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ORIGINAL RESEARCH

Type 2 diabetes in a central Indian population: association with PPARG2 P12IA allele but not ENPP1 K121Q

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Background: It is known that genetic and environmental factors may influence susceptibility to type 2 diabetes and its complications.

Objective: In the investigation reported here we selected the peroxisome proliferator-activated receptor (PPAR) G2 (rs1801282) and ENPP1, also called *PC-1* (rs1044498) gene polymorphisms to determine whether there is a genetic association between these, obesity, and type 2 diabetes. We also examined and environmental factors influencing type 2 diabetes.

Design and methods: The study was carried out on a central Indian population of 190 diabetics and 210 healthy controls. Anthropometric data were collected during sample collection. A genetic polymorphism study of PPARG2 and ENPP1 was undertaken using a polymerase chain reaction restriction fragment length polymorphism method and the observed genotype frequencies, allele frequencies, and carriage rates of the PPARG2 and ENPP1 polymorphisms were recorded.

Results: The patterns of genotype and allele distribution in both groups suggested a significant association between PPARG2 Pro12Ala major allele A carriage (AA carriage) and type 2 diabetes. Further, the results also show the protective effect of the minor allele G. Overall, we found that the distribution of ENPP1 K121Q genotypes was not significantly different between healthy controls and diabetic patients. Thus, ENPP1 polymorphism was not found to be associated with type 2 diabetes in a central Indian population. Body mass index was also found to be significantly higher in female diabetic patient group than in female healthy controls ($P = 0.0388$), while there was no significant difference in body mass index for males in the case group compared with in the control group.

Conclusion: Our study indicates that PPARG2 and obesity have a strong association with type 2 diabetes but ENPP1 polymorphism lacks any association.

Keywords: obesity, BMI, polymorphism, restriction fragment length polymorphism, peroxisome proliferator-activated receptors

Introduction

Diabetes is a metabolic disorder characterized by hyperglycemia and associated with abnormal lipid and protein metabolism. It has now become a global health problem and is the world's sixth leading cause of death. Although the causes of diabetes are not yet clear, many habits (lifestyle factors) as well as genetic susceptibility are now known to cause this disease.[1] Type 2 diabetes (T2D) is a complex metabolic disorder resulting from the interplay of genetic and environmental factors including lifestyle and dietary habits.[1]

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor subfamily of transcription factors. PPARs form heterodimers with retinoid X receptors and these heterodimers regulate transcription of various genes.

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PPARs have been shown to cause T2D with severe insulin resistance and thus it has been indicated that PPARG2 is essential for insulin action and glucose homeostasis.[2,3] A polymorphism in the 12 exon of the *PPARG2* gene has been found to be associated with decreased risk of diabetes, meaning that a minor allele has a protective effect. Yen et al identified a Pro12-to-ala (P12A) change in the *PPARG2* gene that may modify susceptibility to T2D mellitus and obesity.[2] A meta-analysis of 301 published genetic association studies covering 25 different population studies have reported association of PPARG2 with diabetes type 2 susceptibility.[3] Many case-control studies have been undertaken and have established the significant protective effect of the less common allele (minor allele) Ala.

ENPP1 (PC1) encodes for a transmembrane glycoprotein, which interacts with the α-subunit of the insulin receptor and thus inhibits subsequent insulin signaling.[4] A missense mutation at the 121 codon has been found to be significantly associated with increased diabetes risk and, in many studies, the mutant allele has been found to be associated with obesity and many other related metabolic disorders.[5]

K121Q predisposes to insulin resistance and related abnormalities because the 121Q variant binds the insulin receptor more strongly than the K121 variant.[5] A K121Q variant has been previously described in the *PC1* gene (rs1044498), and it was demonstrated that this variant was strongly associated with insulin resistance in 121 healthy non-obese, non-diabetic Caucasians in an Italian population.[6]

In the investigation reported here, we elected to study the association of ENPP1 and PPARG2 polymorphism and obesity with T2D.

