JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Role of peroxisome proliferator-activated receptor gamma gene polymorphisms in type 2 diabetes mellitus patients of West Bengal, India

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Keywords
Fasting sugar, Peroxisome proliferator activated receptor gamma gene, Type 2 diabetes mellitus

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J Diabetes Invest 2014; 5: 188–191

doi: 10.1111/jdi.12130

ABSTRACT

Aims/Introduction: Peroxisome proliferator activated receptor gamma (*PPARG*) is a nuclear hormone receptor of the ligand-dependent transcription factor involved in adipogenesis, and a molecular target of the insulin sensitizer, thiazolidinediones. The present study aimed to investigate whether the *PPARG* gene is associated with type 2 diabetes mellitus and its related traits within the population of West Bengal, India.

Materials and Methods: The study participants (200 type 2 diabetes mellitus and 200 normal individuals) were chosen randomly, and the variants were screened by direct sequencing.

Results: The results showed that rs1801282 (odds ratio 0.66; 95% confidence interval 0.15–2.96; $P = 0.57$) and rs3856806 (odds ratio 1.23; 95% confidence interval 0.73–2.06; $P = 0.44$) variants of the *PPARG* gene were not associated with type 2 diabetes mellitus.

Conclusions: The results showed that the *PPARG* gene was not associated with type 2 diabetes mellitus in our study population. As the lack of association might come from the small sample size, further studies with larger sample size are required to verify the present observation.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders that is characterized by hyperglycemia. Defects in insulin secretion, insulin action or both are responsible for hyperglycemia[1].

Diabetes is a major health problem within certain ethnic groups where both diabetic patients and the risk of associated complications are increasing at an alarming rate[2]. Indians are one of the ethnic groups that are considered to be a high-risk population for diabetes[3,4]. Compared with Europeans, Indians have greater insulin resistance capability[5–7], which promotes susceptibility to diabetes[8–10]. This is indicative of a strong genetic predisposition to type 2 diabetes mellitus in Indians.

Heritability studies have shown that genetic factors also affect the predisposition to type 2 diabetes mellitus[11].

The peroxisome proliferator activated receptor gamma (*PPARG*) gene is a member of the nuclear hormone receptor superfamily that regulates the transcription of several genes involved in glucose metabolism, adipocyte differentiation, lipid oxidation, angiogenesis and inflammation[12]. *PPARG* shows different protein isoforms generated by different promoters and alternative splicing[13]. The functional role of *PPARG* has been well documented, and its alterations have been widely associated with type 2 diabetes and obesity[14,15].

To date, most of the mutation analysis has focused on the coding region of the *PPARG* gene[16]. In different independent studies, the association of the *PPARG* gene with type 2 diabetes mellitus has been consistently reported[17–27]. In the present study, we screened the coding regions of the *PPARG* gene, and examined the association of the *PPARG* gene with type 2 diabetes mellitus in the population of West Bengal, India.

MATERIALS AND METHODS

Study Participants

In the present study, 200 type 2 diabetes mellitus patients were recruited from SSKM Hospital & Institute of Post Graduate Medical Education & Research, Kolkata, India and 200 controls were collected from the same community. The cases were aged 40 years or older, with mean age of 49.7 ± 10.1 years (mean ± standard deviation) at the time of investigation. After

