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

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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. 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. Indians are one of the ethnic groups that are considered to be a high-risk population for diabetes. Compared with Europeans, Indians have greater insulin resistance capability, which promotes susceptibility to diabetes. 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.

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. PPARG shows different protein isoforms generated by different promoters and alternative splicing. The functional role of PPARG has been well documented, and its alterations have been widely associated with type 2 diabetes and obesity. To date, most of the mutation analysis has focused on the coding region of the PPARG gene. In different independent studies, the association of the PPARG gene with type 2 diabetes mellitus has been consistently reported. 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
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).

Statistical Analysis
Hardy–Weinberg equilibrium (HWE) in cases and controls was tested using the χ²-test. The genotype and allele frequencies in type 2 diabetes mellitus cases were compared with control subjects using the χ²-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 0.89/0.10 v 0.88/0.12, respectively. The adjusted ORs under a recessive model could not provide any strong association for rs1801282 (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.
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. In different independent studies, the protective association of the less common “Ala” allele has been consistently reported.

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. 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.

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**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.