Materials and methods

Study population

The study population consisted of 400 unrelated subjects and comprised 190 T2D patients and 210 ethnically matched healthy controls of Indo-European ethnicity. Cases included consecutive patients who attended the Department of Medicine at Shyam Shah Medical College, Rewa, India; Ayurveda Medical College, Rewa, India; Ranbaxy Pathology Regional Collection Centre, Rewa, India; and the District Hospital, Satna, India. T2D was diagnosed in accordance with World Health Organization criteria.[7] Pregnant women, those under the age of 18 years, and any patients with type 1 diabetes were excluded from the study.

Anthropometry

Height and weight were measured with subjects wearing light clothes and without shoes in a standing position, as per standard guidelines. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured with subjects in a standing position, midway between iliac crest and lower costal margin, and hip circumference was measured at its maximum. Waist-to-hip ratio (WHR) was calculated using waist and hip circumferences. Systolic and diastolic blood pressures were measured twice in the right arm – with subjects seated and having rested for at least 5 minutes beforehand – using a standard sphygmomanometer. The average of the two readings was used.

Biochemical analysis

The biochemical parameters related to T2D were estimated for both case and control subjects. Measurement of serum levels of total cholesterol, triglycerides, glycated hemoglobin (HbA_{1c}), high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, urea, uric acid, C-reactive protein, and creatinine were measured using spectrophotometric methods with a COBAS Integra® 400 Plus (Roche Diagnostics, Mannheim, Germany) automated clinical chemistry analyzer.

Blood collection and plasma/serum separation

Venous blood samples were obtained from the subjects after fasting for 12 hours overnight and collected in vacutainers with and without (biochemical profiling samples) appropriate anticoagulants. The plasma and serum from the respective vacutainers were immediately separated by centrifuging the tubes at 1000 rpm for 10 minutes at 4°C.

DNA isolation

Genomic DNA was extracted from whole blood using a modified version of the salting-out procedure described by Miller et al.[8]

Detection of ENPP1 and PPARG2 single-nucleotide polymorphisms using polymerase chain reaction (PCR) restriction fragment length polymorphism

The P12A (substitution of A base to C at 12 exon) polymorphism of the *PPARG2* gene was amplified by PCR. The oligonucleotide sequences (primers; see following) were designed to amplify the wild-type gene but lacked a restriction site for the BstU1 enzyme; however, as the alanine allele contains a restriction site, it cleaved to the 227 and 43 base pair (bp) fragments.[9]

Forward primer: 5′-GCCAATTCAAGCCCAGTC-3′.

Reverse primer: 5′GATATGTTTGCAGACAGTGTATCAGTGAAGGAATCGCTTTCCG3′.

The K121Q (substitution of A base to C at 121 codon) polymorphism of the *ENPP1* gene was amplified by PCR. The oligonucleotide sequences (primers; see following) were designed to amplify the wild-type gene but lacked a restriction site for the AvaII enzyme; however, the mutant allele contains a restriction site.[10]

Forward primer: 5′-GCAATTCTGTGTTCACTTTGGA-3′.

Reverse primer: 5′-GAGCACCTGACCTTGACACA-3′.

PCR mix preparation

The PCR was carried out at a final volume of 25 µL, which contained 100 ng of genomic DNA (4–5 µL), 2.5 µL of 10X Taq polymerase buffer (10 mM Tris HCl pH 8.8, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.005% Tween® 20, 0.005% Tergitol-type NP-40; final concentration 1×; Genetix Biotech Asia, New Delhi, India), 1 µL of 10 mM deoxynucleotide triphosphates (Bangalore Genei, Bangalore, India), 1 µL of 25 pmol/µL of forward and reverse primers specific for respective genes and 1 µL of 1 U/µL RedTaq® DNA polymerase (Bangalore Genei).

