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PPARG genotypes are not a major modifiers of chronic kidney disease progression among the diabetic nephropathy patients

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Abstract

Aim: Diabetic nephropathy (DN), classically defined by the presence of proteinuria is one of the major late microvascular complications of type 1 and type 2 diabetes mellitus and leading to a decline in renal function. The present study is aimed to understand the potential modifier effect of *PPARG* gene on the advancement of chronic kidney disease in DN.

Methods: A total of 187 DN patients (101 male and 86 female) with persistent urine albuminuria (>300 mg/L) were included in the study. The KASPar SNP genotyping method (KBioscience, Herts., UK) was adopted for genotyping three *PPARG* gene polymorphisms (rs10865710: -681C>G; rs1801282: Pro12Ala; rs3856806: 1431C>T). The interaction between *PPARG* genotypes and poor glycemic status or hyperlipidemia in chronic kidney disease (CKD) progression was analyzed using Mantel-Haenszel stratified analysis. We performed a multivariate logistic regression analysis to identify the adjusted effects of risk factors on CKD progression in DN.

Results: In univariate analysis, the hyperlipidemia, glycemic control, duration of diabetes mellitus and the PPARG polymorphisms did not show a significant association with the advancement of CKD. In multivariate analysis, none of the SNPs of *PPARG* showed significant association with CKD risk. No confounding effect of *PPARG* genotypes was observed.

Conclusions: Our results suggest that *PPARG* gene is not a major risk factor for susceptibility to the progression of CKD in South Indian DN patients.

Keywords: Diabetic nephropathy, PPARG, Pro12Ala, Chronic kidney disease, Diabetic kidney disease

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Introduction

Diabetic nephropathy (DN), classically described by the presence of proteinuria, is one of the major late microvascular complications of type 1 and type 2 diabetes and leading to a decline in renal function (1). In fact, epidemiological studies have linked DN with long-standing severe hyperglycemia and its complications such as production of advanced glycation end products, reactive oxygen species, anomalous activation of signaling cascades (protein kinase C) and abnormal stimulation of hemodynamic regulation systems (2). Hence the etiology of DN is multifactorial, including both genetic and environmental factors. The variability seen in the incidence and prevalence of DN corresponds with multi-genetic predisposition to the development of DN. Although the role of genetic susceptibility to the development of DN is evidenced by family aggregation (3), no major gene locus that contributes to its susceptibility has yet been identified (4).

Peroxisome proliferator-activated receptor gamma (PPARγ) is an important transcription factor for lipid and glucose metabolism. *PPARG* mRNA has been identified in renal medullary collecting duct, renal glomeruli and renal micro-vasculature (5). *PPARG* is known to modulate insulin resistance, blood glucose, blood pressure, plasma adiponectin level, circulating non-esterified fatty acid and insulin-desensitizing cytokines (6-10). *PPARG* is involved in renal hemodynamic and water and sodium transport. As *PPARG* shows renoprotective effects, the *PPARG* agonists have been evaluated for their renoprotective effects using animal models of diabetes and chronic kidney diseases (CKDs).

Objectives

The *PPARG* gene is more than 100 kb long and located on 3q25, and is composed of 9 exons. Several studies have

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Implication for health policy/practice/research/ medical education

PPAR agonists are potential renoprotective therapeutic agents that would prevent the development or the progression of diabetic nephropathy. This study helps in identifying the exact role of PPARG polymorphism to predict the progression of chronic kidney disease in diabetic nephropathy.

investigated the association between *PPARG* SNPs and DN risk, but the results are inconclusive. In the present study the role of *PPARG* SNPs was investigated to unravel the *PPARG* gene modifier effect for CKD progression in patients with DN.

Materials and Methods

In the present study, only 187 DN patients (101 male and 86 female) with persistent urine albuminuria (>300 mg/L) in two consecutive measurements were included. Department Nephrology of Sri Ramachandra University, Chennai is the main source of DN patients. The CKD stages of all the DN patients were assessed based on recommendations of the National Kidney Foundation (11). Further, DN patients were divided into two groups such as early stages (CKD 1-3 stages) and advanced (CKD 4 and 5 stages) stages (12). About 3 mL of peripheral blood samples was collected from all patients, and DNA was extracted using the standard protocol (13).

Three SNPs of *PPARG* (rs10865710: -681C>G; rs1801282: Pro12Ala; rs3856806: 1431C>T) were analysed using the Fluorescent Resonance Energy Transfer (FRET)-based KASPar methodology. Briefly, 20 ng of genomic DNA is amplified using a PCR reaction containing 1× KASP reaction mix, 12 µM each allele-specific forward primer and 30 µM reverse primer (KBioscience, Hoddesdon, UK). PCR amplifications were performed in 5 µL reactions. The fluorescent endpoint readings were measured using the ABI7900 SDS software (ABI Prism 7900, Foster City, CA, USA).

Ethical issues

The research followed the tenets of the Declaration of Helsinki. Institutional ethical committee of Sri Ramachandra University, Chennai, India, has approved the study protocol. Informed consent was obtained before commencing the study.

