Original Article

Peroxisome Proliferator-Activated Receptor α and γ Gene Polymorphisms among South Indian Patients with Diabetic Dyslipidaemia

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Abstract

Background: Peroxisome proliferator-activated receptors (PPAR) α and γ genes play an important role in dyslipidaemia of T2DM. **Aims:** To estimate the frequency distribution of PPAR α and γ gene polymorphisms in South Indian T2DM patients with dyslipidaemia compared to healthy controls. Normative frequencies of SNPs were established and compared with data for 1000 genome populations. **Methods:** Eligible 382 cases and 336 age and sex-matched controls were enrolled. Six SNPs in PPARα [rs1800206 C>G (Leu162Val), rs4253778 G>C, rs135542 T>C] and PPARγ [rs3856806 (C>T), rs10865710 (C>G), rs1805192 C>G (Pro12Ala)] genes were selected for genotyping. **Results:** The allele and gene frequencies did not significantly differ between the diabetic dyslipidaemia cases and healthy controls. However, they were significantly different from that of 1000 genome populations except for rs1800206 C>G (Leu162Val) and rs1805192 C>G (Pro12Ala). **Conclusion:** The studied polymorphisms in PPARα and PPARγ genes are not associated with diabetic dyslipidaemia among South Indian patients.

Keywords: Diabetic dyslipidaemia, Minor allele frequency, PPARα/γ gene, Single-nucleotide polymorphisms

Introduction

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycaemia resulting from differential defects in either insulin action or secretion. The effect of chronic hyperglycaemia is directly associated with the dysfunctions of the organs like kidneys, heart, eyes, nerves and blood vessels.[1] The prevalence of diabetes worldwide in 2019 was predicted to be 9.3% (463 million people) and is projected to increase to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. In India alone, 77 million were affected with diabetes in 2019, and it is expected to rise to 101 million by 2030 and 134.2 million by 2045. This is a major public health challenge as it is increasing in epidemic proportion.[2] Diabetic dyslipidaemia is one of the major factors contributing to complications like cardiovascular diseases (CVD) and cerebrovascular accidents (CVA). Despite the progress in the management of diabetes, there is an alarming rise in mortality and morbidity due to diabetic complications.[3] Peroxisome proliferator-activated receptors (PPARs) are a family of three nuclear hormone receptors. They are of three sub-types, namely PPAR α, PPAR β/δ and PPAR γ, each binding with different ligands and having different target gene/s, biological functions and roles. The human PPAR α gene is located on chromosome 22 at the position of 22q12-q13.1. PPAR α shares a common characteristic with the other members of the PPAR family and has five domains, namely A/B, C, D, E, and F.[4] PPARα influences the metabolism of carbohydrates and intracellular lipids with the direct transcriptional control of genes implicated in mitochondrial β-oxidation pathways and peroxisomal, uptake of fatty acid and triglyceride catabolism.[5] It also regulates the expression of proteins

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involved in the transport and β-oxidation of free fatty acids (FFAs). The PPAR γ is situated at chromosome 3p25, which encodes a nuclear transcription factor implicated in the expression of many genes. PPAR γ gene contains nine exons, which helps regulate the transcription of numerous genes concerned with adipocyte separation and insulin-mediated glucose uptake in several tissues.[6,7] PPAR γ regulates glucose metabolism by decreasing FFA and improving insulin action.[8,9] Although PPAR variants have been studied extensively in other populations,[10] the data on PPAR gene polymorphisms in diabetes, dyslipidaemia, and metabolic syndrome are meagre in India, especially in South India. Only a few SNPs were studied concerning PPAR gene variants in Indians. Hence, through the present study, we aimed to find the association between the PPARα and PPAR γ gene polymorphisms with diabetic dyslipidaemia in South Indian type 2 diabetes mellitus (T2DM) patients as compared to healthy controls. The frequencies of genetic polymorphisms were also compared with the data of 1000 genome populations.