PCR thermal program

For ENPP1, after an initial denaturation of 5 minutes at 94°C, the samples were subjected to 35 cycles at 94°C for 1 minute, at 55°C for 40 seconds, and 72°C for 40 seconds, with a final extension of 10 minutes at 72°C in a thermal cycler. A 100 bp ladder with amplified product was run under 1% agarose gel electrophoresis. A 238 bp product was generated after PCR.

For PPARG2, after an initial denaturation of 5 minutes at 95°C, the samples were subjected to 35 cycles at 95°C for 1 minute, at 58°C for 45 seconds, and 72°C for 45 seconds, with a final extension of 10 minutes at 72°C in a thermal cycler. A 100 bp ladder with amplified product was run under 2.5% agarose gel electrophoresis. A 270 bp product was generated after PCR.

Restriction digestion of ENPP1 and PPARG2

The digestion of amplicons containing the homozygous genotype (QQ) resulted in two bands of 148 and 90 bp. The heterozygous genotype (KQ) was represented by three fragments of 238, 148, and 90 bp. Electrophoresis using 2.5% agarose gels was used to analyze the samples to determine the genotype pattern of the genes (Figure 1). The amplified product size of 270 bp was digested by the specific restriction enzyme, BstU1, for 16 hours at 37°C. The wild-type genotype was not digested, whereas the mutated homozygous genotype was cut as a doublet of 227 and 43 bp. The heterozygous genotype (KQ) was represented by three fragments of 270, 227, and 43 bp. Samples were analyzed by electrophoresis using 2.5% agarose gels to determine the genotype pattern of the gene.

The 270 bp PPARG2 amplified product was digested by the specific restriction enzyme, BstU1. The wild-type genotype was not digested, whereas the mutated homozygous genotype was cut as a doublet of 227 and 43 bp. The heterozygous genotype (KQ) was represented by three fragments of 270, 227, and 43 bp. Samples were analyzed by electrophoresis using 2.5% agarose gels to determine the genotype pattern of the gene. The results were documented by digital camera and further recorded by gel documentation system (Figure 2).

Statistical analysis

Biochemical tests were performed on the blood samples to determine clinical parameters and the findings were tabulated. Statistical analysis was performed using Student's *t*-test and the *P* values obtained suggested the level of significant change. Genotype, allele frequency, and carriage rate were analyzed using the chi-square test and Fisher's exact test, with *P* values, odds ratios (ORs), and confidence intervals recorded. All analyses were undertaken using Prism software (v 5.0; GraphPad, San Diego, CA, USA).

Results

Anthropometric results

The descriptive data and comparison of anthropometric parameters of diabetic patients versus controls are presented

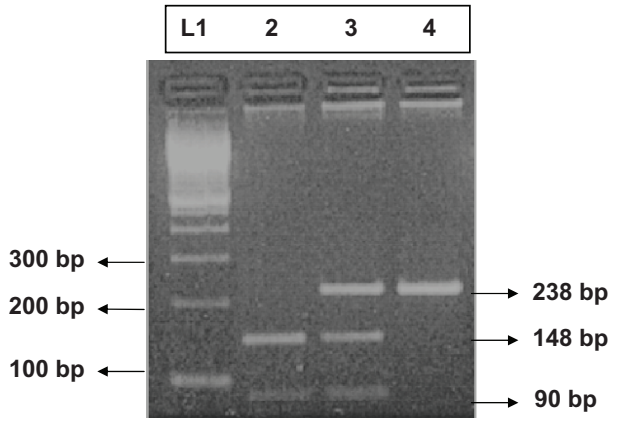

Figure 1 Representative gel electrophoresis picture of ENPP1 K121Q polymorphism.
Notes: Lane 1: 100 bp molecular marker; Lane 2: homozygous mutant genotype; Lane 3: heterozygous genotype; Lane 4: wild-type genotype.

