Published in final edited form as:

Metabolism. 2010 April ; 59(4): 492–501. doi:10.1016/j.metabol.2009.07.043.

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

Dharambir K. Sanghera^{1,*}, F. Yesim Demirci², Latonya Been¹, Lyda Ortega¹, Sarju K. Ralhan³, Gurpreet S. Wander³, Narinder K. Mehra⁴, Jairup Singh⁵, Christopher E. Aston¹, John J. Mulvihill¹, and Ilyas M. Kamboh²

¹ Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK

² Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA

³ Hero DMC Heart Institute, Ludhiana, Punjab, India

⁴ All India Institute of Medical Sciences and Research, New Delhi, India

⁵ Guru Nanak Dev University, Amritsar, Punjab, India

Abstract

We have examined the association of 14 tagging single nucleotide polymorphisms (tagSNPs) in peroxisome proliferator activated-receptor gamma transcripts 1 and 2 (*PPARG1* & 2) and 5 tagSNPs in adiponectin (*ADIPOQ*) genes for their effect on type 2 diabetes (T2D) risk in Asian Indian Sikhs. A total of 554 T2D cases and 527 normoglycemic (NG) controls were examined for association with T2D and other sub-phenotypes of T2D. With the exception of a strong association of *PPARG2*/Pro12Ala with T2D [OR 0.13, 95%CI (0.03–0.56), $p=0.0007$], 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 two marginally associated SNPs (P/rs11715073 and P/rs3892175) in combination with Pro12 Ala (P/rs1801282) revealed a strong association of one ‘risk’ (CGC) ($p=0.003$; permutation $p=0.015$) and one ‘protective’ (CAC) ($p=0.001$; permutation $p=0.005$) 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 ($p=0.262$). Additionally, two-site haplotype analysis in the *ADIPOQ* locus using only two marginally associated SNPs (AD/rs182052 and AD/rs7649121) revealed a significant protective association of the GA haplotype with T2D ($p=0.009$; permutation $p=0.026$). Multiple linear regression analysis also revealed significant association of an *ADIPOQ* variant (AD/rs12495941) with total body weight ($p=0.010$), waist ($p=0.024$) and hip ($p=0.021$), 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 risk for the development of T2D in Indian Sikhs. Identification of causal SNPs in these important biological and positional candidate genes would help determine true physiological significance of these loci in T2D and obesity.

*Corresponding Author: Dharambir K. Sanghera, PhD, Department of Pediatrics, Section of Genetics, University of Oklahoma Health Sciences Center, 975 NE 10th, BRC-254A, Oklahoma City, OK- 73104, USA, Phone: 405-271-8001 ext. 43064, Fax: 405-271-6027.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Asian Indians, 25% of the global population, make up the greatest proportion of diabetics in the world [1]. The underlying reasons of the high prevalence of 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 sub-continent, have a high prevalence of a characteristic metabolic syndrome, including elevated plasma triglycerides, low levels of high-density lipoprotein cholesterol (HDL-C), high prevalence of insulin resistance, and a tendency towards 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 two positional and biological candidate genes-peroxisome proliferator activated-receptor gamma (*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 genome-wide 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 genome-wide association studies (GWAS) in Caucasians [9–11], except in the French GWAS [12]. Their latter study of obese and non-obese Europeans from France and Switzerland suggests the positive association of *PPARG2* (Pro12Ala) on T2D in obese individuals ($\text{BMI} \geq 30 \text{ kg/m}^2$) [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 triglycerides (LOD 3.0) near the *PPARG* locus in our genome-wide 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 to 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 two 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, while *pparg2* is exclusively expressed in adipose tissues [17]. The *ppargs* are also targets of the thiazolidinediones (TZDs), a class of anti-diabetic 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 three separate genome-wide linkage scans on T2D conducted in French [23], US Caucasians [24] and Pima Indians [25]. Patients with coronary heart disease, T2D, and obesity have reduced plasma levels of adiponectin, the protein product of *ADIPOQ* [26]. 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) [27]. 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 normo-glycemic (NG) (258 male, 269 female) subjects were used in this investigation. The T2D cases were 25 or older with mean age (mean $\pm$ SD) of 55.5 ± 11.1 yrs. at the time of recruitment. The details of diagnostic criteria and recruitment of T2D and NG subjects have been described elsewhere [14,27,28]. T2D was diagnosed based on the guidelines of the American Diabetes Association [29]. T2D was defined as (1) a fasting plasma glucose level ≥ 7.0 mmol/l after a minimum 12-h fast or (2) a 2-h post glucose level [2-h oral glucose tolerance test (OGTT)] ≥ 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 ≥ 5.6 mmol/l but ≤ 7.0 mmol/l or a 2-h OGTT $\geq (7.8$ mmol/l) but $< (11.1$ mmol/l). NG was defined as a fasting glycemia ≤ 5.6 mmol/l or a 2-h glucose < 7.8 mmol/l. In the absence of medical record information, we confirmed self-reported T2D cases by performing a 2-h OGTT following the criteria of the World Health Organization (WHO) (75 g oral load of glucose). Control status was based on self reports, medical records, and fasting glucose levels. The 2-h OGTT was also performed on selected controls who presented impaired fasting glucose (IFG) (>5.6 mmol/l) at the time of recruitment. The 2-h OGTT was used to detect the presence of IGT. Subjects with IFG and/or IGT were excluded from analysis. BMI was calculated as [weight (kg)/height (meter)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 [27]. The average age of NG subjects (mean $\pm$ SD) was 51.5 ± 14.0 yrs. The majority 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 (T1D), or a family member with T1D, rare forms of T2D called maturity-onset diabetes of young (MODYs), or secondary diabetes (e.g., 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 life-long 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). Calibration of the glucometer was routinely verified using test strips provided by the manufacturer. Blood pressure was measured twice by an Omron blood pressure machine (# HEM-705 CP) [30] in sitting position with the left arm resting on a table, legs uncrossed, and feet flat. Quantitation of lipids [total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, very low-density lipoprotein (VLDL) cholesterol, triglycerides (TG)], and insulin levels were estimated in serum. Lipids were quantified using standard enzymatic methods (Roche, Basel, Switzerland). Insulin was measured by radio-

