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ORIGINAL ARTICLE

Association of the *PPARGC1A* Gene Polymorphism With Diabetic Nephropathy in an Asian Indian Population (CURES-41)

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Abstract

Background: The aim of this study was to evaluate the association of polymorphisms of the peroxisome proliferator-activated receptor gamma (*PPARG*) gene and peroxisome proliferators-activated receptor gamma co-activator 1 alpha (*PPARGC1A*) gene with diabetic nephropathy (DN) in Asian Indians.
Methods: Six common polymorphisms, 3 of the *PPARG* gene [−1279G/A, Pro12Ala, and His478His (C/T)] and 3 of the *PPARGC1A* gene (Thr394Thr, Gly482Ser, and +A2962G) were studied in 571 normal glucose-tolerant (NGT) subjects, 255 type 2 diabetic (T2D) subjects without nephropathy, and 141 DN subjects. Genotypes were determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and direct sequencing. Logistic regression analysis was performed to assess the covariables associated with DN.
Results: Among the 6 polymorphisms examined, only the Gly482Ser of the *PPARGC1A* gene was significantly associated with DN. The genotype frequency of Ser/Ser genotype of the *PPARGC1A* gene was 8.8% (50/571) in NGT subjects, 7.8% (20/255) in T2D subjects, and 29.8% (42/141) in DN subjects. The odds ratios (ORs) for DN for the susceptible Gly/Ser and Ser/Ser genotype after adjusting for age, sex, body mass index, and duration of diabetes were 2.14 [95% confidence interval (CI), 1.23–3.72; $P = 0.007$] and 8.01 (95% CI, 3.89–16.47; $P < 0.001$), respectively. The unadjusted OR for DN for the XA genotype of the Thr394Thr polymorphism was 1.87 (95% CI, 1.20–2.92; $P = 0.006$) compared to T2D subjects. However, the significance was lost ($P = 0.061$) when adjusted for age, sex, BMI, and duration of diabetes. The +A2962G of *PPARGC1A* and the 3 polymorphisms of *PPARG* were not associated with DN.
Conclusion: The Gly482Ser polymorphism of the *PPARGC1A* gene is associated with DN in Asian Indians.

Introduction

Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) worldwide, and it is estimated that 20% of type 2 diabetic (T2D) patients reach ESRD during their lifetime.[1] The pathogenesis of diabetic nephropathy (DN) has many genetic and environmental factors contributing to its development and progression. Epidemiological studies have clearly established that only a subgroup of individuals with diabetes is at risk of DN.[2] Several genes have been identified that may increase the risk of DN in Europeans[3]; however, there is a need to look at DN susceptibility genes in non-European populations.

The risk of developing DN has been linked to different chromosomes, including chromosome 3,[4] to which the peroxisome proliferators-activated receptor (*PPAR*) gene has been mapped, particularly to the PPAR gamma (PPARG) nuclear receptor, which is mainly expressed in adipose tissue. It is also expressed in renal glomeruli[5] and thus could play a significant role in DN. Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PPARGC1A), a co-activator of nuclear receptors, was discovered as a molecular switch that turns on several key components of the adaptive thermogenic program in brown fat, including the stimulation of fuel intake, mitochondrial fatty acid oxidation, and heat

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production.[6] The *PPARGC1A* gene encodes a coactivator that interacts with PPARG.[7] *PPARGC1A* is strongly expressed in kidney, brown adipose tissue (BAT), heart, skeletal muscle, and brain, all of which are highly oxidative tissues.[6]

Recently, we demonstrated that a silent *PPARGC1A* gene polymorphism (Thr394Thr) is associated with T2D mellitus in Asian Indians,[8] but the Pro12Ala of the *PPARG* gene, which is protective against diabetes in Europeans, was not protective in Asian Indians.[9] We have now extended the study to investigate the possible association of *PPARG* and *PPARGC1A* gene variants with DN. The variants selected for the study were −1279G/A in the promoter, Pro12Ala at exon B, and the silent His478His at exon 6 of the *PPARG* gene and the Thr394Thr at exon 8, Gly482Ser at exon 8, and +A2962G in the 3′-untranslated region (3′-UTR) of the *PPARGC1A* gene. To our knowledge, this is the first report on the association of *PPARGC1A* gene polymorphisms with DN, particularly in Asian Indians.