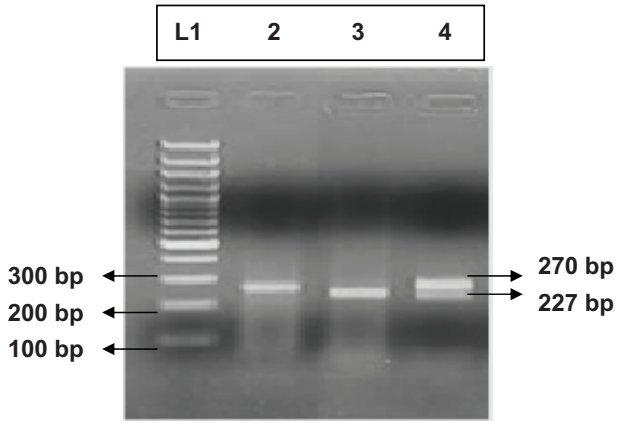

Figure 2 Representative gel electrophoresis picture of PPARG2 Pro12Ala polymorphism.
Notes: The expected product sizes were: normal homozygote, 270 bp; Pro12Ala homozygote, 227 and 43 bp; and Pro12Ala heterozygote, 270, 227, and 43 bp, respectively. Lane 1: marker; Lane 2: Pro homozygote; Lane 3: Ala homozygote; Lane 4: heterozygote. The 43 bp fragments are not shown.

in Table 1. As expected, both female and male diabetic patients weighed markedly more than healthy controls ($P = 0.0024$ and $P = 0.0157$, respectively) and women in this group had higher BMIs than women in the control group ($P = 0.0388$). Moreover, female diabetic subjects had a greater waist circumference than controls ($P < 0.0001$) and both female and male diabetics had a greater WHR ($P < 0.0001$ and $P = 0.0147$, respectively) than control subjects. There were no other significant anthropometric differences between the groups (Table 1).

Table 1 Comparison of anthropometric parameters of diabetic patients and controls

Characteristics	Cases	Controls	*P*
n (men/women)	190 (126/64)	210 (114/96)	
Age (years)	52.5 ± 12.5	53.0 ± 14.2	0.7100
Height (m)	160.50 ± 13.40	162.2 ± 12.000	0.1815
Weight (kg)			
Women	62.5 ± 5.70	60 ± 4.50	0.0024**
Men	68 ± 5.60	66.0 ± 7.1	0.0157*
Body mass index (kg/m^2)			
Women	26.4 ± 3.1	25.1 ± 4.3	0.0388*
Men	24.6 ± 4.7	24.1 ± 5.1	0.4301
Waist circumference (cm)			
Women	92.5 ± 6.2	84.5 ± 6.7	<0.0001***
Men	90.0 ± 7.0	89.0 ± 6.0	0.2383
Hip (cm)			
Women	95.0 ± 5.0	96.5 ± 6.0	0.178
Men	91.0 ± 4.0	90.5 ± 5.5	0.4183
Waist–hip ratio			
Women	0.97 ± 0.05	0.88 ± 0.08	<0.0001***
Men	0.99 ± 0.05	1.00 ± 0.03	0.0147*

Notes: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Biochemical and clinical findings

The descriptive data and comparison of biochemical parameters for diabetic patients versus controls are presented in Table 2. As expected, the diabetic patients had markedly higher levels of fasting plasma glucose ($P < 0.0001$), HbA_{1c} ($P < 0.0001$), and post-prandial glucose ($P < 0.0001$) compared with those of controls. Nominal differences were also observed for low-density lipoprotein cholesterol ($P = 0.0462$), triglycerides ($P = 0.0024$), and systolic blood pressure ($P = 0.0447$). Creatinine values, blood urea levels, high-density lipoprotein cholesterol levels, and diastolic pressure were not significantly different between the two groups. All clinical test results are tabulated in Table 2.

