that this SNP belongs to block 1(D' 0.70-0.72) from which 2 other SNPs (AD/182052 and

AD/7649121) in haplotype combination (GA) revealed a significant association (P = .009, Bonferroni P = .0125, permutation P = .026) with T2D (Table 5). It is possible that these 3 SNPs from the 5′ region are in strong LD with causal variant that is yet unknown. Perhaps their association with T2D is mediated through obesity and may promote insulin resistance as described by Cauchi et al [13]. Perhaps large sample of healthy controls from the same population would help confirm these results.

In summary, even after performing a comprehensive screening using a tagSNP approach, *PPARG2*/Pro12Ala alone remains the strongest predictor of the development of T2D in Indian Sikhs. It would be of interest to note that the same variant/Pro12Ala in *PPARG2* was not associated with T2D in South Indians from Chennai [52] as well as in South Indians living in Singapore [53]. The exceptional findings in Asian Indians are important in view of the extensive diversity existing between different Indian ethnicities [54,55]. Two *ADIPOQ* variants also revealed significant association with T2D in specific haplotype combination. Apparently, the relatively small size of our cohort is a limitation in study. Although our data have successfully replicated the association of *PPARG2*/Pro12Ala (rs1801282), the possibility of false-positive or false-negative results in the remaining tagSNPs cannot be ruled out because of the limited statistical power of this cohort to detect moderate effects of these variants. Our carefully ascertained, relatively homogenous case-control cohort belongs to an endogamous community of urban Asian Sikhs. Therefore, the possibility of spurious association arising because of population substructure is less likely. However, it is possible that some levels of population stratification could have been introduced because of difference in spouse controls and randomly selected controls. It is also possible that the causal SNPs with stronger effects may be population specific and could have been missed during the selection of tagSNPs that was based on HapMap data on whites for both *PPARG* and *ADIPOQ*. Therefore, further confirmation of these results on a larger ethnicity-based data set would be important to rule out the possibility of false-negative or false-positive results. Future studies involving dense SNP panels and deep sequencing would be necessary to discover putative functional variants in these genes causing T2D and obesity susceptibility.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2009.07.043.

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