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

\textbf{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 \textit{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).

\textbf{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.

\textbf{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 \textit{PPARG} polymorphisms and the CKD status was analyzed using univariate logistic regression. The interaction between \textit{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).

\textbf{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 \textit{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 \textit{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 \textit{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 \textit{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).

\begin{table}
\caption{Baseline characters of the diabetic nephropathy subjects}
\begin{tabular}{|l|c|}
\hline
\textbf{Variable} & \textbf{Measure} \\
\hline
Age (y) & 56.3±12.4 \\
<45 & 33 (17.6) \\
>45 & 154 (82.4) \\
\hline
Sex & \\
Male & 101 (54.0) \\
Female & 86 (46.0) \\
\hline
RBS & 182.8±91.3 \\
\hline
Good glycemic control & 115 (61.5) \\
Poor glycemic control & 72 (38.5) \\
\hline
Serum creatinine & 3.2±2.2 \\
\hline
CKD & \\
Early stages & 98 (52.4) \\
Advanced stages & 89 (47.6) \\
\hline
Duration of diabetes (y) & \\
5-9 & 72 (38.5) \\
10-14 & 52 (27.8) \\
>15 & 63 (33.7) \\
\hline
Hyperlipidemia & \\
No & 100 (51.5) \\
Yes & 87 (46.5) \\
\hline
Smoking & \\
No & 101 (54.0) \\
Yes & 86 (46.0) \\
\hline
Alcohol & \\
No & 104 (55.6) \\
Yes & 83 (44.4) \\
\hline
Family h/o DM & \\
No & 93 (49.7) \\
Yes & 94 (50.3) \\
\hline
\end{tabular}
\end{table}
Table 2. Interaction of poor glycemic control and hyperlipidemia with CKD progression in different PPARG genotypes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Early vs. advanced</th>
<th>OR (95% CI)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Poor glycemic control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rs10865710</td>
<td>CC</td>
<td>0.62 (0.28-1.36)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>0.93 (0.33-2.59)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>3.13 (1.38-10.25)</td>
<td></td>
</tr>
<tr>
<td>M-H combined</td>
<td></td>
<td>0.82 (0.45-1.48)</td>
<td></td>
</tr>
<tr>
<td>Rs1801282</td>
<td>CC</td>
<td>0.71 (0.35-1.42)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>1.11 (0.35-3.54)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M-H combined</td>
<td></td>
<td>0.82 (0.46-1.49)</td>
<td></td>
</tr>
<tr>
<td>Rs3856806</td>
<td>CC</td>
<td>0.64 (0.31-1.32)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>1.43 (0.48-4.25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>1.00 (0.03-29.8)</td>
<td></td>
</tr>
<tr>
<td>M-H combined</td>
<td></td>
<td>0.82 (0.46-1.49)</td>
<td></td>
</tr>
<tr>
<td><strong>Hyperlipidemia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rs10865710</td>
<td>CC</td>
<td>1.08 (0.50-2.33)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>1.13 (0.52-2.67)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>3.13 (1.38-10.25)</td>
<td></td>
</tr>
<tr>
<td>M-H combined</td>
<td></td>
<td>1.28 (0.72-2.29)</td>
<td></td>
</tr>
<tr>
<td>Rs1801282</td>
<td>CC</td>
<td>1.18 (0.60-2.32)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>1.40 (0.45-4.38)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M-H combined</td>
<td></td>
<td>1.27 (0.71-2.36)</td>
<td></td>
</tr>
<tr>
<td>Rs3856806</td>
<td>CC</td>
<td>1.12 (0.56-2.25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>1.97 (0.66-5.88)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>0.33 (0.01-11.4)</td>
<td></td>
</tr>
<tr>
<td>M-H combined</td>
<td></td>
<td>1.27 (0.71-2.36)</td>
<td></td>
</tr>
</tbody>
</table>

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

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

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

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

<table>
<thead>
<tr>
<th>Factors</th>
<th>OR (95%CI)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: male vs female</td>
<td>1.54 (0.84-2.82)</td>
<td>0.161</td>
</tr>
<tr>
<td>Age: ≥45 vs &lt;45</td>
<td>0.51 (0.23-1.13)</td>
<td>0.097</td>
</tr>
<tr>
<td>Onset of DM: 10-14 y vs &lt;10 y</td>
<td>1.15 (0.54-2.44)</td>
<td>0.713</td>
</tr>
<tr>
<td>Onset of DM: ≥15 y vs &lt;10 y</td>
<td>1.41 (0.69-2.91)</td>
<td>0.347</td>
</tr>
<tr>
<td>Glycemic control: poor vs good</td>
<td>0.82 (0.44-1.55)</td>
<td>0.549</td>
</tr>
<tr>
<td>Hyperlipidemia Yes vs No</td>
<td>1.19 (0.65-2.17)</td>
<td>0.570</td>
</tr>
<tr>
<td>rs10865710: GC vs CC</td>
<td>0.82 (0.34-1.95)</td>
<td>0.647</td>
</tr>
<tr>
<td>rs10865710: GG vs CC</td>
<td>0.95 (0.24-3.70)</td>
<td>0.939</td>
</tr>
<tr>
<td>rs1801282: GC vs CC</td>
<td>1.05 (0.39-2.86)</td>
<td>0.923</td>
</tr>
<tr>
<td>rs1801282: GG vs CC</td>
<td>0.52 (0.02-11.5)</td>
<td>0.681</td>
</tr>
<tr>
<td>rs1856806: TC vs CC</td>
<td>0.98 (0.43-2.20)</td>
<td>0.956</td>
</tr>
<tr>
<td>rs1856806: TT vs CC</td>
<td>0.90 (0.12-6.83)</td>
<td>0.923</td>
</tr>
</tbody>
</table>

*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 LVKS 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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**References**