Materials and Methods

The study was approved by the scientific advisory committee and institutional ethics committee. The study population consists of 718 participants comprising 382 cases with diabetic dyslipidaemia and 336 healthy controls. Cases of diabetic dyslipidaemia aged between 30 and 60 years were recruited from the Department of Endocrinology (during the study period 2017–2021). Three hundred and thirty-six attendees of unrelated patients and other healthy volunteers of South Indian ethnicity, who were 30–60 years old, gender and age-matched (±3 years) without pregnancy or malignancy with HbA1c <6.5% without any antidiabetic drugs were recruited as controls. The South Indian population was selected based on the criteria of those residing in South India for the past three generations and speaking anyone of South Indian languages, namely Tamil, Telugu, Kannada and Malayalam, as their mother tongue. Type 2 diabetes, for study purposes, was defined as a state requiring regular antidiabetic oral drugs to maintain blood glucose levels in a normal range. Diabetic dyslipidaemia was considered as having a variable combination of total cholesterol (TC) >200 mg/dl, low-density lipoprotein-cholesterol (LDL-C) >100 mg/dl, triglyceride (TG) >150 mg/dl, and high-density lipoprotein-cholesterol (HDL-C) <40 mg/dl (male)/HDL-C <50 mg/dl (female). Glycated haemoglobin (HbA1c) >10%, other types of diabetes and those with other endocrine disorders were excluded.

Written informed consent was obtained from all the participants before the initiation of the study. Socio-demographic details, physical examination and past and personal history were gathered, followed by the collection of blood samples (after a minimum of 8 h of fasting) for measuring biochemical parameters and lipid profiles. In addition, 5 ml of venous blood was collected for DNA extraction in a tube containing 10% liquid EDTA (Na2EDTA) and was centrifuged at 3000 rpm for 10 minutes. Plasma was separated and discarded from the whole blood, and the buffy coat was kept at -80° C until DNA extraction.

SNP selection, genomic DNA extraction and genetic analysis

We selected six single-nucleotide polymorphisms (SNPs) within PPARα and PPAR γ genes from the National Centre for Biotechnology Information (NCBI) SNP database (https://www.ncbi.nlm.nih.gov/snp/) based on the following criteria: 1. previous association with diabetes and lipid abnormalities both combined or in isolation and 2. minor allele frequency (MAF) >0.05%. Table 1 describes the details of the selected SNPs, including allelic variants and their features. PPAR α and γ genes are located at chromosome positions 22q12 and 3p25, respectively. DNA was isolated using the manual phenol-chloroform method. The quantity and quality of the extracted DNA were analysed using a multi-analyser (Infinite 200: Tecan, Austria). The DNA samples were diluted to an optimal concentration of 50 ng/µl and were stored at 4°C overnight before genotyping. The genotyping of selected SNPs was done using real-time PCR (AB 7300, USA: Applied Biosystems). TaqMan SNP assay kits were used for genotyping per the manufacturer's protocol. The PCR reaction was carried out using 10 µl as a final volume containing extracted DNA of 2.5 µl, 5 µl of TaqMan master mix (2x), 0.25 µl TaqMan assay kits (40x), and milli-Q water of 2.25 µl. The genotyping analysis was carried out in duplicate using TaqMan SNP genotyping assays.

Table 1: Description of the six single-nucleotide polymorphisms in peroxisome proliferator-activated receptor genes

Gene rsID	SNP	Position in chromosome	Nucleotide substitution	Variant type	Amino acid substitution	Assay ID
PPAR α						
rs1800206	Leu162Val	chr22:46218377	C>G	Missense variant	p.Leu162Val	C_8817670_20
rs4253778	7G>C	chr22:46234737	G>C	Intron variant	NA	C_2985251_20
rs135542	Intron T>C	chr22:46160138	T>C	Intron variant	NA	C_2988823_20
PPAR γ						
rs3856806	C161T	chr3:12434058	C>T	Synonymous variant	p.His477His	C_11922961_30
rs10865710	C681G	chr3:12311699	C>G	Intron variant	NA	C_9384417_10
rs1805192	Pro12Ala	chr3:12379739	C>G	Missense variant	p.Pro12Ala	C_26856791_20

Statistical analysis

The data were analysed using Statistical Package for Social Sciences SPSS 19.0.0 (SPSS Inc., Chicago, IL, USA). Baseline characteristics have been presented as mean and standard deviation (SD) for continuous variables and were tested using a two-sample *t*-test. Categorical variables were tested using the Chi-square test. Allele and genotype frequencies were calculated for each polymorphism, and a Chi-square test was performed to investigate deviation from Hardy–Weinberg (HW) equilibrium. Genetic models were used to determine the association between PPAR α/γ polymorphisms and diabetic dyslipidaemia. Allelic and genotypic frequencies of South Indian populations were compared with 1000 genome project data using the Chi-square test. The odds ratio (OR) with a 95% confidence interval (CI) was analysed for the association with diabetic dyslipidaemia. A two-sided p-value <0.05 was considered statistically significant. Estimation of linkage disequilibrium (LD) between polymorphisms was performed using the SNPStats web tool (https://www.snpstats.net/start.htm).