immuno assay (Diagnostic Products, Cypress, USA). All quantitative parameters were determined per manufacturer's instructions using a Hitachi 902 auto-analyzer (Roche, Basel, Switzerland). Homeostasis model assessment (HOMA) for 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. [31].

2.3. SNP Genotyping

DNA was extracted from buffy coats using QiaAmp blood kits (Qiagen, Chatworth, CA) or by the salting out procedure [32]. The tagSNPs were selected using HapMap database (<http://hapmap.org>). The HapMap data from Caucasians was chosen to provide the closest possible SNP coverage since allelic distributions of the Asian Indian population from North India are similar to populations of European ancestries [33], HapMap's tagSNP browser generates a list of maximally informative SNP markers. TagSNPs with minor allele frequency (MAF) $\geq 10\%$, and which passed the HWE test ($p < 0.05$), were chosen using a pair-wise r^2 cut off 0.8. This is more conservative and thus selects more SNPs than the haplotype R^2 method [34]. We included SNPs in the 5'UTR regions and the 2–3 Kb 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 two exonic SNPs- a common non-synonymous coding SNP Pro12Ala (rs1801282) from exon B and a non-synonymous 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, USA). TaqMan genotyping reactions and fluorescence detection were performed on an ABI prism 7900HT sequence detection system (SDS) (ABI, Foster City, USA) using 20 ng of genomic DNA following manufacturer's instructions. Genotypes were scored by analyzing data on both real-time as well as allele discrimination assay platforms using SDS software provided by the 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 $< 0.2\%$. Genotyping success rate ranged between 93%–99% for all the investigated SNPs.

2.4. Statistical Analysis

Departure from Hardy-Weinberg equilibrium (HWE) in cases and controls was tested using Pearson chi-square goodness-of-fit test. The genotype and allele frequencies in T2D cases were compared to those in control subjects using chi-square test or Fisher's exact probability test when expected cell values were less than 3. Pair wise linkage disequilibrium (LD) analysis in this sample was performed using HAPLOVIEW version 4.0 (<http://www.broad.mit.edu/mpg/haploview/>) [35]. 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, gender, 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's test for the continuous traits (e.g., fasting insulin, fasting glucose, HOMA-IR, HOMA-B, triglycerides, 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's correlation and step-wise multiple linear regression with an overall 5% level of significance. Analyses were adjusted for the confounding effects of age, BMI, sex, medication, and also for 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 v15.0. Haplotype-based association (with disease status) and permutation tests were performed using HAPLOVIEW (version 4.0) that uses an accelerated expectation maximization (EM) algorithm to estimate the haplotype frequencies [35]. 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). 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 gender. The genomic positions and genotypic frequencies of the investigated SNPs within *PPARG* and *ADIPOQ* are shown in Table 2 & 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% CI: 0.03–0.56; $p = 0.0007$). The remaining two 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; $p = 0.010$) for P/rs11715073 under a recessive model; and 0.69 (95% CI: 0.52–0.92; $p = 0.010$) for P/rs3892175 under a log-additive model (Table 2). Of the five tagSNPs analyzed in the *ADIPOQ* gene, two (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; $p = 0.027$) for rs182052 and 1.36 (95% CI: 1.03–1.79; $p = 0.029$) 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 $p = 0.0012$ in the *PPARG* locus. None of the SNPs in the *ADIPOQ* locus remained significant with a significance threshold of 0.0033 after adjusting for multiple testing.

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 fourteen-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 three-site haplotype analysis using two SNPs from *PPARG1* that showed marginal trends (P/rs11715073, 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 most common haplotype CGC was significantly higher in T2D cases compared to NG controls (77% vs. 71%; $p = 0.003$, permutation test $p = 0.015$; Bonferroni $p = 0.008$), while a protective association was seen with the rare (CAC) haplotype showing significantly lower frequency in cases compared to controls (0.4% vs. 2%; $p = 0.001$, permutation test $p = 0.005$; Bonferroni $p = 0.008$) (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) ($p=0.262$) by applying conditional haplotype analysis using PLINK.

