Role of Genetic Polymorphism Peroxisome Proliferator–Activated Receptor-γ2 Pro12Ala on Ethnic Susceptibility to Diabetes in South-Asian and Caucasian Subjects

Evidence for heterogeneity

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OBJECTIVE — To determine whether the peroxisome proliferator–activated receptor (PPAR)-γ Pro12ala polymorphism modulates susceptibility to diabetes in South Asians.

RESEARCH DESIGN AND METHODS — South Asians ($n = 697$) and Caucasians ($n = 457$) living in Dallas/Forth Worth, Texas, and South Asians living in Chennai, India ($n = 1,619$), were enrolled for this study. PPAR-γ Pro12Ala was determined using restriction fragment–length polymorphism. Insulin responsiveness to an oral glucose tolerance test (OGTT) was measured in nondiabetic subjects.

RESULTS — The Caucasian diabetic subjects had significantly lower prevalence of PPAR-γ 12Ala when compared with the Caucasian nondiabetic subjects (20 vs. 9%, $P = 0.006$). However, there were no significant differences between diabetic and nondiabetic subjects with reference to the Pro12Ala polymorphism among the South Asians living in Dallas (20 vs. 23%) and in India (19 vs. 19.3%). Although Caucasians carrying PPAR-γ Pro12Ala had lower plasma insulin levels at 2 h of OGTT than the wild-type (Pro/Pro) carriers (76 ± 68 and 54 ± 33 μU/ml, respectively, $P = 0.01$), no differences in either fasting or 2-h plasma insulin concentrations were found between South Asians carrying the PPAR-γ Pro12Ala polymorphism and those with the wild-type genotype at either Chennai or Dallas.

CONCLUSIONS — Although further replication studies are necessary to test the validity of the described genotype-phenotype relationship, our study supports the hypothesis that the PPAR-γ Pro12Ala polymorphism is protective against diabetes in Caucasians but not in South Asians.

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Abbreviations: CURES, Chennai Urban Rural Epidemiology Study; HOMA-IR, homeostasis model assessment for insulin resistance; OGTT, oral glucose tolerance test; PPAR, peroxisome proliferator–activated receptor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Type 2 diabetes is highly prevalent in individuals of South-Asian origin (from India, Pakistan, and Bangladesh) when compared with individuals of European descent (Caucasians) (1–4). Genetic causes may account for predisposition to insulin resistance and diabetes in South Asians (4,5). Therefore, evaluation of genetic defects that modulate the insulin signaling pathway and determine predisposition to insulin resistance and type 2 diabetes in this population may shed light on genetic causes of insulin resistance and diabetes. Several candidate genes for defects in insulin signaling have been postulated, and a few studies report positive associations between polymorphisms of genes involved in the insulin signaling pathway and insulin resistance (6). We recently reported a positive association between ENPP1-K121Q polymorphism and type 2 diabetes both in Caucasians and in South Asians studied at Dallas and at Chennai, India (7). On the other hand, some genetic polymorphisms have also been associated to improved insulin sensitivity and protection from type 2 diabetes. Among the latter is peroxisome proliferator–activated receptor (PPAR)-γ Pro12Ala (8–12). The present study was carried out to determine whether this polymorphism contributes to modulate ethnic susceptibility to diabetes in South Asians. To accomplish this goal, we assessed the frequency of PPAR-γ X12Ala in South Asians and in Caucasians with and without diabetes. The study was extended to include both South Asians living in Dallas and South Asians living in India. In addition, insulin responsiveness to an oral glucose load was measured in nondiabetic subjects with PPAR-γ X12Ala versus the wild type of these three study groups.