Sample size

There is sparse information on the frequency rates of PPAR α and γ gene polymorphisms in South Indians. For addressing the primary objective and assuming the frequency rate of 0.5 with 5% absolute precision and 95% confidence interval, 384 diabetic dyslipidaemia patients are required across frequency rates of 0.2–0.97.

Ethical clearance statement

The study was approved by Institutional Ethics Committee (Human studies), JIPMER Puducherry, vide letter no JIP/IEC/2017/0123 on 08/05/2017. Written informed consent was obtained for participation in the study and use of the patient data for research and educational purposes. The procedures follows the guidelines laid down in Declaration of Helsinki 2008.

Results

Clinical Characteristics

A total of 382 diabetic dyslipidaemia and 336 apparently healthy controls were analysed in this case-control study based on eligibility criteria. The average values of baseline characteristics, including weight ($P < 0.001$), body mass index (BMI) ($P = 0.024$), and systolic blood pressure (SBP) ($P = 0.002$), were significantly higher in patients than in controls. However, no difference was found with respect to age, gender, height, and diastolic BP. The mean HbA1c of diabetic dyslipidaemia cases was significantly higher compared to healthy controls (8.01 ± 0.91% vs. 5.60 ± 0.41%, $P < 0.001$), as shown in Table 2. The mean lipid profile among cases was 202.47 ± 45.27 mg/dl in total cholesterol, 189.74 ± 86.88 mg/dl in triglyceride, 125.89 ± 40.4 mg/dl in LDL-C, and 43.95 ± 10.91 mg/dl in HDL-C. Lipid profile was done only for diabetic dyslipidaemia cases and not for the apparently healthy control group presuming they have normal lipid profiles. The duration of diabetes was 6.9 ± 5.30 years.

Table 2: General characteristics of the study participants

Variables	Diabetic dyslipidaemia ($n=382$)	Healthy controls ($n=336$)	*P*
	Mean±SD	Mean±SD	
Age (years)	48.56±6.14	47.90±5.17	0.128
Gender (M:F)[b]	145:237	130:206	0.877
Height (cm)	154.49±8.56	154.92±12.44	0.59
Weight (kg)	65.59±12.28	61.10±11.34	<0.001
BMI (kg/m^2)	27.43±4.85	25.71±4.0	0.024
Systolic BP (mmHg)	130.67±17.58	126.37±16.80	0.002
Diastolic BP (mmHg)	81.04±10.43	80.95±10.48	0.904
HbA1c (%)	8.01±0.91	5.60±0.41	<0.001
Total cholesterol (mg/dl)	202.47±45.27	NA	_
Triglyceride (mg/dl)	189.74±86.88	NA	_
LDL-C (mg/dl)	125.89±40.4	NA	_
HDL-C (mg/dl)	43.95±10.91	NA	_

The parameters are represented as mean±SD for age, height, weight, BMI, systolic BP, diastolic BP, and HbA1c and compared using Student's *t*-test, [b]Chi-square test. A *P* value less than 0.05 was considered statistically significant. NA: Not available

The mean age at sampling was 48.5 ± 6.14 years, and the age at diabetes onset was 41.6 ± 5.39 years. There was a significant difference between age at diabetes onset and age at sampling ($P < 0.001$) among cases. Among 382 diabetic dyslipidaemia patients, 270 (71%) of them were on 10 mg atorvastatin, 79 (21%) on 20 mg atorvastatin, and 33 (8%) of the diabetic dyslipidaemia patients were on 40 mg atorvastatin. Six SNPs were genotyped, and their details are given in Tables 3 and 4.