For *ADIPOQ* a five-site haplotype analysis showed, the frequency of the most common haplotype (GTACG) was significantly lower in T2D cases (17%) compared to NG controls (20%) ($p=0.039$), however, this was not significant with permutation testing (1000 permutations; $p = 0.135$) (online Table 2s). A two-site haplotype analysis using only the two 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 to controls (62% vs. 67%; $p = 0.009$, permutation $p = 0.026$; Bonferroni $p = 0.0125$).

3.3. Association of PPARG and ADIPOQ Variants with Quantitative Sub-phenotypes of T2D

Indians generally have a strong tendency towards 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 towards central adiposity [27]. 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 (CHD), 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 ($p=0.04$) in controls, total weight ($p=0.01$) in cases and a consistence association of obesity-related variables like weight (R-square = 0.010, $p= 0.010$), waist circumference (R-square = 0.005, $p = 0.024$), hip circumference (R-square = 0.006, $p = 0.021$) but not with BMI (R-square = 0.001, $p = 0.104$) was seen in the combined cohort after adjusting for age, sex, disease stratus and medication (Table 6). Applying a conservative Bonferroni adjustment for considering nine independent variables requires a level of significance of 0.0056; 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), $p=0.0007$], 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 towards association with T2D in this population. However, in our follow-up haplotype analysis using these three SNPs suggested a strong evidence of association with T2D susceptibility with the most common haplotype (CGC) ($p = 0.003$; permutation $p = 0.015$) due to unique combination of three common risk alleles of these three 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 ($p=0.262$) when we analyzed these haplotype combinations of three 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. Additionally, the empirical p value for the CGC haplotype by permutation distribution ($p = 0.015$) under the null hypothesis of no association of CGC haplotype is dependent upon their observed association which was significant in these SNPs. Whereas, the permutation p value for these three variants carrying CGC combination in 14 SNP haplotype was not significant in 1000 permutations ($p = 0.257$). 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 ($D' = 0.87$, $r^2 = 0.30$) (see online Figure 1s).

PPARG has been shown to increase or decrease the transcription of target genes [36,37]. The *PPARG* target genes including *TNF α* , leptin, resistin, *ADIPOQ*, *IL-6*, and *PAI -1* encode proteins or peptides that participate in the pathogenesis of insulin resistance [38–42]. Interestingly, adiponectin is one of those insulin-sensitizing target proteins whose expression is increased upon activation of *PPARG* by TZDs [38] and increased expression of adiponectin improves the insulin sensitivity [43]. 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 *PPARG*2-Ala allele carriers in a Finnish study, when weight loss was induced by heavy exercise [44]. Thus, it is possible that the *PPARG*2 (Pro12Ala) associated increase in T2D risk could be associated with lower adiponectin levels. However, due to 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 (PPRE), binding sites for *PPARG* [45]. 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 PPRE. Genotyping of additional SNPs in the *ADIPOQ* and *PPARG* genes along with the data on serum adiponectin levels may help to detect causative variants. Interestingly, our preliminary results on gene-gene interaction using PIA v. 2.0, MDR v. 1.2.4, and logistic regression analysis tools have consistently identified the 3-SNPs- two from *PPARG*1 (P/rs2972164, P/rs4135247) and one from *ADIPOQ*-(AD/rs3821799) showing significant interaction ($p = 0.00009$) in three-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 $\alpha = 0.05$ in all *ADIPOQ* and *PPARG* variants except P/rs10510419 under dominant and recessive models (online Table 4s). Our sample also had 62–80% power to detect significant association with T2D in AD/rs182052 and P/rs3892175 using log-additive model, however, the power was <40% for remaining three SNPs (P/12497191, and 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 ($p=0.0012$).

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 [21,46–49]. However, unlike other studies, the association of this variant (AD/rs12495941) with obesity-related traits was independent of BMI in Khatri Sikhs. BMI 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 [50–52]. 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 two other SNPs (AD/182052 and AD/7649121) in haplotype combination (GA) revealed a significant association ($p=0.009$; Bonferroni $p = 0.0125$; permutation $p = 0.026$) with T2D (Table 5). It is possible that these three SNPs from the 5' region are in strong LD with causal variant which 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 [53] as well as in South Indians living in Singapore [54]. The exceptional findings in Asian Indians are important in view of the extensive diversity existing between different Indian ethnicities [55,56]. Two *ADIPOQ* variants also revealed significant association with T2D in specific haplotype combination. Apparently, relatively small size of our cohort is a limitation in study. Though our data has successfully replicated the association of *PPARG2*/Pro12Ala (rs1801282), possibility of false positive or false negative results in the remaining tagSNPs cannot be ruled out because of 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 due to population sub-structure is less likely. However, it is possible that some levels of population stratification could have been introduced due to 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 which was based on HapMap data on Caucasians for both *PPARG* and *ADIPOQ*. Therefore, further confirmation of these results on a larger ethnicity-based dataset 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grant number KO1 TW006087 from Fogarty International Center, National Institute of Health, USA and seed grant from University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA. Authors thank all the participants of SDS and are grateful for their contribution in this study. Technical help provided by Nirbhay Singh from Hero DMC Heart Institute, India is duly acknowledged.