RESEARCH DESIGN AND METHODS

— To determine whether the frequency of the candidate genetic polymorphisms was different in South Asians and in Caucasians, we recruited unrelated South Asians and Caucasians living in Dallas by public advertisement and offering free screening for cardiovascular risk factors at the University of Texas Southwestern Lipid and Heart Disease Risk Management Clinic in Dallas, Texas. To determine the frequency of the candidate genetic polymorphism in a nonmigrant population living in India, the study included a group of South Asians enrolled in the Chennai Urban Rural Epidemiology Study (CURES) (13). Institutional review board approval was obtained for the study, and informed consent was obtained from all study subjects. Each of the participants was administered a health questionnaire. A blood sample was then drawn from each participant and immediately refrigerated. After separation of plasma and serum, aliquots were frozen at −80°C. Blood samples were collected for biochemical and genetic studies. The presence of diabetes was documented either by the use of hypoglycemic agents or by fasting plasma glucose ≥126 mg/dl and/or plasma glucose at time 2 h of an oral glucose tolerance test (OGTT) ≥200 mg/dl. To further evaluate the impact of the studied mutations on insulin resistance, we invited the volunteers to participate in the second part of the study, which included more detailed anthropometric measurements (skinfolds, waist and hip circumferences, and underwater weighing in subjects studied at Dallas) and an OGTT. Methods for these procedures are reported elsewhere (7). Subjects who were diagnosed to have diabetes based on the World Health Organization criteria (fasting plasma glucose ≥126 mg/dl and/or 2-h plasma glucose ≥200 mg/dl) were added to the diabetic group. All the subjects who had type 2 diabetes were defined by the absence of ketosis and adequate insulin reserve as demonstrated by response to oral hypoglycemic agents.

Biochemical parameters

Fasting plasma glucose (glucose oxidase–peroxidase method), serum cholesterol (cholesterol oxidase–peroxidase–amidopyrine method), serum triglycerides (glycerol phosphate oxidase–peroxidase–amidopyrine method), and HDL cholesterol (direct method–polyethylene glycol–pretreated enzymes) were measured using a Hitachi-912 Autoanalyzer (Hitachi, Mannheim, Germany). In all the samples from the Dallas cohort, insulin was measured by radioimmunoassay at Linco Research (St. Louis, MO), while at Chennai, it was measured using the Dako enzyme-linked immunosorbent assay kit (Dako Diagnostics). For quality assurance, in a subsample of 120 subjects from the Chennai cohort (30 nondiabetic subjects with wild-type genotype, 30 nondiabetic subjects with Pro12Ala, 30 type 2 diabetic patients with wild-type genotype, and 30 type 2 diabetic patients with Pro12Ala), insulin was also measured using the same Linco insulin kit used for the Dallas samples.

Bioelectric impedance measurements at Chennai for the subjects were made using a Beurer body fat analyzer, which incorporates weighing scales and measures both weight and bioimpedance (Beurer BF 60; Beurer, Ulm, Germany). Impedance measurements allow assessment of the fat-free mass and, by difference with body weight, assessment of body fat percentage.

Dual-energy X-ray absorptiometry and computed tomography scan

Both these procedures were done for a subset of South Asians from Chennai (n = 164) on the same day by two different observers at the Bharat Scans, Chennai, a specialized center for imaging and radiological studies. Both observers and the radiologist who interpreted the scans were unaware of the clinical status of the study subjects. Subcutaneous and visceral fat were measured using a Helical computed tomography scan (General Electric, Milwaukee, WI). Dual-energy X-ray absorptiometry was used to measure total body fat, abdominal fat, nonabdominal fat, and central abdominal fat. The machine used was a Lunar Prodigy (Model 8743-BX/1L; Lunar, Madison, WI).

DNA amplification by PCR

Fasting blood samples were drawn into 10-ml vacuum tubes containing EDTA. Genomic DNA was isolated from whole blood using commercial DNA isolation kits from QIAGEN (Chatsworth, CA).

Assay of PPAR-γ Pro12Ala polymorphisms

PCR amplification of the segment with the Pro12Ala polymorphism was carried out in a volume of 25 μl, containing 100 ng genomic DNA, 5 pmol of each primer, and *Taq*DNA polymerase. The PCR conditions were as follows: denaturation at 94°C for 3 min followed by 40 cycles of denaturation for 30 s, annealing at 53°C for 30 s, extension at 72°C, and final extension at 72°C for 9 min. The polymorphism was genotyped using the following primers: forward: 5′-GCCAATTCAAGC CCAGTC3′, reverse: 5′-GATATGTTTGC AGACAGTGTATCAGTGAAGGAATCG CTTTCCG-3′. The PCR product size is 273 bp. Restriction fragment–length polymorphism was detected after digestion overnight with 2 units of *Bst*UI.