Genotype distribution and allele frequencies of PPAR α and γ genes polymorphisms

The observed genotype and allele frequencies of the SNPs in the PPAR α (rs1800206, rs4253778, rs135542) gene are given in Table 3. All the SNPs included in this study were in HWE ($P > 0.05$). The different models assessed the selected polymorphisms (overdominant, co-dominant, dominant, and recessive). Since the mutant G/G genotype of PPAR α C>G (Leu162Val) polymorphism was not detected, data from C/G (Leu/Val) and G/G (Val/Val) were pooled and analysed. The distribution of the PPAR γ gene SNPs (rs3856806, rs10865710, rs1805192) among cases and controls is given in Table 4. There was no significant difference in the distribution of case and control subjects across different genetic models among the studied PPAR α and γ gene polymorphisms. The results were the same even after adjustment for age of onset of diabetes, BMI, and BP. The frequency distribution of the wild homozygous C/C (Pro/Pro) variant of the PPAR γ gene rs1805192 SNP was 100%, and none of the study patients had either heterozygous C/G (Pro/Ala) or homozygous mutant G/G (Ala/Ala) variants.

Linkage disequilibrium analysis

The LD was not observed between the SNPs of PPAR α [rs1800206 and 4253778 (D' =0.52, r2 = 0.26); rs1800206 and

Table 3: Genotype and allele frequencies of three single-nucleotide polymorphisms in peroxisome proliferator-activated receptor α gene and their association with the diabetic dyslipidaemia

PPAR α	Genotypes/alleles	Case *n* (%)	Control *n* (%)	Crude OR (95% CI), *P*	Adjusted OR (95% CI), *P*
rs1800206 C>G	CC	372 (97.4)	325 (97)	1	1
(Leu162Val)	CG	10 (2.6)	11 (3)	1.26 (0.53–3.0), 0.6	0.83 (0.25–2.80), 0.77
	GG	0 (0)	0 (0)		
	Allele				
	C	754 (98.6)	661 (98)	1	
	G	10 (1.4)	11 (2)	1.25 (0.56–2.91), 0.52	--
rs4253778 G>C					
Codominant	GG	336 (88)	307 (91.4)	1	1
	GC	44 (11.5)	29 (8.6)	0.72 (0.44–1.18), 0.12	0.60 (0.29–1.27), 0.17
	CC	2 (0.5)	0 (0)	0 (0–2.37), 0.5	NA
	Allele				
	G	716 (93.7)	643 (96)	1	
	C	48 (6.3)	29 (4)	0.67 (0.42–1.08), 0.1	--
Dominant	GG	336 (88)	307 (91.4)	1	1
	GC+CC	46 (12)	29 (8.6)	0.69 (0.42–1.13), 0.13	0.57 (0.27–1.19), 0.13
Recessive	CC	2 (0.5)	0 (0)	1	1
	GG+GC	380 (99.5)	336 (100)	NA	NA
Overdominant	GC	44 (11.5)	29 (8.6)	1	1
	GG+CC	338 (88.5)	307 (91.4)	0.73 (0.44–1.19), 0.20	0.60 (0.29–1.27), 0.18
rs135542 T>C					
Codominant	TT	281 (73.6)	254 (75.6)	1	1
	TC	90 (23.5)	77 (22.9)	0.95 (0.67–1.34), 0.42	0.92 (0.55–1.56), 0.64
	CC	11 (2.9)	5 (1.5)	0.50 (0.17–1.47), 0.21	0.47 (0.09–1.2.40), 0.25
	Allele				
	T	652 (85)	585 (87)	1	
	C	112 (15)	87 (13)	0.86 (0.64–1.17), 0.35	--
Dominant	TT	281 (73.6)	254 (75.6)	1	1
	TC+CC	101 (26.4)	82 (24.4)	0.90 (0.64–1.26), 0.53	0.88 (0.53–1.45), 0.61
Recessive	CC	11 (2.9)	5 (1.5)	1	1
	TT+TC	371 (97.1)	331 (98.5)	0.51 (0.18–1.48), 0.20	0.48 (0.10–2.43), 0.37
Overdominant	TC	90 (23.6)	77 (22.9)	1	1
	TT+CC	292 (76.4)	259 (77.1)	0.96 (0.68–1.36), 0.84	0.94 (0.56–1.58), 0.82

P<0.05 was considered statistically significant, adjusted OR with age at diabetes onset, BMI, and BP

rs135542 (D' =0.40, r2= -0.02); rs4253778 and rs135542 (D' =0.10, r2= -0.01)] and PPAR γ [rs3856806 and rs10865710 (D' =0.29, r2 = 0.27)] genes on chromosome 22 and chromosome 3, respectively. Therefore, haplotype analysis was not done.