References

1. Basnayak B, Rajapaksa LC. Cardiovascular and infectious diseases in South Asia: the double whammy. *British Medical Journal* 2004;328:781. [PubMed: 15070612]
2. Mather HM, Keen H. The Southhall Diabetes Survey: prevalence of known diabetes in Asians and Europeans. *British Medical Journal (Clin Res Ed)* 1985;291:1081–1084.
3. McKeigue PM, Pierpoint T, Ferrie JE, Marmot MG. Relationship of glucose intolerance and hyperinsulinaemia to body fat pattern in south Asians and Europeans. *Diabetologia* 1992;35:785–791. [PubMed: 1511807]
4. McKeigue PM, Miller GJ, Marmot MG. Coronary heart disease in south Asians overseas: a review. *Journal of Clinical Epidemiology* 1989;42:597–609. [PubMed: 2668448]
5. Kooner JS, Baliga RR, Wilding J, Crook D, Packard CJ, Banks LM, et al. Abdominal obesity, impaired nonesterified fatty acid suppression, and insulin-mediated glucose disposal are early metabolic abnormalities in families with premature myocardial infarction. *Arteriosclerosis, thrombosis, and vascular biology* 1998;18:1021–1026.
6. Zimmet PZ. Kelly West Lecture 1991. Challenges in diabetes epidemiology--from West to the rest. *Diabetes Care* 1992;15:232–252. [PubMed: 1547680]
7. Parikh H, Groop L. Candidate genes for type 2 diabetes. *Rev Endocr Metab Disord* 2004;5:151–176. [PubMed: 15041791]

8. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, et al. The common PPARGgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nature Genetics* 2000;26:76–80. [PubMed: 10973253]
9. Saxena R, Voight BF, Lyssenko V, Burt NP, de Bakker PI, Chen H, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science (New York, NY)* 2007;316:1331–1336.
10. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science (New York, NY)* 2007;316:1341–1345.
11. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–678. [PubMed: 17554300]
12. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 2007;445:881–885. [PubMed: 17293876]
13. Cauchi S, Nead KT, Choquet H, Horber F, Potoczna N, Balkau B, et al. The genetic susceptibility to type 2 diabetes may be modulated by obesity status: implications for association studies. *BMC Medical Genetic* 2008;9:45.
14. Sanghera DK, Ortega L, Han S, Singh J, Ralhan SK, Wander GS, et al. Impact of nine common type 2 diabetes risk polymorphisms in Asian Indian Sikhs: PPARG2 (Pro12Ala), IGF2BP2, TCF7L2 and FTO variants confer a significant risk. *BMC Medical Genetics* 2008;9:59. [PubMed: 18598350]
15. Zhu Y, Qi C, Korenberg JR, Chen XN, Noya D, Rao MS, et al. Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPAR gamma) gene: alternative promoter use and different splicing yield two mPPAR gamma isoforms. *Proceedings of the National Academy of Sciences of the United States of America* 1995;92:7921–7925. [PubMed: 7644514]
16. Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R, et al. The organization, promoter analysis, and expression of the human PPARGgamma gene. *J Biol Chem* 1997;272:18779–18789. [PubMed: 9228052]
17. Braissant O, Foulfelle F, Scotto C, Dauca M, Wahli W. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* 1996;137:354–366. [PubMed: 8536636]
18. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *Journal of Biological Chemistry* 1995;270:12953–12956. [PubMed: 7768881]
19. Iwamoto Y, Kosaka K, Kuzuya T, Akanuma Y, Shigeta Y, Kaneko T. Effects of troglitazone: a new hypoglycemic agent in patients with NIDDM poorly controlled by diet therapy. *Diabetes Care* 1996;19:151–156. [PubMed: 8718436]
20. Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, et al. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. *Diabetes* 2002;51:536–540. [PubMed: 11812766]
21. Menzaghi C, Ercolino T, Di Paola R, Berg AH, Warram JH, Scherer PE, et al. A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome. *Diabetes* 2002;51:2306–2312. [PubMed: 12086965]
22. Bouatia-Naji N, Meyre D, Lobbens S, Seron K, Fumeron F, Balkau B, et al. ACDC/adiponectin polymorphisms are associated with severe childhood and adult obesity. *Diabetes* 2006;55:545–550. [PubMed: 16443793]
23. Vionnet N, Hani EH, Dupont S, Gallina S, Francke S, Dotte S, et al. Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. *American Journal of Human Genetics* 2000;67:1470–1480. [PubMed: 11067779]
24. Vionnet N, Hani El H, Dupont S, Gallina S, Francke S, Dotte S, et al. Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. *American Journal of Human Genetics* 2000;67:1470–1480. [PubMed: 11067779]