Statistical analysis and calculations

Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated from fasting insulin and glucose concentrations (14). Matsuda insulin sensitivity index was calculated from plasma glucose and insulin concentrations during the OGTT (15). Continuous demographic variables were compared between South Asians and Caucasians using the Mann-Whitney *U* test. Frequencies of mutation were compared between ethnic groups using the Fisher's exact test. Two-way ANOVA models were used to assess effects of ethnicity, polymorphisms, and the interaction between ethnic group and polymorphisms. Multiple comparisons of these group means were made with the least-squares contrasts of the ANOVA models. Because of skewness, triglycerides and insulin were log transformed before analysis. Statistical analysis was performed using SAS version 8.02 (SAS Institute, Cary, NC).

RESULTS

— A total of 1,619 South Asians were recruited in Chennai within the CURES epidemiological study (13); 697 subjects of South-Asian origin and 457 of European descent were recruited in Dallas. The general characteristics of diabetic and nondiabetic subjects are reported in Table 1. The prevalence of hypertension in type 2 diabetic subjects was 37% in South Asians living in Chennai, 45% in South Asians living in Dallas, and 15% in Caucasians living in Dallas. The corresponding figures for nondiabetic subjects were 8% (South Asians living in Chennai), 15% (South Asians living in Dallas), and 5% (Caucasians living in Dallas). Body fat measurements by computed tomography and dual-energy X-ray absorptiometry revealed that diabetic subjects had significantly higher visceral fat (P = 0.005) and central abdominal fat (P = 0.011) than nondiabetic subjects. Visceral fat and central abdominal fat also

Table 1—*General characteristics of the study population*

	South Asians living in Chennai	South Asians living in Dallas	Caucasians living in Dallas	*P*
Nondiabetic subjects				
n (M/F)	820 (299/521)	616 (363/253)	334 (168/166)	<0.0001
Age (years)	41 ± 13	42 ± 13	37 ± 14†	<0.0001
BMI (kg/m^2)	23.4 ± 4.7	24.9 ± 3.7*	25.4 ± 5.4*	<0.0001
Glucose (mg/dl)	85 ± 8	96 ± 23*	88 ± 12†	<0.0001
Total abdominal fat (cm^2)	332.0 ± 135.8	—	—	—
Visceral fat (cm^2)	119.5 ± 53.5	—	—	—
Subcutaneous abdominal fat (cm^2)	208.7 ± 118.6	—	—	—
Central abdominal fat (g)	1,368.4 ± 510.1	—	—	—
Diabetic subjects				
n (M/F)	799 (348/451)	81 (46/35)	123 (88/35)	<0.0001
Age (years)	52 ± 11	56 ± 10*	59 ± 8*	<0.0001
BMI (kg/m^2)	25.1 ± 4.2	25.6 ± 3.7	33.4 ± 7.3†	<0.0001
Glucose (mg/dl)	162 ± 68	161 ± 65	172 ± 74	0.40
Total abdominal fat (cm^2)	371.4 ± 113.6	—	—	—
Visceral fat (cm^2)	140.5 ± 40.6	—	—	—
Subcutaneous abdominal fat (cm^2)	230.1 ± 97.5	—	—	—
Central abdominal fat (g)	1,547.7 ± 371.7	—	—	—

Data are means ± SD unless otherwise indicated. *P* values were determined using ANOVA. SI conversion factors are as follows: 0.0555 mmol/l for glucose, 0.0259 mmol/l for cholesterol, and 0.0113 mmol/l for triglycerides. *$P < 0.05$ vs. the South Asians living in Chennai; †$P < 0.05$ vs. the South Asians living in Dallas.

showed a strong correlation with each other ($P < 0.0001$). Waist circumference and sagittal abdominal diameter showed a strong correlation with visceral fat ($P < 0.01$) and central abdominal fat ($P < 0.0001$) in both diabetic and nondiabetic subjects.

There were 1% of South Asians (living in either Dallas or Chennai) and 1% of Caucasians with homozygosity of the PPAR-γ 12Ala polymorphism. The allele frequency of PPAR-γ 12 Ala was 10% in the Caucasian group, 11% in the South-Asian group from Dallas, and 10% in the South-Asian group from Chennai. The genotype frequencies were in Hardy-Weinberg equilibrium in each subgroup. Because of the small number of homozygotes in the sample, individuals homozygous (Ala/Ala) and heterozygous for PPAR-γ 12Ala (Pro/Ala) were grouped together for analysis and identified as X/Ala. As depicted in Fig. 1, the frequency of at least one copy of PPAR-γ 12Ala was comparable among the nondiabetic subjects of the three-study group (19% for the South Asians living in Chennai, 21% for the South Asians living in Dallas, and 21% for the Caucasians living in Dallas). Among the Caucasians, a significant decrease in frequency of PPAR-γ 12Ala was found in the diabetic subgroup when compared with the nondiabetic subjects (20 vs. 9%, $P = 0.006$). On the other hand, no statistically significant differences in PPAR-γ Pro12Ala frequency were found between diabetic and nondiabetic subjects among the South Asians studied in Dallas or Chennai (20 vs. 23% for the Dallas cohort; 19 vs. 19.3% for the Chennai cohort).