Received 29 November 2012; revised 26 June 2013; accepted 30 June 2013

scrutinizing medical records for symptoms, medication and measuring fasting glucose levels, the diagnosis of type 2 diabetes mellitus was established following the American Diabetes Association recommended guidelines. A medical record representing either: (i) a fasting plasma glucose level ≥126 mg/dL after a minimum 12-h fast; or (ii) a 2-h post glucose level (2-h oral glucose tolerance test [OGTT]) ≥200 mg/dL on more than one occurrence with symptoms of diabetes. The 2-h OGTTs were carried out following the criteria of the World Health Organization (WHO; 75-g oral load of glucose). Body mass index (BMI) was measured using the following formula: BMI = weight (kg)/height $(m)^2$. Participants with type 1 diabetes and secondary diabetes (e.g., hemochromatosis, pancreatitis) were excluded from the study. The selection of controls was based on a fasting glycemia <110 mg/dL or a 2-h glucose <140 mg/dL. The average age of the controls (mean ± standard deviation) was 51.5 ± 7.5 years. The clinical characteristics of the participants used for the present investigation are summarized in Table 1. All the participants provided written informed consent for the investigation. The study was approved by the Ethics Committee of the Institute of Post Graduate Medical Education & Research, Kolkata.

Metabolic Assays

Low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), total cholesterol and triglycerides (TG), and glycosylated hemoglobin (HbA1c) were estimated. Lipids (LDL, VLDL, cholesterol, TG) were quantified using a Hitachi-912 Autoanalyzer (Hitachi, Mannheim, Germany). HbA1c levels were determined by high-pressure liquid chromatography using the Variant Machine (Bio-Rad, Hercules, CA, USA). Creatinine was quantified by using kinetic colorimetric assay (Roche, Basel, Switzerland).

Genotyping

Peripheral blood samples were collected from the patients and normal individuals. Genomic deoxyribonucleic acid (DNA) was isolated from leukocytes using QIAamp Blood Kit (QIAGEN, Hilden, Germany). To identify sequence variants, the coding region was amplified by polymerase chain reaction (PCR) and sequenced. All the primers for the coding region of the *PPARG* gene are shown in the Supporting Information, Table S1. PCR amplification was undertaken in a 25-μL volume containing 100 ng of DNA, 0.5 μL of each primer (10 mmol/L), 0.5 μL of deoxyribonucleotide triphosphate mix (10 mmol/L; Invitrogen, Carlsbad, CA, USA), 1 μL magnesium chloride (50 mmol/L), 2.5 μL of 10× buffer and 0.4 μL of Taq Polymerase (5 units/μL; Invitrogen). The PCR conditions were as follows: denaturation at 94°C for 3 min followed by 44 cycles of denaturation for 30 s, annealing at 58°C for 45 s, extension at 72°C for 45 s, and final extension at 72°C for 5 min. Sequencing was carried out using the Big Dye Terminator kit (Applied Biosystems, Foster City, CA, USA) on an automated DNA capillary sequencer (Model 3700; Applied Biosystems).

Table 1 | Clinical characteristics of the study population

Parameter	Diabetic participants n = 200	Control participants n = 200	*P*-value
Age (years)	49.7 ± 10.1	51.5 ± 7.5	0.016
BMI (kg/m^2)	22.85 ± 3.28	21.66 ± 3.12	0.0002
Cholesterol (mg/dL)	200.63 ± 46.14	185.38 ± 27.32	0.0008
Triglyceride (mg/dL)	207.36 ± 81.05	118.5 ± 22.39	<0.0001
LDL (mg/dL)	127.17 ± 43.87	115.23 ± 26.44	0.002
HDL (mg/dL)	42.6 ± 6.21	44.04 ± 5.42	0.0276
VLDL (mg/dL)	32.78 ± 9.34	26.84 ± 8.19	<0.0001
Creatinine (mg/dL)	0.87 ± 0.14	0.81 ± 0.12	<0.0001
HbA1c (%)	8.96 ± 1.62	5.5 ± 0.24	<0.0001
Fasting sugar (mg/dL)	180.39 ± 71.91	102.54 ± 10.87	<0.0001
PP sugar (mg/dL)	266.7 ± 65.23	118.65 ± 22.53	<0.0001

Data presented as mean ± standard deviation. BMI, body mass index; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density liporprotein; PP, post-prandial; VLDL, very low-density lipoprotein.