Comparison of genotype and allele frequencies of the South Indian population in comparison with 1000 genome populations

The genotype and allele frequencies of PPAR α gene SNP, rs1800206, were not different from the 1000 genome populations. Our study's allele frequency of rs4253778 was significantly different from the African, American, and European populations. The allele and genotype frequencies of SNP rs135542 were significantly different from all 1000 genome populations except for allele frequencies in the African and European populations [Table 5]. For the PPAR γ gene polymorphisms, the genotype frequency of rs3856806 was significantly different from East Asian and African populations. In the case of rs10865710, allele and genotype frequencies distribution were significantly different from the ethnic 1000 genome populations except those of African and South Asian ancestry. The frequency distribution of rs1805192 was not different from that seen with 1000 genome populations [Table 6].

Discussion

In the present study, we assessed the frequency of PPAR α (rs1800206 C>G (Leu162Val), rs4253778 G>C and rs135542 T>C)) and PPAR γ (rs3856806 C>T, rs10865710 C>G, and rs1805192 C>G (Pro12Ala)) gene polymorphisms in patients with diabetic dyslipidaemia in comparison with healthy controls. Our study did not show the association of the studied polymorphisms with the risk of diabetic dyslipidaemia in the South Indian population.

In our study, rs1800206 C>G (Leu162Val) of the PPARα gene failed to be associated with diabetic dyslipidaemia among the South Indian population. A similar study by Lacquemant

Table 4: Genotype and allele frequencies of three single-nucleotide polymorphisms in peroxisome proliferator-activated receptor γ gene and their association with the diabetic dyslipidaemia

		PPAR γ			
Model	**Genotypes/Alleles**	**Case *n* (%)**	**Control *n* (%)**	**Crude OR (95% CI), *P***	**Adjusted OR (95% CI), *P***
rs3856806 C>T					
Codominant	CC	299 (78.3)	260 (77.4)	1	1
	CT	81 (21.2)	71 (12.1)	1.01 (0.70–1.44), 0.41	1.26 (0.73–2.19), 0.71
	TT	2 (0.5)	5 (1.5)	2.87 (0.55–14.94), 0.26	1.16 (0.12–11.66), 0.28
	Allele				
	C	679 (88.9)	591 (88)	1	
	T	85 (11.1)	81 (12)	1.0 (0.79–1.50), 0.62	--
Dominant	CC	299 (78.3)	260 (77.4)	1	1
	CT+TT	83 (21.7)	76 (22.6)	1.05 (0.74–1.50), 0.77	1.26 (0.73–2.16), 0.41
Recessive	TT	2 (0.5)	5 (1.5)	1	1
	CC+CT	380 (99.5)	331 (98.5)	2.87 (0.55–14.89), 0.18	1.11 (0.11–10.87), 0.93
Overdominant	CT	81 (21.2)	71 (21.1)	1	1
	CC+TT	301 (78.8)	265 (78.9)	1.0 (0.70–1.43), 0.98	1.26 (0.73–2.18), 0.41
rs10865710 C>G					
Codominant	CC	282 (73.8)	254 (75.6)	1	1
	CG	96 (25.1)	78 (23.2)	0.90 (0.64–1.27), 0.83	1.26 (0.75–2.11), 0.49
	GG	4 (1.1)	4 (1.2)	1.11 (0.27–4.49), 0.87	0.47 (0.07-3.34), 0.53
	Allele				
	C	660 (86.4)	586 (87)	1	
	G	104 (13.6)	86 (13)	0.93 (0.69–1.25), 0.69	--
Dominant	CC	282 (73.8)	254 (75.6)	1	1
	CG+GG	100 (26.2)	82 (24.4)	0.91 (0.65–1.28), 0.59	1.20 (0.72–1.98), 0.48
Recessive	GG	4 (1.1)	4 (1.2)	1	1
	CC+CG	378 (99)	332 (98.8)	1.14 (0.28–4.59), 0.86	0.44 (0.06–3.15), 0.42
Overdominant	CG	96 (25.1)	78 (23.2)	1	
	CC+GG	286 (74.9)	258 (76.8)	0.90 (0.64–1.27), 0.55	1.27 (0.76–2.13), 0.36
rs1805192 C>G					
(Pro12Ala)	CC	382 (100)	336 (100)	---	---
	CG	0	0		
	GG	0	0		
	Allele				
	C	764 (100)	672 (100)	---	---
	G	0	0		