Statistical analysis

The genotype distribution for each SNP was evaluated for Hardy-Weinberg equilibrium by using chi-square goodness-of-fit test. Allele frequencies were determined by direct gene counting method. The association between *PPARG* polymorphisms and the CKD status was analyzed using univariate logistic regression. The interaction between PPARG genotypes and poor glycemic status or hyperlipidemia in CKD progression was analyzed using Mantel-Haenszel stratified analyses. All the statistical analysis was carried out using the IBM SPSS Statistics V 18.0 (IBM Corporation, Armonk, New York, USA).

Results

Clinical characteristics of DN patients are given in Table 1. The mean age of the study participants was 56.3 ± 12.4 years and 154 (82.4) of them are above 45 years of age. All polymorphisms followed Hardy-Weinberg equilibrium. The odds ratios and 95% confidence intervals for various risk factors and *PPARG* genotypes were depicted in Figure 1. Male gender, duration of diabetes, hyperlipidemia, smoking and alcoholism showed a trend of increased risk of CKD but the *PPARG* variants showed a trend of decreased risk of CKD. However these associations are not statistically significant in univariate analysis (Figure 1). No evidence of heterogeneity of the effect of hyperlipidemia or poor glycemic control on CKD progression was observed among different genotypes of *PPARG* SNPs (Table 2). This indicated lack of potential confounding effect on the relationship between progression of CKD and hyperlipidemia or progression of CKD and poor glycemic control. In multivariate analysis, none of the *PPARG* SNPs showed significant association with increased or decreased CKD risk, when corrected for other risk factors like age, male gender, hyperlipidemia, duration of diabetes mellitus and glycemic control (Table 3).

Tables 1. Baseline characters of the diabetic nephropathy subjects

Variable	Measure
Age (y)	56.3±12.4
<45	33 (17.6)
>45	154 (82.4)
Sex	
Male	101 (54.0)
Female	86 (46.0)
RBS	182.8±91.3
Good glycemic control	115 (61.5)
Poor glycemic control	72 (38.5)
Serum creatinine	3.2±2.2
CKD	
Early stages	98 (52.4)
Advanced stages	89 (47.6)
Duration of diabetes (y)	
5-9	72 (38.5)
10-14	52 (27.8)
>15	63 (33.7)
Hyperlipidemia	
No	100 (53.5)
Yes	87 (46.5)
Smoking	
No	101 (54.0)
Yes	86 (46.0)
Alcohol	
No	104 (55.6)
Yes	83 (44.4)
Family h/o DM	
No	93 (49.7)
Yes	94 (50.3)

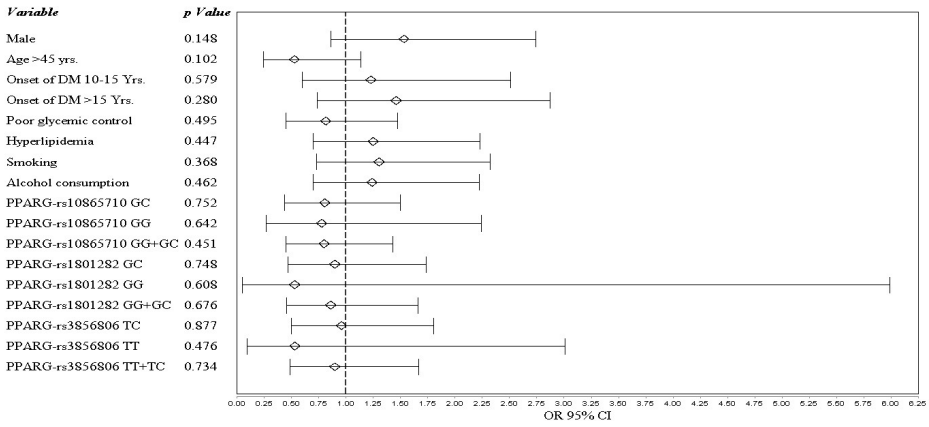

Figure 1. Effects of risk factors and PPARG polymorphisms their association with CKD stages in DN patients.

Table 2. Interaction of poor glycemic control and hyperlipidemia with CKD progression in different *PPARG* genotypes

Gene	Genotype	Early vs. advanced OR (95% CI)	P value*
Poor glycemic control			
Rs10865710	CC	0.62 (0.28-1.36)	
	GC	0.93 (0.33-2.59)	
	GG	3.13 (0.38-25.7)	0.335
M-H combined		0.82 (0.45-1.48)	
Rs1801282	CC	0.71 (0.35-1.42)	
	GC	1.11 (0.35-3.54)	
	GG	-	0.521
M-H combined		0.82 (0.46-1.49)	
Rs3856806	CC	0.64 (0.31-1.32)	
	TC	1.43 (0.48-4.25)	
	TT	1.00 (0.03-29.8)	0.481
M-H combined		0.82 (0.46-1.49)	
Hyperlipidemia			
Rs10865710	CC	1.08 (0.50-2.33)	
	GC	1.38 (0.52-3.67)	
	GG	3.13 (0.38-25.6)	0.633
M-H combined		1.28 (0.72-2.29)	
Rs1801282	CC	1.18 (0.60-2.32)	
	GC	1.40 (0.45-4.38)	
	GG	-	0.707
M-H combined		1.27 (0.71-2.26)	
Rs3856806	CC	1.12 (0.56-2.25)	
	TC	1.97 (0.66-5.88)	
	TT	0.33 (0.01-11.4)	0.521
M-H combined		1.27 (0.71-2.26)	

M-H: Mantel-Haenszel; OR: odds ratio; CI: confidence interval.
*Homogeneity test.