Statistical Analysis

Hardy–Weinberg equilibrium (HWE) in cases and controls was tested using the χ^2-test. The genotype and allele frequencies in type 2 diabetes mellitus cases were compared with control subjects using the χ^2-test. The association between the case–control status and each individual single nucleotide polymorphism (SNP) were measured by the odds ratio (OR) and its corresponding 95% confidence limits. Association analyses measured the dominant, recessive and co-dominant effect for each polymorphism using SNPassoc version 1.8-1 software (Catalan Institute of Oncology, Barcelona, Spain)[28]. The impact of *PPARG* polymorphisms on quantitative risk variables of type 2 diabetes mellitus including fasting insulin, glucose levels and fasting lipid levels was analyzed through multiple linear regression using PLINK version 1.07 software. The mean values of continuous independent variables between cases and controls were compared by using *t*-test. The power was computed for an OR of approximately 0.8 for SNPs, and the power was 35%. The power was estimated using Genetic Power Calculator[29].

RESULTS

In Table 2, the genotypic frequencies and ORs have been summarized. The results showed that just two SNPs (rs1801282 [Pro12Ala] and rs3856806 [His478His]) of the *PPARG* gene were found in this case–control group. The allele frequencies of rs1801282-C and rs1801282-G, and the allele frequencies of rs3856806-C and rs3856806-T polymorphism in the case and control participants were 0.88/0.12 v 0.91/0.09 and 0.90/0.10 v 0.88/0.12, respectively. The adjusted ORs under a recessive model could not provide any strong association for rs18081282 (OR 0.66, 95% confidence interval [CI] 0.15–2.96; $P = 0.57$). In contrast, dominant models also did not suggest any association for rs3856806 (OR 1.23, 95% CI 0.73–2.06 $P = 0.44$). Genotype distribution of rs1801282 of the *PPARG* gene was in Hardy–Weinberg equilibrium in both cases and controls,

Table 2 | Genotypic distribution of the *PPARG* gene

SNP	Genotype	Control (%) (n = 200)	Case (%) (n = 200)	Odds ratio		
				Dominant OR (95% CI)	Recessive OR (95% CI)	Co-dominant OR (95% CI)
				CC vs CG + GG	CC + CG vs GG	CC vs CG, GG
rs1801282	CC	158 (79)	167 (83.5)	0.68 (0.40 – 1.16)	0.66 (0.15 –2.96)	0.69 (0.40 –1.21)
	CG	37 (18.5)	30 (15)	P = 0.15	P = 0.57	0.62 (0.14 –2.78)
	GG	5 (2.5)	3 (1.5)			P = 0.36
				CC vs CT + TT	CC + CT vs TT	CC vs CT, TT
rs3856806	CC	163 (81.5)	157 (78.5)	1.23 (0.73 – 2.06)	1.13 (0.26 – 4.88)	1.23 (0.72 –2.12)
	CT	34 (17)	37 (18.5)	P = 0.44	P = 0.87	1.17 (0.27 –5.07)
	TT	3 (1.5)	6 (3)			P = 0.74

Odds ratios (OR) were adjusted for age, sex and body mass index. CI, confidence interval.

but genotype distribution of rs3856806 of *PPARG* showed a moderate deviation from equilibrium (P = 0.049).

We analyzed the association of these two SNPs of the *PPARG* gene with the type 2 diabetes mellitus-related quantitative phenotypes (height, weight, BMI, creatinine, total cholesterol, LDL cholesterol, VLDL cholesterol, high-density lipoprotein cholesterol and triglycerides) using multiple linear regression analysis in control and case participants after adjusting for the effects of age, sex and BMI. However, multiple linear regression analysis did not show any significant association of these two SNPs (rs1801282 and rs3856806) with related quantitative phenotypes (data not shown).