Detection of genetic polymorphism in PPARG2

The genotype distribution of PPARG2 (rs1801282) was strongly under the Hardy–Weinberg equilibrium ($\chi^2 = 0.28$ and 2.79 for cases and controls, respectively). The observed genotype frequencies, allele frequencies, and carriage rates for PPARG2 P12A polymorphism are tabulated in Table 3. The overall distribution of PPARG2 P12A genotypes was significantly different between the two groups ($\chi^2 = 7.253$, $P = 0.0266$). More healthy subjects had the less common (mutant) genotype GG than diabetic subjects (3.9% vs 0.52%). Similarly, the common (wild-type) genotype AA was present at a significantly lower frequency in the control group compared with in the case group (73.3% vs 82.11%). An OR of 0.1336 for less common (rare) genotype GG indicates a strong protective effect of this mutant-type genotype in our population, whereas an OR of 1.668 in the diabetic patients

Table 2 Comparison of biochemical and clinical findings between diabetic patients and controls

Characteristics	Cases	Controls	*P*-value
FPG (mg/dL)	143.3 ± 17.6	92.44 ± 7.5	<0.0001***
Post-prandial glucose (mg/dL)	211.7 ± 44.7	108.5 ± 12.1	<0.0001***
HbA_{1c} (%)	6.9 ± 0.8	5.3 ± 0.6	<0.0001***
HDL-C (mmol/L)	112.2 ± 14.8	109.8 ± 11.6	0.0705
LDL-C (mg/dL)	42.1 ± 4.3	41.3 ± 3.7	0.0462*
TG (mg/dL)	131.1 ± 13.2	126.9 ± 14.2	0.0024**
Systolic BP (mmHg)	130.20 ± 8.1	128.8 ± 5.7	0.0447*
Diastolic BP (mmHg)	87.1 ± 5.8	86.5 ± 6.0	0.3109
Blood urea (mg/dL)	9.1 ± 1.6	8.8 ± 1.8	0.0801
Creatinine (mg/dL)	1.08 ± 0.14	1.06 ± 0.10	0.0986

Notes: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Values expressed as mean ± SD are taken at one point of time during treatment and will not indicate a life long trend of the concentrations in the given patients.
Abbreviations: BP, blood pressure; FPG, fasting plasma glucose; HbA_{1c}, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

group indicates a positive association of the common type genotype AA with T2D. There was an OR of 0.5643 for the minor allele G, indicating its protective association. The carriage rate of allele G was slightly higher in the control group than in the case group, whereas the carriage rate of allele A was higher in the case group than in the control group ($\chi^2 = 3.281$, $P = 0.0701$), but the values were not significant, although the carriage of the G allele had an OR of 0.6489, indicating the protective role of this less common allele in our population. The pattern of genotype and allele distribution in the case and control groups suggests a significant association of PPARG2 P12A genotype AA and major allele A frequency ($P = 0.0415$; OR = 1.668, 95% confidence interval [CI] 1.031–2.699 and $P = 0.01$; OR = 1.772, 95% CI 1.144–2.746, respectively) with T2D and also shows the protective effect of the PPARG2 P12A (rs1801282) G allele frequency (OR = 0.5643, 95% CI 0.3642–0.8744) (Table 3).

Detection of genetic polymorphism in ENPP1

The distribution of the ENPP1 polymorphism (rs1044498) was consistent with the Hardy–Weinberg equilibrium in diabetic patients ($\chi^2 = 3.26$) as well as in healthy controls ($\chi^2 = 2.75$). No significant level of change was seen in the distribution of ENPP1 K121Q genotypes in the healthy control group as compared with the diabetic patient group, although there was a slightly greater frequency of the common KK genotype in the control group compared with in the case group (69.5% vs 63.7%). The QQ genotype was present at a low frequency in both the case and control group (1.58% and 0.96%, respectively). The overall genotype was statistically nonsignificant. The major allele K was found at a slightly lower frequency in the case group (63.68%) than in the control group (69.52%), whereas allele Q was present at a slightly higher frequency in the case group compared with the control group (18.94% vs 15.71%), but the difference was nominal and not statistically significant ($\chi^2 = 1.461$, $P = 0.2268$). An OR of 1.254 for the rare allele Q shows the moderate effect of the minor allele in T2D. The carriage rate of allele Q was slightly higher in the diabetic group as compared with the healthy control group (36.32% vs 30.48%), whereas the carriage rate of allele K was approximately similar in both groups, with no significant difference observed. The OR of minor allele Q carriage was 1.199, which does not suggest any association of Q allele carriage with disease susceptibility (Table 4).