P<0.05 was considered statistically significant, adjusted OR with age at diabetes onset, BMI, and BP

et al. [11] also showed no association between G/G (Val/Val) genotype and T2DM in the South Indian population. The present non-association of the PPAR α Val162 allele follows a similar non-association with obesity (OR = 1.02 and 95% CI = 0.55 to 1.86), metabolic syndrome MetS (OR = 0.95 and 95% CI = 0.41 to 2.16), and obesity with MetS (OR = 1.64 and 95% CI = 0.34 to 7.80) of the Malaysian population. [12] In a Brazilian study (n = 222), the carrier of the Val allele was not associated with T2DM (OR = 1.1 and 95% CI = 0.52 to 2.54). [13] This study's minor allele frequency (MAF) of G (V162) was 0.015, similar to 0.02 documented in Singaporean Indians. [12] In contrast, a much higher MAF of 0.16 was reported in the Chinese Hans population by Gu *et al*. [14] However, the PPARα Val162 allele has been associated with lipid concentrations in T2DM patients. [15] Similarly, Andrulionyte *et al*. reported an association of the Val162 allele with the increased level of insulin and plasma glucose in T2DM subjects (OR = 1.9 and 95% CI = 1.5 to 3.58). [16] Gu *et al*. [17] showed a positive association of the G/G (Val/Val) carrier genotype with dyslipidaemia (OR = 2.38 and 95% CI = 0.43 to 13.31) in the Chinese population. In another study, C> G (Leu162Val) of rs1800206 was significantly associated with dyslipidaemia (OR = 2.1 and 95% CI = 1.0 to 4.47) among the Cuiaba population. [18] In a Lithuanian study, the authors reported an association of CG (Leu/Val) genotype with elevated TG levels (OR = 2.67 and 95% CI = 1.15 to 6.16) among dyslipidaemia subjects. [19]

In the rs4253778 variant, the intron 7G>C is in the non-coding region. Previous studies have specified its potential biological

Table 5: Comparison of allele and genotype frequencies of SNPs of PPARα gene in the study population and 1000 genome projects

SNP	South Indian	Global	AFR	AMR	EAS	EUR	SAS
Genotype/allele							
PPARα	*n*=336	*n*=5008	*n*=1322	*n*=694	*n*=1008	*n*=1006	*n*=978
rs1800206 C>G (Leu162Val)							
CC	96.7	95	98.9	93.4	100	88.7	95
CG	3.3	4.3	0.1	6.3	0	10.9	4.5
GG	0	0.7	0	0.3	0	0.4	0.5
Allele							
C	98.4	97.7	99.4	96.5	100	94.2	97.5
G	1.6	2.3	0.6	3.5	0	5.8	2.5
rs4253778 G>C							
GG	91.4	65.1	5.7	70.3	99.8	65.6	84.9
GC	8.6	22	39.5	25.4	0.2	30.4	14.7
CC	0	12.9**	54.8**	4.3**	0	4**	0.4
Allele							
G	95.7	72.5	25.5	75.5	99.9	80.8	92.2
C	4.3	27.5**	75.5**	24.5**	0.1	19.2**	7.8
rs135542 T>C							
TT	75.6	67.7	52.3	65.7	85.9	59.4	74.8
TC	22	26.9	39.5	30.6	13.1	31.6	22.1
CC	2.4	5.4	8.2**	3.7	1	9*	3.1
Allele							
T	86.6	74	72.1	81.1	92.5	75.2	85.9
C	13.4	26*	27.9*	18.9	7.5	24.8*	14.1