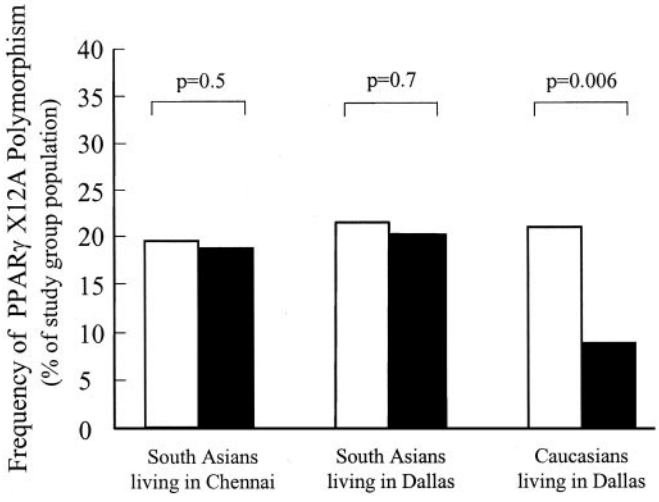

Figure 1—*Frequency of PPAR-γ variants in Caucasians, South Asians living in Dallas, and South Asians living in Chennai. □, nondiabetic participants; ■, type 2 diabetic patients.*

A total of 976 South Asians (820 living in Chennai and 156 living in Dallas) and 151 Caucasians, who had no diabetes, underwent OGTT. Within each study group, there were no differences in general characteristics, including BMI, body fat content, and waist circumference between the carriers of the polymorphism and the wild-type genotype (Table 2). Because the Chennai cohort was the largest, we also compared general characteristics and measures of insulin resistance in this cohort separately for the heterozygous (58 males and 89 females) and homozygous (6 males and 5 females) subjects. No differences were found for any of the variables analyzed (data not shown).

To assess any association between PPAR-γ Ala carrier and improved insulin sensitivity, we compared subgroups with wild-type PPAR-γ and 12Ala carriers within the same main study groups (South Asians living in Chennai, South Asians living in Dallas, and Caucasians

Table 2—*Clinical profile of nondiabetic subjects who underwent an OGTT*

	South Asians living in Chennai		South Asians living in Dallas		Caucasians living in Dallas	
	Wild-type PPAR-γ (Pro/Pro)	X/Ala carriers	Wild-type PPAR-γ (Pro/Pro)	X/Ala carriers	Wild-type PPAR-γ (Pro/Pro)	X/Ala carriers
n (M/F)	662 (235/427)	158 (64/94)	120 (80/40)	36 (23/13)	118 (47/71)	35 (15/18)
Age (years)	41 ± 13	42 ± 12	33 ± 11	33 ± 11	30 ± 8	29 ± 5
BMI (kg/m^2)	23.4 ± 4.5	23.5 ± 5.1	23.8 ± 3.7	24.3 ± 3.4	24.9 ± 5.9	25.4 ± 4.1
Body fat (% body wt)	32.7 ± 9.7	33.2 ± 9.2	26.7 ± 7.6	26.9 ± 8.2	26.8 ± 9.8	27.6 ± 8.8
Waist (cm)	84 ± 12	84 ± 13	83 ± 11	83 ± 11	84 ± 14	82 ± 11
Systolic blood pressure (mmHg)	119 ± 16	119 ± 14	115 ± 13	113 ± 12	114 ± 13	116 ± 11
Diastolic blood pressure (mmHg)	75 ± 10	74 ± 9	72 ± 11	69 ± 11	70 ± 12	69 ± 8
Glucose (mg/dl)	85 ± 6	85 ± 8	91 ± 12	97 ± 11	92 ± 9	90 ± 6
Baseline insulin (μU/ml)	9 ± 6	9 ± 6	16 ± 22	18 ± 25	12 ± 9	11 ± 6
HOMA-IR (index)	1.9 ± 1.4	1.9 ± 1.3	3.3 ± 6.1	3.3 ± 4.3	2.9 ± 2.5	2.4 ± 1.4
2-h OGTT insulin (μU/ml)	54 ± 46	57 ± 46	104 ± 90	101 ± 55	76 ± 68	54 ± 33*
Insulin sensitivity index (Matsuda)	11.4 ± 9.4	11.6 ± 10.6	4.9 ± 3.3	4.3 ± 1.8	6.2 ± 3.7	7.0 ± 5.4