Subjects and Methods

Subjects

A total of 826 subjects, 571 normal glucose tolerant (NGT) control group subjects and 255 T2D subjects, were selected from phase 2 and phase 3 of the Chennai Urban Rural Epidemiology Study (CURES), the details of which are published elsewhere.[10] Briefly, 26,001 adult subjects (>20 years of age) were recruited in phase 1 of CURES using a systematic random sampling method covering the whole of Chennai (formerly Madras) city in Southern India. In phase 2 of the CURES, all self-reported diabetic subjects ($n = 1529$) were invited to our center for detailed studies, of whom 255 randomly selected individuals without microalbuminuria or proteinuria were included in this study. The control group of NGT subjects ($n = 571$) was randomly selected from phase 3 of the CURES, where every tenth subject from phase 1 (excluding those with self-reported diabetes) was invited to undergo an oral glucose tolerance test. All NGT subjects had fasting plasma glucose of less than 100 mg/dL and a 2-h plasma glucose value of 140 mg/dL or less.[11] Subjects having a 2-h plasma glucose value greater than or equal to 200 mg/dL were considered to be T2D subjects.[11] Subjects with DN ($n = 141$) were selected from Dr. Mohan's Diabetes Specialities Centre, a tertiary centre for diabetes in Chennai. In all subjects, albumin excretion measured by immunoturbidometric assay was ≥300 μg/mg of creatinine in at least 2 out of 3 fasting urine collections over a period of 3 months.[12]

Anthropometric measurements, including weight, height, and waist circumference, were obtained using standardized techniques. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters (kg/m^2). The systolic (SBP) and diastolic blood pressures (DBP) (mmHg) were measured using a mercury sphygmomanometer.

Biochemical analyses were done on a Hitachi-912 Autoanalyzer (Hitachi, Germany) using kits supplied by Roche Diagnostics (Mannheim, Germany). Fasting plasma glucose (GOD-POD method), serum cholesterol (CHOD-PAP method), and serum triglycerides (GPO-PAP method) were measured. Glycosylated hemoglobin (HbA1c) was estimated by high-performance liquid chromatography using the variant machine (Bio-Rad, Hercules, CA). Serum creatinine was measured using the Jaffe method (coefficient of variation, 5.7%). Macroalbumin concentration was measured in a fasting urine sample using an immunoturbidometric assay (Hitachi 902 autoanalyser; Roche Diagnostics), as previously reported.[2] The mean inter- and intraassay coefficients of variation were 3.5 and 4.2%, respectively.