25. Lindsay RS, Hanson RL, Wiedrich C, Knowler WC, Bennett PH, Baier LJ. The insulin gene variable number tandem repeat class I/III polymorphism is in linkage disequilibrium with birth weight but not Type 2 diabetes in the Pima population. *Diabetes* 2003;52:187–193. [PubMed: 12502511]
26. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochemical and biophysical research communications* 1999;257:79–83. [PubMed: 10092513]
27. Sanghera DK, Bhatti JS, Bhatti GK, Ralhan SK, Wander GS, Singh JR, et al. The Khatri Sikh Diabetes Study (SDS): study design, methodology, sample collection, and initial results. *Human Biology* 2006;78:43–63.
28. Sanghera DK, Nath SK, Ortega L, Gambarelli M, Kim-Howard X, Singh JR, et al. TCF7L2 polymorphisms are associated with type 2 diabetes in Khatri Sikhs from North India: genetic variation affects lipid levels. *Annals of Human Genetics* 2008;72:499–509. [PubMed: 18397358]
29. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2004;27 (Suppl 1):S5–S10. [PubMed: 14693921]
30. Mogensen CE, Neldam S, Tikkanen I, Oren S, Viskoper R, Watts RW, et al. Randomised controlled trial of dual blockade of renin-angiotensin system in patients with hypertension, microalbuminuria, and non-insulin dependent diabetes: the candesartan and lisinopril microalbuminuria (CALM) study. *British Medical Journal* 2000;321:1440–1444. [PubMed: 11110735]
31. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–419. [PubMed: 3899825]
32. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* 1988;16:1215. [PubMed: 3344216]
33. Bamshad M, Kivisild T, Watkins WS, Dixon ME, Ricker CE, Rao BB, et al. Genetic evidence on the origins of Indian caste populations. *Genome Research* 2001;11:994–1004. [PubMed: 11381027]
34. Halldorsson BV, Istrail S, De La Vega FM. Optimal selection of SNP markers for disease association studies. *Hum Hered* 2004;58:190–202. [PubMed: 15812176]
35. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics (Oxford, England)* 2005;21:263–265.
36. Corton JC, Anderson SP, Stauber A. Central role of peroxisome proliferator-activated receptors in the actions of peroxisome proliferators. *Annu Rev Pharmacol Toxicol* 2000;40:491–518. [PubMed: 10836145]
37. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, et al. The nuclear receptor superfamily: the second decade. *Cell* 1995;83:835–839. [PubMed: 8521507]
38. Rangwala SM, Lazar MA. Peroxisome proliferator-activated receptor gamma in diabetes and metabolism. *Trends in pharmacological sciences* 2004;25:331–336. [PubMed: 15165749]
39. De Vos P, Lefebvre AM, Miller SG, Guerre-Millo M, Wong K, Saladin R, et al. Thiazolidinediones repress ob gene expression in rodents via activation of peroxisome proliferator-activated receptor gamma. *Journal of Clinical Investigation* 1996;98:1004–1009. [PubMed: 8770873]
40. Teboul L, Febbraio M, Gaillard D, Amri EZ, Silverstein R, Grimaldi PA. Structural and functional characterization of the mouse fatty acid translocase promoter: activation during adipose differentiation. *The Biochemical Journal* 2001;360:305–312. [PubMed: 11716758]
41. Takata Y, Kitami Y, Yang ZH, Nakamura M, Okura T, Hiwada K. Vascular inflammation is negatively autoregulated by interaction between CCAAT/enhancer-binding protein-delta and peroxisome proliferator-activated receptor-gamma. *Circulation Research* 2002;91:427–433. [PubMed: 12215492]
42. Liu HB, Hu YS, Medcalf RL, Simpson RW, Dear AE. Thiazolidinediones inhibit TNFalpha induction of PAI-1 independent of PPARgamma activation. *Biochemical and Biophysical Research Communications* 2005;334:30–37. [PubMed: 15990085]
43. Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. *Endocrine Review* 2005;26:439–451.
44. Mousavinasab F, Tahtinen T, Jokelainen J, Koskela P, Vanhala M, Oikarinen J, et al. Effect of the Pro12Ala polymorphism of the PPARgamma2 gene on serum adiponectin changes. *Endocrine* 2005;27:307–309. [PubMed: 16230789]