Discussion

T2D is a well-established multifactorial disorder that is strongly associated with genetic as well as lifestyle and environmental factors. Both genetic and environmental factors are necessary for T2D. Although the etiology of T2D is not very clear, many studies and family histories suggest the transmission of T2D in families; this, as well as the 100% susceptibility of diabetes in twins, strongly supports a genetic basis for the disease.[1]

BMI is a tool used to document obesity. In our investigation, we found that BMI was significantly higher in diabetic females, while no such finding was observed in males. The BMI averages of diabetic females was 26.4 as compared with 25.1 in healthy females ($P = 0.0388$). WHR was also higher in both males and females in the case group. A sedentary lifestyle is key to the rise in the prevalence of both obesity and diabetes.[11] In the past decade, we have witnessed epidemics of both T2D and obesity. The prevalence of T2D has increased by 33% in the USA, with 62% of Americans classified as obese (BMI $\geq$ 30 kg/m^2) or overweight (BMI 25.0–29.9 kg/m^2).[12] The recent increase in the prevalence of obesity is closely paralleled by the increase in the prevalence of diabetes. Indeed, this new unprecedented phenomenon has been referred to as "diabesity." There is a strong relationship

Table 3 Fisher exact test values for PPARG2 P12A polymorphism

PPARG2 genotype	Cases (N = 190), n (%)	Controls (N = 210), n (%)	*P*	Odds ratio (95% CI)
AA	156.0 (82.11)	154.0 (73.30)	0.0415*	1.668 (1.031–2.699)
AG	33.0 (17.37)	48.0 (22.90)	0.2127	0.7094 (0.4326–1.1630)
GG	1.0 (0.52)	8.0 (3.90)	0.0390*	0.1336 (0.01654–1.07900)
Alleles				
A	345 (90.79)	356 (84.50)	0.01**	1.772 (1.144–2.746)
G	35 (9.21)	64 (15.50)		0.5643 (0.3642–0.8744)
Carriage rate				
A	189 (99.40)	202 (96.20)	0.0789	1.541 (0.963–2.466)
G	34 (17.89)	56 (26.70)		0.6489 (0.4055–1.0380)

Notes: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. "%" indicates genotype allele frequency and carriage rate expressed as a percentage.

Table 4 Fisher exact test values for ENPP1 polymorphism

ENPP1 genotype	Cases (N = 190), n (%)	Controls (N = 210), n (%)	*P*	Odds ratio (95% CI)
KK	121 (63.68)	146 (69.52)	0.2427	0.7687 (0.5066–1.166)
KQ	66 (34.74)	62 (29.52)	0.2841	1.271 (0.8340–1.936)
QQ	3 (1.58)	2 (0.96)	0.6717	1.668 (0.2757–10.10)
Allele				
K	308 (81.05)	354 (84.29)	0.2608	0.7976 (0.5524–1.152)
Q	72 (18.94)	66 (15.71)		1.254 (0.8684–1.810)
Carriage rate				
K	187 (98.42)	208 (99.05)	0.3689	0.8339 (0.5627–1.236)
Q	69 (36.32)	64 (30.48)		1.199 (0.8091–1.777)

Notes: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. "%" indicates genotype allele frequency and carriage rate expressed as a percentage.

between obesity and the risk for diabetes.[12] In India, our data also suggest that obesity and higher BMI can be important factors affecting susceptibility to T2D.