Genotyping

Ethylenediaminetetraacetic acid (EDTA) anticoagulated venous blood samples were collected from all study subjects, and the genomic DNA was isolated from whole blood by proteinase K digestion followed by phenol–chloroform extraction.[13] The 6 polymorphisms were genotyped by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and confirmed by direct sequencing. The sequences of the primers to genotype were: 1279 G→A polymorphism of the *PPARG* gene were 5′-TGCCATCGTGTCTGG ATTAC-3′ and 5′-CCTGTCAATCATGGTGCAAG-3′.[14] The sequences of primers to genotype the Pro12Ala polymorphism of *PPARG* were: 5′-GCCAATTCAAGCCCAGTC-3′ and 5′-GATATGTTTGCAGACAGTGTATCAGTGAAGGAATCG CTTTCCG-3′.[9] The primer sequences used to genotype the His478His polymorphism of *PPARG* were 5′-TGTG AAGCCCATTGAAGACA-3′ and 5′-GAGCGGGTGAA GACTCATGT-3′.[15] The PCR products were digested overnight with 3 Units of *Nla*III for the −1279 G→A polymorphism, 2 Units of *Bst*U1 for the Pro12Ala polymorphism, and 2 Units of *Nla*III for the His478His polymorphism. The primers used to genotype the Thr384Thr polymorphism of *PPARGC1A* were 5′-GCCAGTCAATTAATTCCAAACC-3′ and 5′-TTGGAGCTGTTTTCTTGTGC-3′.[8] The Gly482Ser polymorphism of *PPARGC1A* was genotyped using primers 5′-CAAGTCCTCAGTCCTCAC-3′ and 5′-GGGGTCTTTGAG AAAATAAGG-3′, whereas those used for the +A2962G polymorphism were 5′-CAATAACAACAATGGTTTACATGA-3′ and 5′-CGAACAT TTTGAAGTTCTAGG TTTTACG-3′.[8] RFLP was detected after overnight digestion of the PCR products with 2 Units of *Msp*I for the Thr394Thr polymorphism, 2 Units of *Hpa*II for the Gly482Ser polymorphism, and 2 Units of *Mlu*I for the +A2962G polymorphism of the *PPARGC1A* gene. The RFLP products were resolved electrophoretically on a 3% agarose gel. The assays were performed by a technician who was blinded to the phenotype. To assure that the genotyping was of adequate quality, we performed random duplicates in 20% of the samples and found 100% concordance in genotyping. The overall call rate for the genotyping was >96%. To confirm the DNA sequence, direct sequencing was performed by the Big Dye Terminator method using the ABI Prism 310 Genetic Analyzer (Applied Biosystems).

Statistical analysis

Statistical Package for Social Sciences, Windows version 10.0 (SPSS, Chicago, IL), was used for statistical analysis. The effects of the 6 polymorphisms on quantitative and categorical variables were analyzed. Agreement with Hardy–Weinberg equilibrium (HWE) expectations was tested using a chi-squared goodness-of-fit test. The chi-squared test was used to compare the proportions of genotypes or alleles. One-way analysis of variance (ANOVA) was used to compare genotype class for continuous variables. Data for

continuous variables were expressed as mean ± standard deviation (SD). Logistic regression analysis with and without adjustment for age, sex, BMI, and diabetes duration was performed using DN as the dependent variable and the genotypes as the independent variable. *P* values less than 0.05 were considered statistically significant.

Results

Study subjects

The clinical and biochemical characteristics of the study subjects are shown in Table 1. Subjects with DN were significantly older (57.1 ± 11.4 years) compared to those with NGT (43.3 ± 13.6 years) and T2D (51.8 ± 11.4 years, $P < 0.001$). Age and sex-adjusted mean ± SD values of BMI, blood pressure, fasting plasma glucose, serum triglycerides, HbA1c, and serum creatinine were significantly higher in the DN subjects ($P < 0.0001$).

PPARG gene polymorphisms

The association of the *PPARG* gene polymorphisms with T2D and DN risk was examined. Genotype frequency distributions of the 3 polymorphisms did not deviate from HWE among the study participants. None of the polymorphisms of the *PPARG* gene [−1279G/A, Pro12Ala, and His478His] were associated with T2D or with DN.

PPARGC1A gene polymorphisms

Thr394Thr polymorphism of the PPARGC1A gene. The minor ‘A’ allele frequency of the Thr394Thr polymorphism of the *PPARGC1A* gene was 8% in NGT subjects, 12% in T2D subjects, and 19% in DN subjects and showed a significant association with T2D ($P = 0.01$) and with DN ($P = 0.032$ vs. T2D and $P < 0.001$ vs. NGT group) (Table 2). The Thr394Thr genotype was also associated with T2D ($P = 0.001$) and with DN ($P = 0.02$ vs. T2D and $P < 0.001$ vs. NGT group). The unadjusted odds ratio (OR) for DN of the XA variant of Thr394Thr of the *PPARGC1A* gene was 1.87 [95% confidence interval (CI), 1.20–2.92; $P = 0.006$] compared with T2D subjects. However, the significance was lost ($P = 0.061$) when adjusted for potential confounders such as age, sex, BMI, and diabetes duration. Compared to the NGT subjects, the OR was 3.49 (95% CI, 2.31–5.27; $P < 0.001$), which remained significant even after adjusting for age, sex, and BMI (OR_{DN}, 2.24; 95% CI, 1.40–3.56; $P = 0.001$) (Table 3).