45. Iwaki M, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M, et al. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes* 2003;52:1655–1663. [PubMed: 12829629]
46. Stumvoll M, Tschrirer O, Fritsche A, Staiger H, Renn W, Weisser M, et al. Association of the T-G polymorphism in adiponectin (exon 2) with obesity and insulin sensitivity: interaction with family history of type 2 diabetes. *Diabetes* 2002;51:37–41. [PubMed: 11756320]
47. Hu FB, Doria A, Li T, Meigs JB, Liu S, Memisoglu A, et al. Genetic variation at the adiponectin locus and risk of type 2 diabetes in women. *Diabetes* 2004;53:209–213. [PubMed: 14693717]
48. Vimalaswaran KS, Radha V, Ramya K, Babu HN, Savitha N, Roopa V, et al. A novel association of a polymorphism in the first intron of adiponectin gene with type 2 diabetes, obesity and hypoadiponectinemia in Asian Indians. *Human Genetics* 2008;123:599–605. [PubMed: 18465144]
49. Kotani K, Saiga K, Kurozawa Y, Sakane N, Tsuzaki K, Hamada T. Adiponectin I164T gene polymorphism and the obesity-related effects on the Japanese female population. *Clinica Chimica Acta* 2007;384:182–183.
50. McKeigue PM, Shah B, Marmot MG. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* 1991;337:382–386. [PubMed: 1671422]
51. Chandalia M, Abate N, Garg A, Stray-Gundersen J, Grundy SM. Relationship between generalized and upper body obesity to insulin resistance in Asian Indian men. *Journal of Clinical Endocrinology Metabolism* 1999;84:2329–2335. [PubMed: 10404798]
52. Chambers JC, Eda S, Bassett P, Karim Y, Thompson SG, Gallimore JR, et al. C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. *Circulation* 2001;104:145–150. [PubMed: 11447077]
53. Radha V, Vimalaswaran KS, Babu HN, Abate N, Chandalia M, Satija P, et al. Role of genetic polymorphism peroxisome proliferator-activated receptor-gamma2 Pro12Ala on ethnic susceptibility to diabetes in South-Asian and Caucasian subjects: Evidence for heterogeneity. *Diabetes Care* 2006;29:1046–1051. [PubMed: 16644635]
54. Tai ES, Corella D, Deurenberg-Yap M, Adiconis X, Chew SK, Tan CE, et al. Differential effects of the C1431T and Pro12Ala PPARGgamma gene variants on plasma lipids and diabetes risk in an Asian population. *Journal of Lipid Research* 2004;45:674–685. [PubMed: 14729856]
55. Papiha SS. Genetic variation in India. *Hum Biol* 1996;68:607–628. [PubMed: 8908794]
56. Papiha SS. The first 25 years of human genetics in Newcastle. *Human Biology* 1996;68:603–606.

Table 1
Clinical characteristics of study population stratified by disease status and gender (Mean $\pm$ SD)

	Gender	NG ^{ϕ}		T2D ^{$\ddagger$}		p Value ^{**} (2-tailed)
		N=527 (258M/269F)		N=554 (309M/245F)		
Age (yrs)	M*	51.8 $\pm$ 15.6		56.1 $\pm$ 11.1		<0.0001
	F	51.5 $\pm$ 13.3		55.2 $\pm$ 11.1		0.001
Age at Diagnosis (yrs)	M	--		47.3 $\pm$ 11.0		--
	F	--		48.2 $\pm$ 10.6		--
Duration of Diabetes (yrs)	M	--		8.9 $\pm$ 7.4		--
	F	--		7.4 $\pm$ 6.0		--
BMI	M	26.7 $\pm$ 4.2		26.6 $\pm$ 4.4		0.817
	F	27.5 $\pm$ 4.8		29.0 $\pm$ 5.4		0.001
WHR ^{$\ddagger$}	M	0.97 $\pm$ 0.08		0.99 $\pm$ 0.08		1.44 $\times 10^{-3}$
	F	0.91 $\pm$ 0.07		0.93 $\pm$ 0.07		1.32 $\times 10^{-5}$
Fasting Glucose (mmol/l)	M	5.4 $\pm$ 0.6		9.4 $\pm$ 3.3		4.57 $\times 10^{-44}$
	F	5.5 $\pm$ 0.6		9.6 $\pm$ 3.1		4.09 $\times 10^{-49}$
Fasting Insulin(pmol/ml)	M	93.0 $\pm$ 95.6		72.5 $\pm$ 82.9		0.008
	F	93.6 $\pm$ 96.0		70.5 $\pm$ 74.4		0.003
Total Cholesterol (mg/dl)	M	178.7 $\pm$ 50.6		181.1 $\pm$ 56.9		0.612
	F	179.8 $\pm$ 42.9		192.6 $\pm$ 54.3		0.004
LDL-Cholesterol (mg/dl)	M	103.0 $\pm$ 35.4		100.7 $\pm$ 39.0		0.474
	F	105.3 $\pm$ 37.5		111.9 $\pm$ 40.6		0.065
Triglycerides (mg/dl)	M	173.6 $\pm$ 91.8		184.2 $\pm$ 109.4		0.237
	F	154.6 $\pm$ 77.0		174.7 $\pm$ 94.0		0.011

* M - male, F- female;

^{$\ddagger$} waist to hip ratio;