Discussion

Analysis of three SNPs within the *PPARG* gene did not show any significant association with CKD progression in DN patients. Earlier studies performed in diabetic animals and *in vitro* cells also provided evidence for the beneficial action of *PPARG* in diabetic kidney disease (14, 15). As *PPARG* receptors are localized in the endothelium and vascular smooth muscle cell, improvement in hemodynamic profiles upon treatment using *PPARG* agonists could reflect not only improvement in endothelial function but also direct vasodilator effects (16). Rosiglitazone, a PPAR agonist improved hemodynamic status in type 2 diabetic patients by reducing endothelial dysfunction and microalbuminuria (17).

Analysis of 30 polymorphisms of 26 candidate genes in Japanese CKD patient revealed that the *PPARG* gene is one of the susceptibility loci for hypertension induced CKD (18). Further, no significant associations between the *PPARG* SNPs and the risk of CKD were documented in Japanese Multi-Institutional Collaborative Cohort Study (19). Although *PPARG* C161T polymorphism was not associated with the renal survival rate in histologically confirmed immunoglobulin A nephropathy (IgAN) patients, further stratified analysis showed better renal survival in individuals with mutant genotypes and without hypertension (20). The *PPARG*-681G allele was associated with increased height and plasma low-density lipoprotein cholesterol concentrations in a French population (21).

Pro12Ala polymorphism of *PPARG* gene is one of the most extensively studied functional polymorphism. The Ala12 allele is associated with decreased binding affinity to promoters and thereby reduces its expression. Numerous studies have evaluated the association between *PPARG* Pro12Ala and DN, such studies have also been somewhat disappointing with respect to lack of consistency of findings. Protective effect of the Ala12 allele against DN was demonstrated in studies using Berlin (22) and Brazilian patients with type 2 diabetes (23). Further, Ala12 allele carriers had reduced prevalence of microalbuminuria and this effect is overshadowed by duration of diabetes and systolic blood pressure in the Oji-Cree population of Canada (24). In contrast this, Han Chinese (25), African-Americans (26), and Turkish (27), and Indian populations (28) showed no association between *PPARG* Pro12Ala and DN. Comparing gene expression in mesenchymal

Table 3. Adjusted effects of risk factors on CKD stages in diabetic nephropathy

Factors	OR (95%CI)	*P* value[a]
Sex: male vs female	1.54 (0.84-2.82)	0.161
Age: ≥45 vs <45	0.51 (0.23-1.13)	0.097
Onset of DM: 10-14 y vs <10 y	1.15 (0.54-2.44)	0.713
Onset of DM: ≥15 y vs <10 y	1.41 (0.69-2.91)	0.347
Glycemic control: poor vs good	0.82 (0.44-1.55)	0.549
Hyperlipidemia Yes vs No	1.19 (0.65-2.17)	0.570
rs10865710: GC vs CC	0.82 (0.34-1.95)	0.647
rs10865710: GG vs CC	0.95 (0.24-3.70)	0.939
rs1801282: GC vs CC	1.05 (0.39-2.86)	0.923
rs1801282: GG vs CC	0.52 (0.02-11.5)	0.681
rs3856806: TC vs CC	0.98 (0.43-2.20)	0.956
rs3856806: TT vs CC	0.90 (0.12-6.83)	0.923

[a]Wald's test.

stem cells isolated from bone marrow and adipose tissues of CKD and control rats demonstrated up-regulation of *PPARG* in both groups (29). A recent study revealed that the *PPARG* Pro12Ala polymorphism is not associated with all-cause mortality in patients with type 2 diabetes mellitus (30).

Conclusion

In summary, we observed that the SNPs of *PPARG* gene were not implicated in the advancement of CKD in DN. However, further complementary studies that include larger sample sizes and well characterized functional SNPs is necessary to clarify the role of the *PPARG* gene in the development of CKD in DN in the study population.

Authors' contribution

PS, ER and LVKS defined the research theme. RVM designed methods and experiments as well as conducting the laboratory experiments. RVM and LVKSB analyzed the data, interpreted the results and wrote the paper. All authors have contributed to, seen and approved the manuscript.

Conflicts of interest

There are no conflicts of interests.

Ethical considerations

The authors of this manuscript declare that they all have followed the ethical requirements for this communication. Also, Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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