Gly482Ser polymorphism of the PPARGC1A gene. The frequency distribution of the Gly/Ser genotype of the Gly482Ser polymorphism of the *PPARGC1A* gene was significantly higher in DN subjects than T2D and NGT subjects ($P < 0.001$) (Table 2). The minor Ser allele frequency was also significantly higher in DN (54%) when compared to T2D (26%) and NGT (28%) ($P < 0.001$) subjects. However, there was no association of the Gly/Ser genotype with T2D.

The unadjusted ORs for DN for the Gly/Ser genotype and Ser/Ser genotypes of the Gly482Ser polymorphism were 3.18 (95% CI, 1.93–5.23; $P < 0.001$) and 9.42 (95% CI, 4.87–18.21; $P < 0.001$), respectively, when compared to T2D subjects. Even after adjusting for age, sex, BMI, and diabetes duration, there was a significant association of the Gly/Ser and Ser/Ser genotypes with DN (OR_{DN}, 2.14; 95% CI, 1.23–3.72; $P = 0.007$; and OR_{DN}, 8.01; 95% CI, 3.89–16.47; $P < 0.001$; respectively) in comparison with T2D subjects. The unadjusted OR of the Gly/Ser and Ser/Ser genotypes for DN when compared to NGT subjects was 2.98 (95% CI, 1.88–4.71; $P < 0.001$) and 8.13 (95% CI, 4.68–14.12; $P < 0.001$), respectively. After adjusting for age, sex, and BMI, the respective odds ratios were: OR_{DN} 2.360; 95% CI, 1.42–3.91; $P = 0.001$; and OR_{DN} 5.97; 95% CI, 3.22–11.05; $P < 0.001$ (Table 3).

Table 4 compares the genotype and minor allele frequencies in subjects with NGT and T2D with and without various microangiopathies namely, nephropathy, retinopathy, and neuropathy. It can be seen that there is a significant

Table 1. Clinical and Biochemical Characteristics of the Study Subjects

	NGT subjects (n = 571)	*T2D subjects (n = 255)*	*DN Subjects (n = 141)*	*ANOVA P value*
Men/women	219/352	130/125	79/62	<0.001
Age (years)	43.3 ± 13.6	51.8 ± 11.4	57.1 ± 11.4	<0.001
Body mass index (kg/m^2)	24.7 ± 4.6	26.4 ± 4.2	26.6 ± 4.3	<0.001[a]
Duration of diabetes (years)	—	6.6 ± 5.8	14.5 ± 9.5	<0.001
Systolic blood pressure (mmHg)	119 ± 15	127 ± 16	140 ± 21	<0.001[a]
Diastolic blood pressure (mmHg)	75 ± 9	77 ± 11	80 ± 10	<0.001[a]
Fasting plasma glucose (mg/dL)	89 ± 8	164 ± 63	178 ± 72	<0.001[a]
Glycosylated hemoglobin (%)	5.8 ± 0.4	8.8 ± 1.9	9.1 ± 2.2	<0.001[a]
Serum cholesterol (mg/dL)	187 ± 36	202 ± 38	188 ± 70	<0.001[a]
Serum triglycerides (mg/dL)	120 ± 64	174 ± 92	181 ± 102	<0.001[a]
Serum creatinine (mg/dL)	0.87 ± 0.1	0.90 ± 0.1	0.99 ± 0.33	<0.001[a]
Hypertension	144 (25.2%)	122 (47.8%)	108 (76.5%)	<0.001
Obesity	155 (27.1%)	102 (40.0%)	58 (41.1%)	<0.001
Dyslipidemia	26 (4.5%)	135 (52.9%)	74 (52.4%)	<0.001
Ischemic heart disease	12 (2.1%)	29 (11.3%)	10 (7.0%)	<0.001

Data are presented as mean ± SD.