The *PPARG2* gene, which is abundantly expressed in adipose tissue, has several variants. One of the most common variants – with a minor allele frequency of 10% in Caucasians – is the P12A substitution at codon 12 in PPARG2. This polymorphism has been shown to be associated with reduced ability to trans-activate responsive promoters and thus with lower PPARG2 transcriptional activity.[13] The importance of PPARG2 in lipid, glucose, and energy metabolism is well established. Since *PPARG2* promotes adipocyte differentiation, it is an attractive candidate gene for states of altered triglyceride storage, such as obesity or conditions associated with underweight. Further, as the Pro12 allele is present in at least 80% of humans, the percentage of the population at risk of T2D associated with this polymorphism is as high as 25%.[14] More consistently, the P12A polymorphism has been associated with a lower risk of T2D in a meta-analysis of genome-wide association studies.[15] This gene has been confirmed as T2D susceptibility factor and is one of almost 20 T2D susceptibility loci that have been identified over the last few years.[16]

In our investigation, we found that the minor allele G appeared with a significantly lower frequency in diabetic subjects compared with in healthy subjects, whereas the major allele A was present at a significantly higher frequency in diabetic subjects. These finding suggest that the minor allele G may have a protective effect against the pathophysiology of T2D. Despite our study having a small sample size, our results revealed that the PPARG2 Pro12Ala polymorphism could be protective against T2D. Our study supports various prior studies in different populations.[17–19] For example, the protective effect of the less common allele Ala occurred at a lower frequency in patients than in controls in a Punjabi population in India.[17] In our population, Ala occurred at a little higher frequency than in a Punjabi Sikh population,[17] thus is more protective in our population. The results of other studies, such as one in a Japanese population[18] and another in a Finnish population,[19] are consistent with our findings. In a study with a large Caucasian sample, an OR of 0.81 for the Ala allele revealed a significant protective association of this allele.[20] Another meta-analysis of eight studies on Japanese populations reported an identical OR of 0.81 for the same allele.[9] When only the largest studies (with >500 cases) were considered, the association remained stable worldwide with an OR 0.84, 95% CI of 0.79–0.90,[16] and an OR of 0.65 for the less common Ala allele in an East Asian population.[21] Our results are consistent with these previous studies and demonstrate the association of the major allele A of PPARG2 P12A with T2D, results are not consistent in all other studies. Several studies have suggested that the Ala allele is associated with a higher risk of diabetes, including a study of a Canadian Oji-Cree population,[22] while a south Indian study has indicated that the Ala allele does not have any protective effects.[23] North Indian and south Indian populations have shown different results for the association of PPARG2 with T2D, but these findings may be due to the diversity of these two Indian populations and their different origins.[24] Our results strongly support the results obtained for a north Indian population,[17] indicating the similarity between the north and Vindhyan-region (central Indian) populations. In the present study, we found that the Ala allele was associated with a significant protective effect and our findings are in line with most worldwide population studies, including those of Caucasians, north Indians, and Japanese.

The *ENPP1* gene is known as a susceptible gene because of its affinity in binding with the α-subunit of the insulin receptor and may inhibit tyrosine kinase activity, which is essential for glucose metabolism.[4] Metformin, a biguanide oral antidiabetic agent, has been shown to affect insulin resistance by decreasing the enzymatic activity of

over-expressed PC-1 molecules in obese type 2 diabetics. In functional studies, the 121Q allele variant binds more strongly to the insulin receptor and inhibits its protein kinase activity more effectively than the K variant.[5]