^{$\ddagger$} type 2 diabetes,

^{ϕ} normo-glycemic,

** difference between T2D cases and controls

Table 2

PPARG (1 and 2) polymorphisms and type 2 diabetes risk in Asian Indian Sikhs

TagSNP	POSITION	GENOTYPE	NG (%)	T2D (%)	MODEL	ODD RATIOS (ORs)* (95%CI)	p VALUE
P/rs 2972164	12309416	CC	273 (54.9)	263 (49.8)	Dominant	1.30 (1.00–1.68)	0.048
		CT	179 (36.0)	219 (41.5)	CC vs. CT + TT		
		TT	45 (9.1)	46 (8.7)			
		C/T ^φ	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)	0.010
		CG	188 (36.7)	184 (34.1)	CC + CG vs. GG		
		GG	42 (7.8)	26 (4.8)			
		C/G [‡]	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)	0.010
		AG	105 (21.3)	82 (16.1)	GG, AG, AA		
		AA	11 (2.2)	6 (1.2)			
		G/A [‡]	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)	0.311
		AG	139 (28.3)	160 (30.8)	GG vs. AG + AA		
		AA	23 (4.7)	21 (4.0)			
		G/A ^φ	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)	0.106
		AG	115 (22.1)	105 (20.3)	AA, AG, GG		
		GG	14 (2.7)	9 (1.7)			
		A/G [‡]	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)	0.0007
		CG	108 (20.9)	103 (19.5)	CC + CG vs. GG		
		GG	14 (2.8)	2 (0.4)			
		C/G [‡]	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)	0.157
		AG	230 (46.9)	252 (48.6)	GG + AG vs. AA		
		AA	108 (22.0)	100 (19.3)			
		G/A [‡]	0.54/0.46	0.56/0.44			

TagSNP	POSITION	GENOTYPE	NG (%)	T2D (%)	MODEL	ODD RATIOS (ORs)* (95%CI)	p VALUE
P/rs 2972162	12399793	CC	194 (38.5)	201 (38.4)	Recessive	0.88 (0.63–1.23)	0.448
		CT	223 (44.2)	241 (46.0)	CC + CT vs. TT		
		TT	87 (17.3)	82 (15.6)			
		C/T ^φ	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)	0.329
		GT	131 (26.0)	133 (26.3)	GG + GT vs. TT		
		TT	20 (4.0)	15 (3.0)			
		G/T [†]	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)	0.148
		AG	159 (31.7)	194 (36.1)	AA vs. AG + GG		
		GG	26 (5.2)	28 (5.2)			
		A/G ^φ	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)	0.433
		AT	130 (25.7)	142 (27.2)	AA, AT, TT		
		TT	14 (2.8)	16 (3.1)			
		A/T ^φ	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)	0.209
		AC	131 (26.5)	156 (30.0)	AA vs. AC + CC		
		CC	18 (3.6)	19 (3.7)			
		A/C ^φ	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)	0.141
		AC	179 (36.6)	167 (32.6)	AA vs. AC + CC		
		CC	26 (5.3)	31 (6.1)			
		A/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)	0.103
		CT	82 (16.6)	105 (19.8)	CC, CT, TT		
		TT	7 (1.4)	12 (2.3)			
		C/T ^φ	0.90/0.10	0.88/0.12			

* ORs were adjusted for age, sex, and BMI; Position of SNPs on chromosome has been taken from NCBI (Genome Build 36.3);

ϕ Least frequent allele to be associated with risk;
 $\ddagger$ Least frequent allele to be associated with protection

Table 3

ADIPOQ polymorphisms and type 2 diabetes risk in Asian Indian Sikhs

TagSNP	POSITION	GENOTYPE	NG (%)	T2D (%)	MODEL	ODD RATIOS (ORs)* (95%CI)	p VALUE
AD/rs 182052	188043476	GG	240 (48.6)	233 (42.1)	Log-Additive	1.23 (1.02–1.48)	0.027
		GA	204 (41.3)	253 (45.7)	GG, GA, AA		
		AA	50 (10.1)	68 (12.3)			
		G/A ^φ	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)	0.222
		GT	228 (45.6)	245(47.9)	GG + GT vs. TT		
		TT	119 (23.8)	108 (21.1)			
		G/T [‡]	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)	0.029
		AT	116 (22.6)	148 (27.1)	AA vs. AT + TT		
		TT	16 (3.1)	23 (4.2)			
		A/T ^φ	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)	0.207
		CT	231 (46.8)	278 (51.1)	CC vs. CT + TT		
		TT	89 (18.0)	92 (16.9)			
		C/T ^φ	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)	0.085
		GA	205 (41.2)	247 (46.0)	GG vs. GA + AA		
		AA	65 (13.1)	61 (11.4)			
		G/A ^φ	0.66/0.34	0.66/0.34			

* ORs were adjusted for age, sex, and BMI; Position of SNPs on chromosome has been taken from NCBI (Genome Build 36.3);

^φ Least frequent allele to be associate with risk;

[‡] Least frequent allele to be associated with protection

Table 4

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

Haplotype Sequence	Haplotype Frequency		Chi Square	p Value	Permutation p-Value
	NG	T2D			
<u>CGC</u>	<u>0.710</u>	<u>0.765</u>	<u>8.75</u>	<u>0.003</u>	<u>0.015</u>
GCG	0.109	0.089	2.52	0.112	0.443
GAC	0.106	0.090	1.59	0.207	0.667
GGC	0.036	0.040	0.26	0.609	0.990
CGG	0.015	0.011	0.812	0.368	0.890
<u>CAC</u>	<u>0.018</u>	<u>0.004</u>	<u>10.75</u>	<u>0.001</u>	<u>0.005</u>

Underlined fonts in haplotypes indicate the combination of 3 risk alleles with more frequent presence in T2D cases than NG controls; Bonferroni p = 0.008