[a]*P* value adjusted for age duration of diabetes and sex.

Abbreviations: NGT, normal glucose tolerance; T2D, type 2 diabetes mellitus without nephropathy; DN, diabetic nephropathy; ANOVA, analysis of variance; SD, standard deviation.

Table 2. Association of the Polymorphism in the *PPARGC1A* Gene With Type 2 Diabetes and Diabetic Nephropathy

Genotype	*NGT subjects (n = 571)*	*T2D subjects (n = 255)*	*DN subjects (n = 141)*
PPARGC1A (Thr394Thr)			
GG	487 (85.3%)	193 (75.7%)	88 (82.4%)
GA	77 (13.5%)	60 (23.5%)	51 (36.2%)
AA	7 (1.2%)	2 (0.8%)	2 (1.4%)
A allele[a]	0.08	0.12	0.19
HWE *P* value	0.08	0.12	0.05
		Genotype P value	*Minor allele P value*
T2D subjects vs. NGT subjects		0.001	0.001
DN subjects vs. T2D subjects		0.006	0.02
PPARGC1A (Gly482Ser)			
Gly/Gly	300 (52.5%)	139 (54.5%)	31 (22.0%)
Gly/Ser	221 (38.7%)	96 (37.6%)	68 (48.2%)
Ser/Ser	50 (8.8%)	20 (7.8%)	42 (29.8%)
Ser allele[a]	0.28	0.26	0.54
HWE *P* value	0.31	0.55	0.73
		Genotype P value	*Minor allele P value*
T2D subjects vs. NGT subjects		0.848	0.689
DN subjects vs. T2D subjects		<0.001	<0.001
PPARGC1A (+A2962G)			
AA	287 (50.3%)	118 (46.3%)	64 (45.4%)
AG	225 (39.4%)	119 (46.7%)	67 (47.5%)
GG	59 (10.3%)	18 (7.1%)	10 (7.1%)
G allele[a]	0.3	0.3	0.3
HWE *P* value	0.14	0.09	0.17
		Genotype P value	*Minor allele P value*
T2D subjects vs. NGT subjects		0.089	0.958
DN subjects vs. T2D subjects		0.985	0.983

[a]Minor allele frequency.

Abbreviations: NGT, normal glucose tolerance; T2D, type 2 diabetes mellitus; DN, diabetic nephropathy; HWE, Hardy–Weinberg equilibrium; Gly, glycine; Ser, serine.

association with nephropathy alone or nephropathy in combination with retinopathy or neuropathy (microangiopathy) but not with retinopathy or neuropathy *per se*.

None of the clinical and biochemical parameters showed any differences in NGT subjects in the different genotypes of Gly/Ser polymorphism. Age-adjusted systolic blood pressure (SBP) (Gly/Gly, 129.17 ± 17.35 mmHg; Gly/Ser, 125.18 ± 15.69 mmHg; Ser/Ser, 121.25 ± 12.49 mmHg; $P = 0.049$) was significantly lower in T2D subjects carrying Gly/Ser and Ser/Ser genotypes. Among the DN subjects, age-adjusted cholesterol (Gly/Gly, 218.52 ± 69.54 mg/dL; Gly/Ser, 173.93 ± 45.82 mg/dL; Ser/Ser, 180.65 ± 96.66 mg/dL; $P = 0.012$) was significantly lower in DN subjects carrying Gly/Ser and Ser/Ser genotypes.

There was no association of the A2962G polymorphism of the *PPARGC1A* gene with either T2D or DN.