Data are means ± SD unless otherwise indicated. Each study group (South Asians living in Chennai, South Asians living in Dallas, and Caucasians living in Dallas) was analyzed separately. *P* values were determined using the Student's *t* test for independent groups. SI conversion factors are as follows: 0.0555 mmol/l for glucose, 0.0259 mmol/l for cholesterol, and 0.0113 mmol/l for triglycerides. *$P < 0.05$ vs. the wild-type Caucasians living in Dallas.

living in Dallas). Plasma insulin levels at the 2-h OGTT increased by a similar degree in the wild-type carrier and carrier of the PPAR-γ 12Ala allele in both the South-Asian populations (54 ± 46 and 57 ± 46 μU/ml, respectively, for the South Asians living in Chennai, and 104 ± 90 and 101 ± 55 μU/ml, respectively, for those living in Dallas) when compared with baseline. However, the 2-h OGTT increase in plasma insulin concentrations was less in the Caucasians who were carriers of the PPAR-γ 12Ala polymorphism when compared with the noncarriers (54 ± 33 and 76 ± 68 μU/ml for the carriers and noncarriers, respectively; $P = 0.01$). No differences in fasting plasma insulin and HOMA-IR were found between carriers and wild-type carriers in the three study cohorts. The plasma insulin levels and the HOMA-IR values were lower in the South Asians at Chennai compared with the South Asians at Dallas. To test whether this is due to differences in assays, we compared plasma insulin concentrations of a subset of 120 subjects from the Chennai cohort using the Dako and Linco kits (used at Dallas) on the same samples. The Dako method used to determine insulin in the whole Chennai cohort underestimated plasma insulin concentrations. The mean difference was 4.16 μU/ml (95% CI 2.8–5.5) and 14.4 μU/ml (11.2–17.7) for the baseline and 2-h insulin values, respectively.

CONCLUSIONS — There are two major findings in this study. First, the frequency of the PPAR-γ X12Ala polymorphism in South Asians is similar to that seen in Caucasians. Second, whereas type 2 diabetes is associated with decreased frequency of PPAR-γ X12Ala in people of European descent, the prevalence of this polymorphism is not decreased in type 2 diabetic subjects of South-Asian descent.

The PPAR-γ 12A polymorphism has been reported to have a "protective" role in diabetes risk (8–12). Although findings have not been uniform (16–19), a meta-analysis by Altshuler et al. (12) determined that the presence of the 12Ala allele confers ~20% reductions in risk for diabetes. If PPAR-γ 12Ala had a protective effect on diabetes risk on various ethnic groups, one would expect lower 12Ala allele frequency in populations characterized by a high prevalence of diabetes, such as the South Asians. Contrary to this expectation, we found a similar 12Ala allele frequency in South Asians and in Caucasians (~10 and 11%, respectively). A recent study on South Asians who migrated to Singapore also reported a 12Ala allele frequency of ~11% (19). Our study was performed both in a migrant population and in a homogeneous population of South Asians residing in India. In both populations, we observed a prevalence of a 12Ala allele similar to that reported from Singapore. The findings in the Caucasian group living in Dallas are in concordance with the published literature (8,9) and confirm an allele frequency of ~10% in this ethnic group. In addition, our data support the hypothesis of a protective role of a 12Ala allele on diabetes risk in the Caucasian population. As shown in Fig. 1, the frequency of 12Ala was significantly lower in the diabetic Caucasians when compared with the nondiabetic Caucasians. On the other hand, we show that South-Asian diabetic and nondiabetic subjects have virtually the same prevalence of 12Ala allele. Thus, our results support the view that the PPAR-γ 12Ala allele does not reduce the risk for diabetes in South Asians. Similar findings were obtained in South Asians, Chinese, and Malays living in Singapore (19).