Table 5

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

Haplotype	Haplotype Frequency		Chi Square	p Value	Permutation p-Value
	NG	T2D			
GA	0.67	0.62	6.77	0.009	0.026
AA	0.19	0.21	1.39	0.238	0.546
AT	0.12	0.14	2.77	0.096	0.240
GT	0.02	0.03	1.61	0.205	0.486

Underlined fonts indicate the haplotype combination of protective alleles showing more frequent presence in NG controls than T2D cases; Bonferroni $p = 0.0125$

Table 6

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

	CONTROLS (n = 500)					CASES (n = 511)				
	GG (153)	GT (228)	TT (119)	p corrected*	GG (158)	GT (245)	TT (108)	p corrected*		
BMI	26.7 (25.9 – 27.4)	27.3 (26.7 – 27.9)	27.4 (26.5 – 28.2)	0.205	27.3 (26.5 – 28.1)	27.6 (27.0 – 28.2)	28.0 (27.0 – 29.0)	0.350		
Weight (kg)	69.6 (67.4 – 71.8)	70.6 (68.9 – 72.4)	72.1 (69.3 – 74.9)	0.113	70.4 (68.4 – 72.4)	71.7 (70.0 – 73.3)	73.4 (70.5 – 76.4)	0.010		
Waist (cm)	88.7 (87.0 – 90.4)	90.5 (89.0 – 92.0)	90.6 (88.3 – 93.0)	0.158	93.2 (91.5 – 94.8)	93.8 (92.5 – 95.1)	95.5 (93.2 – 97.7)	0.117		
Hip (cm)	95.0 (93.5 – 96.5)	96.0 (94.8 – 97.2)	97.3 (95.5 – 99.1)	0.040	97.0 (95.5 – 98.5)	97.6 (96.4 – 98.8)	98.7 (96.5 – 100.8)	0.521		
Fasting glucose (mg/dL)	98.3 (96.1 – 100.5)	98.3 (96.4 – 100.1)	99.1 (96.7 – 101.5)	0.888	183.8 (172.5 – 195.1)	176.9 (168.4 – 185.4)	180.6 (165.4 – 195.9)	0.641		
Fasting Insulin (pmol/L) [†]	56.8 (48.1 – 67.2)	64.3 (56.1 – 73.6)	54.7 (45.2 – 66.2)	0.558	42.8 (36.8 – 49.7)	47.4 (41.8 – 53.8)	40.3 (33.1 – 48.9)	0.764		
Homa IR [†]	1.5 (1.2 – 1.8)	1.6 (1.3 – 1.8)	1.5 (1.2 – 1.9)	0.874	2.2 (1.9 – 2.6)	2.4 (2.1 – 2.8)	1.8 (1.5 – 2.3)	0.242		
Homa B [†]	64.9 (53.7 – 78.4)	70.2 (58.7 – 84.0)	64.2 (51.2 – 80.4)	0.970	17.8 (14.8 – 21.3)	20.1 (17.4 – 23.3)	17.0 (14.0 – 20.6)	0.915		
Triglycerides (mg/dL) [†]	144.2 (133.0 – 156.3)	155.9 (145.9 – 166.7)	137.5 (127.1 – 148.7)	0.504	157.6 (146.3 – 169.8)	157.0 (146.4 – 168.4)	158.3 (143.4 – 174.7)	0.964		
COMBINED (n = 1,011)										
BMI	27.0 (26.4 – 27.5)	27.4 (27.0 – 27.8)	27.7 (27.0 – 28.3)					0.104		
Weight (kg)	70.0 (68.5 – 71.5)	71.2 (70.0 – 72.4)	72.7 (70.7 – 74.7)					0.010		
Waist (cm)	91.0 (89.8 – 92.2)	92.2 (91.2 – 93.2)	93.0 (91.3 – 94.6)					0.024		
Hip (cm)	96.0 (95.0 – 97.1)	96.8 (96.0 – 97.6)	98.0 (96.6 – 99.3)					0.021		
Fasting glucose (mg/dL)	146.3 (137.8 – 154.7)	144.8 (138.2 – 151.4)	144.0 (133.5 – 154.5)					0.697		
Fasting Insulin (pmol/L) [†]	49.1 (43.9 – 54.9)	54.9 (50.0 – 60.2)	47.0 (41.0 – 53.9)					0.544		
Homa IR [†]	1.9 (1.7 – 2.1)	2.0 (1.8 – 2.3)	1.7 (1.5 – 2.0)					0.465		
Homa B [†]	30.9 (26.4 – 36.1)	33.6 (29.4 – 38.5)	30.0 (25.0 – 36.1)					0.917		
Triglycerides (mg/dL) [†]	151.1 (143.0 – 159.6)	156.5 (149.1 – 164.3)	147.2 (138.2 – 156.8)					0.710		

Data represents mean and its 95% confidence interval from ANOVA;

* 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;

[†] log transformed traits; mean values of log-transformed variables are presented as geometric mean and other un-transformed variables are presented as arithmetic mean.