AFR: African, AMR: American, EAS: East Asian, EUR: European, SAS: South Asian, N: Number of participants. Frequencies are presented in percentage.*P<0.05,**P<0.001

importance. The SNPs themselves might not be functional, but they may be in allelic association with an effectual variant in a promoter or enhancer that results in reduced gene expression.[20] Some previous studies found that the C allele of rs4253778 was associated with an increased risk of diabetes (OR = 2.78 and 95% CI = 1.14 to 6.79).[16] A study by Hai *et al*.[21] has shown an association of the 7G>C variant with increased Apo A1/Apo B risk among the Chinese population. Purushothaman *et al*.[22] reported <>intron 7 G>C variant has a positive association with dyslipidaemia in CAD among the South Indian population (OR = 2.9 and 95% CI = 1.5 to 4.39). Furthermore, the intron 7G>C variant of rs4253778 was positively associated with dyslipidaemia (OR = 1.56 and 95% CI = 1.05 to 2.33).[18] Although it seems that the 7G>C variant influences metabolic disturbances, we did not find an association with diabetic dyslipidaemia. A similar finding was reported by Gu *et al*.,[17] who showed no association of intron 7 G>C variant with dyslipidaemia (OR = 0.65 and 95% CI = 0.24 to 1.78). In T2DM subjects, the variant of intron 7 G> C was not associated with T2DM (OR = 0.98 and 95% CI = 0.63 to 1.51) among the Brazilian population.[13] For rs135542 T>C SNP, there was no association between genotype and allele frequencies in different genetic models. Halder *et al*.[23] showed rs135542 to influence habitual physical activity and cardiometabolic risk.

In rs3856806 of PPARγ, 161C>T is a silent C>T substitution in nucleotide 161 of exon 6 that does not cause an amino acid change in the protein.[10] The PPARγ C>T variant has been associated with higher LDL-C risk[24] and serum fetuin.[25] In the Iranian population, the carrier T allele of the PPARγ C>T variant positively correlated with a higher risk of MetS (OR = 2.2 and 95% CI = 1.32 to 3.74).[26] In agreement, Haseeb *et al*.[27] reported C>T variant was associated with higher levels of plasma resistin (P = 0.017) in MetS among the South Indian population. Further, TT carrier genotypes were associated with dyslipidaemia (OR = 2.2 and 95% CI = 1.28 to 3.90) after adjusting for age, gender, body mass index and waist circumference.[17] Our study did not find any association with diabetic dyslipidaemia. Pattanayak *et al*.[28] could not demonstrate the association of the PPARγ C>T variant with T2DM (OR = 1.2 and 95% CI = 0.73 to 2.06). Similarly, the C>T variant of rs3856806 was not associated with diabetic nephropathy[29] and MetS[30] in the South Indian population. Chia *et al*.[12] reported that the C>T variant was not associated with obese MetS (OR = 0.7 and 95% CI = 0.32 to 1.78) after adjusting for age, sex, and ethnicity among the Malaysian population.

The intronic SNP of rs10865710 C>G, included in this study, has been associated with the LDL-C level, as reported by Fan *et al*.[24] In a French study, authors reported a positive association of the C>G variant with increased plasma LDL-C concentration.[31] Our study showed a negative association of the C>G variant with diabetic dyslipidaemia. Similarly, the

Table 6: Comparison of allele and genotype frequencies of SNPs of PPARγ gene in the study population and 1000 genome project

SNP	South Indian	Global	AFR	AMR	EAS	EUR	SAS
Genotype/allele							
PPARγ	*n*=336	*n*=5008	*n*=1322	*n*=694	*n*=1008	*n*=1006	*n*=978
rs3856806 C>T							
CC	77.4	75.6	90.3	78.7	61.9	76.9	71
CT	21.1	22.3	9.7	19.9	33.9	21.9	26.2
TT	1.5	2.1	0*	1.4	4.2*	1.2	2.9
Allele							
C	87.9	87.3	95.2	88.6	78.9	87.9	84
T	12.1	12.7	4.8	11.4	21.1	12.1	16
rs10865710 C>G							
CC	75.6	53.3	61.6	42.4	48.8	55.3	58.7
CG	23.2	38.6	33.9	43.5	40.7	38.4	37
GG	1.2	8.1**	4.5	14.1**	10.5**	6.4**	4.3*
Allele							
C	87.2	73.5	78.5	64.1	69.1	74.5	77.2
G	12.8	26.5*	21.5	35.9**	30.9*	25.5*	22.8
rs1805192 C>G							
(Pro12Ala)							
CC	100	100	100	100	100	100	0
CG	0	0	0	0	0	0	0
GG	0	0	0	0	0	0	0
Allele							
C	100	100	100	100	100	100	100
G	0	0	0	0	0	0	0