The role of the genetic polymorphism of the *ENPP1* gene in susceptibility to T2D has been widely studied but the results are inconsistent across different populations. Many case-control studies have concluded the possible role of the *ENPP1* gene in genetic susceptibility to T2D, while others have shown that the *ENPP1* gene has a possible role in obesity.[27,37] In addition, other studies have revealed that ENPP1 is neither associated with diabetes nor with obesity. Because of the multifactorial nature of the disease, no single gene can be attributed to causing T2D by itself.[28–35]

Our findings show that the genotype pattern and allele frequency of ENPP1 K121Q was not significantly different between case and healthy controls. With an OR of 0.7687, the KK genotype may have some protective role, but neither KK nor other genotypes were significantly different, although the higher allele frequency of the mutant 121Q allele in the diabetic population was observed but the difference was not significant. The frequency of the homozygous mutant 121Q allele was very low in cases as well as in controls. Overall, the allele K was found at a slightly lower frequency in the disease group than in the healthy control group, whereas allele Q was present at a slightly higher frequency in the disease group (18.94% in patients and 15.71% in controls) but the difference was nominal. An OR of 1.254 for the rare allele Q shows little or no effect of the mutant allele in diabetes susceptibility. The carriage rate of allele Q was slightly higher in the diabetic group than in the healthy control group (36.32% vs 30.48%). Previous studies have reported the frequency of 121Q allele carriers in ethnic Chinese to be 18.8%; in Caucasians, 23.2%–36.4%; in South Asian Indians (the Chennai population living in Chennai and Dallas, USA), 27.5%–34.2%; in African-Americans, 67.0%; and in Dominicans, 78.4%. Further, a significant association between the 121Q allele and T2D has been shown in Caucasian populations in the USA and Finland.[10,25,26] In addition to diabetes, the PC-1 Q121 allele has also recently been reported to influence the risk of obesity.[26] Many association studies have been undertaken, but the results have been different in different ethnicities and populations.[25–34]

Other research on Caucasian populations in Sweden and Denmark has shown that the ENPP1 polymorphism is not associated with susceptibility to T2D,[28,29] and this same finding has also been reported in Chinese[30] and North Indian Sikh populations.[31] Moreover, a Spanish cohort study has shown that the ENPP1 K121Q polymorphism is not significantly associated with T2D.[32] Our findings are consistent and in agreement with those reported for work on Caucasian and African-American,[33] Oji-Cree,[34] and Mexican[35] populations. It is possible that the susceptibility induced by the *ENPP1 K121Q* gene polymorphism is modulated by interactions with other ethnic specific genetic or environmental factors.

This study of a central Indian population has shown that the ENPP1 K121Q polymorphism is not associated with susceptibility to T2D, and our results are similar to previous studies of north Indian, Caucasian, Japanese, and Chinese populations.[28–35] The frequency of heterozygous and homozygous minor Q alleles found in our study was closer to that found in Caucasians but lower than that found in a Sikh population.[28,29,31] Although the Sikh population study shows a high level of heterozygous alleles, no statistical difference was seen. Other studies of a south Indian Chennai population and some Caucasian populations have shown that the Q allele could be a risk factor and, in our study, this allele was present at a higher percentage in heterozygous form in diabetes patients, but its effect in or association with T2D was not significantly established. Studies from different population are not similar and many more genes are involved in the pathophysiology of T2D. These discordant results suggest that differences in either the genetic and/or environmental backgrounds of the subjects studied or the recruitment procedures of the populations investigated are important factors in analysis. In the present study, we found that insulin resistance in T2D could be due to PPARG2 polymorphism. ENPP1 could also influence diabetes in other populations but we did not find any such role for the ENPP1 polymorphism in the central Indian population we observed.

An improved understanding of pathophysiology achieved through genetic discovery provides new opportunities for treatment, diagnosis, and monitoring. Studies of risk variants for T2D in healthy populations have shown that most variants act through perturbation of insulin secretion rather than insulin action, establishing inherited abnormalities of beta-cell function or mass (or both) as critical components of the progression to T2D.[36]

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Disclosure

The authors declare no conflicts of interest in this work.

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