Power calculation

We estimated the power of the present study to detect ORs for DN (1.0–4.0) for a range of minor allele frequencies (0.05–0.50) for a sample size of 967 (571 NGT, 255 T2D, and 141 DN subjects). We found that at 80% power, for polymorphisms with allele frequencies ranging from 5% to 50%, we can detect ORs for DN >1.5.

Discussion

The risk of developing DN has been mapped to chromosomes 2q, 3q, 7q, 10q, 14q, 15q, and 18q,[16] and some of the genes in these regions have been evaluated for their contribution to the susceptibility of nephropathy.[3] The present study was undertaken to evaluate the association of *PPARG* and *PPARGC1A* gene polymorphisms with DN in Asian Indians. This is the first report to our knowledge of an association of the *PPARGC1A* gene polymorphism with DN.

The Pro12Ala polymorphism of the *PPARG* gene has been reported to be associated with insulin sensitivity[17] in subjects with T2D. Recently, we reported that the Pro12Ala polymorphism does not protect Asian Indians against diabetes or insulin resistance, in contrast to the findings in Europeans.[9,14] Among the European T2D subjects, *PPARG* Ala12 allele carriers have been reported to have a significantly lower albumin excretion rate and tend to develop overt proteinuria less frequently.[18] The present study shows that *PPARG* gene polymorphisms are not associated with or protective against DN in Asian Indians.

The human *PPARGC1A* gene has been mapped to chromosome 4p15.1, and this region has been linked to increased fasting serum insulin levels in Pima indians[19] and higher SBP in Dutch families.[20] We earlier reported that the Thr394Thr

Table 3. Logistic Regression Showing the Association of the *PPARGC1A* Gene Polymorphism With Type 2 Diabetes and Diabetic Nephropathy

Genotype	*Unadjusted OR (95% CI)*	*P value*	*Adjusted OR[a] (95% CI)*	*P value*
		PPARGC1A (Thr394Thr)		
		T2D subjects vs. NGT subjects		
GG	Reference		Reference	
GA	1.86, (1.28–2.69)	0.001	1.49 (1.00–2.22)	0.046
AA				
		DN subjects vs. T2D subjects		
GG	Reference		Reference	
GA	1.875, (1.20–2.92)	0.006	1.639 (0.98–2.72)	0.061
AA				
		PPARGC1A (Gly482Ser)		
		DN subjects vs. T2D subjects		
Gly/Gly	Reference		Reference	
Gly/Ser	3.18, (1.93–5.23)	<0.001	2.14 (1.23–3.72)	0.007
Ser/Ser	9.42, (4.87–18.21)	<0.001	8.01 (3.89–16.47)	0.001

[a]Odds ratio adjusted for age, sex, BMI, and diabetes duration.

Abbreviations: OR, odds ratio; CI, confidence interval; T2D, type 2 diabetes; NGT, normal glucose tolerance; DN, diabetic nephropathy; Gly, glycine; Ser, serine; BMI, body mass index.

polymorphism of the *PPARGC1A* gene is associated with T2D[8] in Asian Indians. In the present study, a statistical significance was observed in Thr394Thr variants and DN, which was however lost when adjusted for age, sex, BMI, and diabetes duration.

The Gly482Ser polymorphism of the *PPARGC1A* gene has been shown to be associated with T2D diabetes in a European population[21] but not in Asian Indians.[8] The Ser allele was found to be independently associated with increased arterial blood pressure.[22,23] In contrast, some reports have shown an inverse effect on blood pressure in the presence of the Ser482 allele.[24] Although not significant, the SBP showed a trend toward being higher in those with Gly/Ser and Ser/Ser genotypes in our population, and, among the T2D subjects, those with Ser/Ser genotype had significantly lower SBP ($P = 0.049$).