DISCUSSION

The present study evaluated the potential association of *PPARG* gene polymorphisms in patients with type 2 diabetes mellitus in West Bengal, India. The major finding of the present study was that the *PPARG* gene is not associated with diabetes mellitus in West Bengal, India. We found only two SNPs (rs1801282 and rs3856806) in the entire coding region of the *PPARG* gene. In the present study, we investigated the impact of these two polymorphisms on type 2 diabetes mellitus, but no significant association was observed with *PPARG* genotypes with these variables. The genotype frequency of the rs1801282; (Pro12Ala; P = 0.57) was not significantly different between controls and cases. The protective association of the Ala allele was not confirmed in a South Indian population[17]. In different independent studies, the protective association of the less common "Ala" allele has been consistently reported[18–24].

In the present study, genotype and allele frequencies of the rs3856806 variant were also not significantly different between controls and type 2 diabetes mellitus participants. This finding replicates the results of the earlier reports from different ethnic origins[25–27]. Conversely, several studies had shown T minor allele (C>T) of rs3856806 (His478His) to be associated with obese individuals.

In conclusion, the present findings suggest that the *PPARG* gene (rs1801282 and rs3856806) does not show any significant association with type 2 diabetes mellitus in the population of West Bengal, India. Since the lack of association might come from the small sample size, further studies with larger sample size are required to verify the present observation.

ACKNOWLEDGEMENTS

The present study was supported by a BRNS grant from DAE, Government of India. We thank all the participants and are grateful for their contribution to this study. The authors declare that they have no conflicts of interest.

REFERENCES

1. American Diabetes Association (ADA). Diagnosis and classification of diabetes mellitus. *Diabetes care* 2005; 28: S37–S42.
2. Froguel P, Vaxillaire M. Genetic factors in the pathogenesis of type 2 diabetes. In: Pickup JC, Williams G (eds). Textbook of Diabetes. Blackwell Scientific Publishers, Oxford, 1996; 45–58.
3. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995–2025—prevalence, numerical estimates and projections. *Diabetes Care* 1998; 21: 1414–1431.
4. Mohan V. Why are Indians more prone to diabetes? *J Assoc Physicians India* 2004; 52: 468–474.
5. Chandalia M, Abate N, Garg A, *et al.* Relationship between generalized and upper body obesity to insulin resistance in Asian Indian men. *J Clin Endocrinol Metab* 1999; 84: 2329–2335.
6. Ramachandran A, Snehalatha C, Viswanathan V, *et al.* Risk of noninsulin dependent diabetes mellitus conferred by obesity and central adiposity in different ethnic groups: a comparative analysis between Asian Indians, Mexican Americans and whites. *Diabetes Res Clin Pract* 1997; 36: 121–125.
7. Joshi SR. Metabolic syndrome–emerging clusters of the Indian phenotype. *J Assoc Physicians India* 2003; 51: 445–446.