AFR: African, AMR: American, EAS: East Asian, EUR: European, SAS: South Asian, N: Number of participants. Frequencies are presented in percentage. *P<0.05,**P<0.001

C>G variant was not associated with the Apo-1/Apo-B ratio, [21] lipoprotein (a) concentration in MetS in Chinese population. [32] Further, Velayuthan *et al*. [29] reported no significant association of the C>G variant in diabetic nephropathy (OR = 0.82 and 95% CI = 0.45 to 1.48) among South Indian patients. In another study, Gu *et al*. [33] showed that the C>G variant of PPARγ was not associated with hypertriglyceridemia (OR = 0.90 and 95% CI = 0.68 to 1.1).

As regards rs1805192 SNP, the connection between the replacement of the C>G (*Pro12Ala*) allele and the risk of T2DM has been broadly studied throughout the world [10] and previous studies have found an association with higher levels of LDL-C, [24] T2DM [34], and obesity. [35] Zietz *et al*. [36] showed the Pro12Ala variant of PPARγ to be associated with higher levels of total cholesterol and LDL-C in male T2DM among Caucasian subjects. Gu *et al*. [33] reported the association of the Ala/Ala carrier genotype of PPARγ and hypertriglyceridemia (OR = 2.4 and 95% CI = 1.80 to 3.29) in MetS and with dyslipidaemia (OR = 2.9 and 95% CI = 1.70 to 5.14) [17] among Chinese Han population. In our study, no polymorphism was detected in C>G (*Pro12Ala*), and a similar finding was reported by Sarkar *et al*. [37] in the Assamese population in East India with T2DM. Correspondingly, Vimaleswaran *et al*. [30] have shown no association with MetS among the South Indian population. In another study, the carriers Ala allele of the C>G (*Pro12Ala*) variant were not associated with Apo-A1/Apo-B ratio (OR = 0.98 and 95% CI = 0.70 to 1.36). [21] Further, the absence of association of the C>G (*Pro12Ala*) variant with T2DM subjects was reported by Radha *et al*. [38] This contradictory finding might be due to inter-individual inconsistency in DNA repair capacity and variation in the distribution of polymorphic frequencies within populations. The frequency distribution among South Indians significantly differs from all the populations described above, as well as the 1000 genome projects, except for rs1800206 C>G (*Leu162Val*) and rs1805192 C>G (*Pro12Ala*). This result shows that genotype frequencies vary from population to population.

The present study might help increase the awareness of pharmacogenomics in clinical practice in South India. It also provides valuable information on the frequency distribution of studied polymorphisms among the South Indian population. Future research should concentrate on analysing other PPAR α/γ gene SNPs in diabetic dyslipidaemic populations.

Strength of the study

This is the first study to assess the frequency distribution of PPARα/γ gene SNPs in the South Indian population with diabetic dyslipidaemia and healthy controls. The study's case-control design is the strength of the study.

Limitation of the study

First, our findings may not be generalizable to other populations. Second, only six SNPs were chosen from PPAR α/γ genes. The selected SNPs might not sufficiently represent the genetic association between the PPAR α/γ genes and diabetic dyslipidaemia. Additional analyses of the polymorphisms rs135539 of PPARα, rs709158, and rs4684847 of PPARγ would have improved the study's comprehensiveness. Future research should further analyse other PPAR α/γ gene SNPs in diabetic dyslipidaemia populations.

Conclusion

The studied polymorphisms in PPARα and PPARγ genes are not associated with diabetes dyslipidaemia among South Indian patients. However, their frequencies were significantly different from 1000 genome populations except for rs1800206 C>G (*Leu162Val*) and rs1805192 C>G (*Pro12Ala*).

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Conflicts of interest

There are no conflicts of interest.

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