In the present study, the Gly482Ser polymorphism was significantly associated with DN, even after adjusting for age, sex, BMI, and diabetes duration, suggesting that this polymorphism may be a potential genetic marker for DN in this ethnic group. Indeed, the risk of DN in Asian Indians increased 8 times in the presence of Ser/Ser genotype. This study thus adds to the body of existing knowledge of susceptibility genes to DN in Asian Indians.[25–28] Determination of whether this polymorphism is in linkage disequilibrium with a nearby functional variant will require additional studies. Whether this polymorphism directly influences the albumin excretion rate is currently unknown, and this aspect also deserves further functional studies. The possible link through higher SBP in the Gly/Ser and Ser/Ser variants is another possibility that should be confirmed by larger studies.

Table 4. Association of the Gly482Ser Polymorphism With Type 2 Diabetes and Its Complications

Genotype	*Normal glucose tolerant (n = 326)*	*Type 2 diabetes mellitus without complications (n = 187)*	*Retinopathy (n = 40)*	*Nephropathy (n = 41)*	*Neuropathy (n = 68)*	*Retinopathy and nephropathy (n = 20)*	*Retinopathy and neuropathy (n = 21)*	*Nephropathy and neuropathy (n = 21)*	*Microangiopathy (retinopathy, nephropathy, and neuropathy) (n = 59)*
Gly/Gly	182 (55.8%)	101 (54.0%)	23 (57.5%)	15 (36.6%)	38 (55.9%)	5 (25%)	7 (33.3%)	4 (19%)	7 (11.9%)
Gly/Ser	110 (33.7%)	69 (36.9%)	13 (32.5%)	15 (36.6%)	27 (39.7%)	5 (25%)	13 (61.9%)	14 (66.7%)	34 (57.6%)
Ser/Ser	34 (10.4%)	17 (9.1%)	4 (10.0%)	11 (26.8%)	3 (4.4%)	10 (50%)	1 (4.8%)	3 (14.3%)	18 (30.5%)
Ser allele[a]	178 (27.3%)	103 (27.5%)	21 (26.2%)	37 (45.1%)	33 (24.3%)	25 (62.5%)	15 (37.1%)	20 (47.6%)	70 (59.3%)

	Genotype (Gly/Ser) P value	*Minor allele P value*
Type 2 diabetic mellitus vs. normal glucose tolerant	0.732	0.994
Diabetic retinopathy vs. type 2 diabetic mellitus without complications	0.87	0.964
Diabetic nephropathy vs. type 2 diabetic mellitus without complications	0.005	0.03
Diabetic neuropathy vs. type 2 diabetic mellitus without complications	0.466	0.648
Diabetic retinopathy and nephropathy vs. type 2 diabetic mellitus	1.46E-06	0.0043
Diabetic retinopathy and neuropathy vs. type 2 diabetic mellitus	0.084	0.52
Diabetic nephropathy and neuropathy vs. type 2 diabetic mellitus	0.01	0.52
Microangiopathy vs. type 2 diabetic mellitus	8.32E-09	0.000053

[a]Minor allele frequency.

Abbreviations: Gly, glycine; Ser, serine.

It has been shown that there is significant diversity in allele frequencies at many autosomal loci within different castes in South India.[29] To address this issue of population stratification, a cross-validation using genomic control was done.[30] A case–control study at 6 unlinked marker loci believed to be unrelated to the disease under study but known to have allelic diversity among different populations was carried out. The allele frequency difference between NGT/T2D/DN was not statistically significant at any of the 6 loci studied. This indicates that the findings in this study are not likely to be an artefact of population substructuring.

In conclusion, this study reports on the association of the Gly482Ser polymorphism of the *PPARGC1A* gene with DN in an Asian Indian population. In addition, this study also shows that the *PPARG* gene is not associated with or protective against DN in this ethnic group. One of the limitations of this study is that being a cross-sectional study, no cause-and-effect relationship can be established; this issue will need longitudinal follow-up studies. Also it is important to confirm these findings with additional investigations using a larger sample size using high-throughput genotyping methods, which would allow screening of a large number of samples, and thus will be sufficiently powered to decrease the probability of false-positive associations.

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Author Disclosure Statement

No competing financial interests exist.

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