8. McKeigue PM, Pierpoint T, Ferrie JE, *et al.* Relationship of glucose intolerance and hyperinsulinaemia to body fat pattern in South Asians and Europeans. *Diabetologia* 1992; 35: 785–791.
9. Abate N, Chandalia M. The impact of ethnicity on type 2 diabetes. *J Diabetes Complications* 2003; 17: 39–58.
10. Zimmet P, Taylor R, Ram P, *et al.* Prevalence of diabetes and impaired glucose tolerance in the biracial (Melanesian and Indian) population of Fiji: a rural-urban comparison. *Am J Epidemiol* 1983; 118: 673–688.
11. Meigs JB, Cupples LA, Wilson PW. Parental transmission of type 2 diabetes: the Framingham Offspring Study. *Diabetes* 2000; 49: 2201–2207.
12. Francis GA, Fayard E, Picard F, *et al.* Nuclear receptors and the control of metabolism. *Annu Rev Physiol* 2003; 65: 261–311.
13. Mukherjee R, Jow L, Croston GE, *et al.* Identification, characterization, and tissue distribution of human peroxisome proliferator-activated receptor (PPAR) isoforms PPARγ2 versus PPARγ1 and activation with retinoid X receptor agonists and antagonists. *J Biol Chem* 1997; 272: 8071–8076.
14. Rosen ED, Spiegelman BM. PPARγ: a nuclear regulator of metabolism, differentiation, and cell growth. *J Biol Chem* 2001; 276: 37731–37734.
15. Hasstedt SJ, Ren QF, Teng K, *et al.* Effect of the peroxisome proliferator-activated receptor-γ2 Pro12Ala variant on obesity, glucose homeostasis, and blood pressure in members of familial type 2 diabetic kindreds. *J Clin Endocrinol Metab* 2001; 86: 536–541.
16. Knouff C, Auwerx J. Peroxisome proliferator-activated receptor-γ calls for activation in moderation: lessons from genetics and pharmacology. *Endocr Rev* 2004; 25: 899–918.
17. Radha V, Vimaleswaran KS, Babu HN, Abate N, *et al.* Role of genetic polymorphism peroxisome proliferator-activated receptor-gamma2 Pro12Ala on ethnic susceptibility to diabetes in South-Asian and Caucasian subjects: evidence for heterogeneity. *Diabetes care* 2006; 29: 1046–1051.
18. Altshuler D, Hirschhorn JN, Klannemark M, *et al.* The common PPAR gamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 2000; 26: 76–80.
19. Deeb SS, Fajas L, Nemoto M, *et al.* A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 1998; 20: 284–287.
20. Hara K, Okada T, Tobe K, *et al.* The Pro12Ala polymorphism in PPAR gamma2 may confer resistance to type 2 diabetes. *Biochem Biophys Res Commun* 2000; 271: 212–216.
21. Mori H, Ikegami H, Kawaguchi Y, *et al.* The Pro12 → Ala substitution in PPAR-gamma is associated with resistance to development of diabetes in the general population: possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. *Diabetes* 2001; 50: 891–894.
22. Douglas JA, Erdos MR, Watanabe RM, *et al.* The peroxisome proliferator-activated receptor-gamma2 Pro12A1a variant: association with type 2 diabetes and trait differences. *Diabetes* 2001; 50: 886–890.
23. Kawasaki I, Tahara H, Emoto M, *et al.* Impact of Prol2Ala variant in the peroxisome proliferator-activated receptor (PPAR) gamma2 on obesity and insulin resistance in Japanese Type 2 diabetic and healthy subjects. *Osaka City Med J* 2002; 48: 23–28.
24. Sanghera DK, Ortega L, Han S, *et al.* Impact of nine common type 2 diabetes risk polymorphisms in Asian Indian Sikhs: PPARG2 (Pro12Ala), IGF2BP2, TCF7L2 and FTO variants confer a significant risk. *BMC Med Genet* 2008; 9: 59.
25. Meirhaeghe A, Fajas L, Helbecque N, *et al.* A genetic polymorphism of the peroxisome proliferator–activated receptor–gamma gene influences plasma leptin levels in obese humans. *Hum Mol Genet* 1998; 7: 435–440.
26. Poulsen P, Andersen G, Fenger M, *et al.* Impact of two common polymorphisms in the PPARgamma gene on glucose tolerance and plasma insulin profiles in monozygotic and dizygotic twins: thrifty genotype, thrifty phenotype, or both? *Diabetes* 2003; 52: 194–198.
27. Tavares V, Hirata RD, Rodrigues AC, *et al.* Effect of the peroxisome proliferator–activated receptor–gamma C161T polymorphism on lipid profile in Brazilian patients with type 2 diabetes mellitus. *J Endocrinol Invest* 2005; 28: 129–136.
28. Gonzalez JR, Armengol L, Sole X, *et al.* SNPassoc: an R package to perform whole genome association studies. *Bioinformatics* 2007; 23: 644–645.
29. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003; 19: 149–150.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1| Polymerase chain reaction primers for coding region analysis of the *PPARG* gene.