Previous studies have linked the 12Ala allele with better insulin sensitivity in Caucasians (7,8). However, these studies have often been confounded by the presence of decreased BMI in the 12Ala allele carriers, and no body composition studies with direct measures of body fat content and distribution were available. In our study, we have two subgroups of Caucasians with similar body composition and fat distribution. We also included a biomarker of insulin resistance with the measurement of plasma insulin concentrations at 2 h from glucose ingestion during OGTT. This measure is superior to fasting plasma insulin concentration when estimating variability in insulin area under the curve during OGTT and better indicates insulin resistance than fasting insulin concentrations (20). We also compared another index of insulin resistance, the Matsuda index (15), across the study groups. Despite similar glucose loads, the Caucasian group with the 12Ala had a much lower increase in plasma insulin concentrations at 2 h and a trend toward a higher Matsuda index,

thus suggesting better biological activity of insulin in disposal of glucose in peripheral cells. Of note, the association between allele 12Ala and better insulin sensitivity was found despite similarities in body composition and fat distribution. In contrast to the findings of the Caucasian group, the South Asians did not exhibit any difference in 2-h plasma insulin concentrations during the OGTT, HOMA-IR, or Matsuda index. Because of the differences in insulin concentration, it appears that South Asians living in Dallas are more insulin resistant that individuals living in Chennai, despite minor differences in BMI. The reasons for the differences between South Asians living in Chennai and those living in Dallas have not been investigated in this study. Besides the contribution of methodological differences in measuring plasma insulin in the two cohorts, a possible role of differences in physical activity and diet cannot be excluded (21). However, further studies are required that are specifically designed to address the issues of dietary intake, physical activity, and insulin sensitivity in these two populations. Regardless of the reasons for the differences in insulin resistance between the two South-Asian cohorts, the conclusions of our study remain unchanged because the comparisons were made between the Pro/Pro and X/Ala groups at the respective centers using the same insulin assay.

Taken together, these findings suggest a lack of a "protective" role of 12Ala allele on insulin resistance and risk for type 2 diabetes in the South-Asian population. Case-control studies, including ours, are generally underpowered to allow firm conclusions on the association between a given polymorphism and the studied phenotype. Previous meta-analyses have helped in estimating a significant effect of PPAR-γ Pro12Ala on risk reduction for type 2 diabetes in Caucasians with an odds ratio of 0.80 (12). However, not enough studies are available in multiple ethnic groups to determine whether ethnicity is a factor that modulates the effects of this particular polymorphism in predicting risk for diabetes. Although our study will need replication in a larger cohort, the concordance of results in the two South-Asian groups of this study as well as the study from Singapore in South Asians, Chinese, and Malays (19) adds support to the hypothesis that there are ethnic differences in the association between 12Ala and type 2 diabetes. Our data suggest the need for a more systematic evaluation of gene-gene and environment-gene interaction with the inclusion of a larger cohort of South Asians to explain ethnic differences in phenotypic response to a common polymorphism. The ethnic differences in relation to the "protective" role of allele 12Ala for diabetes risk may in fact have both genetic and environmental origins. The similarity of our findings between the South Asians living in Chennai and those living in Dallas would support gene-gene interaction as a major mechanism for the observed ethnic differences. It is possible that genetic interaction between PPAR-γ 12Ala with other polymorphisms involved in the regulation of insulin signaling may determine decreased phenotypic expression of the "protective" effect of the PPAR-γ 12Ala allele. The role of gene-gene interaction and also environment-gene interaction, including diet and exercise, should be systematically studied in the evaluation of the ethnic heterogeneity reported here. On this line, it is of interest that the average BMI was much lower in the South Asians with diabetes than in the Caucasians with diabetes. Contrary to the Caucasian population, little difference was found between diabetic and nondiabetic patients of South-Asian descent. This observation supports the view that excessive insulin resistance and susceptibility to type 2 diabetes in South Asians is likely the result of a particular genetic milieu that differs from that of Caucasians.

We conclude that despite the frequency of the Ala allele at the PPAR-γ Pro12Ala locus being high in individuals of South-Asian descent, this particular polymorphism does not appear to improve insulin sensitivity and/or decrease risk for type 2 diabetes in this ethnic group, as it does in Caucasians. If confirmed by larger studies, the hypothesis generated in this study may help us understand the susceptibility to insulin resistance and excessive risk for type 2 diabetes observed in South Asians.

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