J Prev Epidemiol. 2017;2(1):e06 Original

Journal of Preventive Epidemiology

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Insulin receptor gene polymorphisms modify the progression of kidney failure in diabetic nephropathy patients

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Received: 9 Jan. 2017
Accepted: 27 Mar. 2017
ePublished: 5 Apr. 2017

Keywords: Diabetic nephropathy, Chronic kidney disease, End-stage renal disease

Citation: Velayuthan MR, Elumalai R, Periyasamy S, Lakkakula BVKS. Insulin receptor gene polymorphisms modify the progression of kidney failure in diabetic nephropathy patients. J Prev Epidemiol. 2017;2(1):e06.

Abstract

Introduction: Diabetic nephropathy (DN) is one of the most common causes of end-stage renal disease (ESRD). Development and progression of DN result from a combination of genetic susceptibility and metabolic and hemodynamic abnormalities. Many studies have shown that insulin resistance can also occur in the early stages of renal disease.
Objectives: The aim of the present study is to investigate the role of insulin signalling pathway gene (*INSR, IRS1, IRS2* and *PPARG*) polymorphisms in the progression of chronic kidney disease (CKD) in diabetic patients.
Patients and Methods: A total of 9 single nucleotide polymorphisms (SNPs) were genotyped in 261 individuals with persistent urine albuminuria using fluorescence resonance energy transfer (FRET)-based KASPar method. Genotypes and haplotypes were compared between early and advanced CKD groups. The effect of genotypes and glycemic control on CKD progression was assessed using univariate and multivariate logistic regression. Interaction between CKD groups and glycemic control was studied using Mantel-Haenszel (M-H)stratified analysis.
Results: Of the 9 SNPs analysed, only rs1801278 deviated Hardy-Weinberg equilibrium. The *INSR* rs2059807 showed decreased risk of CKD progression in non-dialysis patients and *INSR* rs1799817 showed heterogeneity in causing CKD progression in the absence of glycemic control. The *IRS1*, *IRS2* and *PPARG* polymorphisms are not associated with the CKD progression or with uncontrolled glycemic status. Pair-wise linkage disequilibrium (LD) between SNPs of *INSR* or *PPARG* did not reveal strong LD.
Conclusion: The results of our study suggest that the *INSR* gene polymorphisms modify the progression of kidney failure in DN patients. Further, no significant association of CKD risk with the polymorphisms in the *IRS1*, *IRS2* and *PPARG* genes was observed.

Introduction

Type 2 diabetes mellitus (T2DM) is a long-term metabolic disorder that is characterized by impairment of insulin secretion and insulin resistance (1). Undiagnosed or poorly controlled diabetes leads to several microvascular and macrovascular complications. The major microvascular complications include diabetic retinopathy, nephropathy, and neuropathy (2). Diabetic nephropathy (DN) is one of the most common causes of end-stage renal disease (ESRD) in elderly people with T2DM. Development and progression of nephropathy in type 2 diabetes patients seems to result from the interaction of genetic susceptibility with metabolic and hemodynamic changes. Review of the epidemiology studies noted that the hereditary factor plays a marked role in the in the pathogenesis of DN (3).

Core tip
In the present study, we investigated the role of insulin signalling pathway gene polymorphisms in the progression of chronic kidney disease (CKD) in diabetic patients. The IRS1, IRS2 and PPARG polymorphisms are not associated with the CKD progression in diabetic patients. However, this study demonstrated that the INSR gene polymorphisms modify the progression of kidney failure in diabetic nephropathy (DN) patients. Our results support the hypothesis of insulin resistance and glucose intolerance contribute to CKD.

Although genetic linkage analysis (4,5) and association studies have implicated several loci and candidate genes in predisposition to chronic kidney disease (CKD) (6,7), genetic contributions of CKD susceptibility remain largely unknown. Epidemiological studies suggest that inherited

factors play a major role in the pathogenesis of DN (3). Although identification of microalbuminuria serves as a predictor of DN, identification of early renal function deterioration is now accepted as an important clinical indicator of renal impairment in type 2 diabetes individuals (8,9). In diabetic patients, identification of structural abnormalities of the kidneys before the appearance of albuminuria indicates that renal injury is initiated in the prediabetes stages (10).

Objectives

Several lines of evidence demonstrated the involvement of multiple factors in the genesis of CKD induced by metabolic disorders. After initial evidence suggesting that the occurrence of insulin resistance (IR) in the pathogenesis of CKD (11), renewed interest in IR as a cause of CKD occurred. Further, insulin resistance in nondiabetic patients with mild renal dysfunction was also documented (12,13). Therefore gene polymorphisms of insulin signalling pathway may be critical factors in the development of microvascular diabetic complications, including nephropathy. In this context, this study is aimed to examine the association between insulin signalling pathway gene polymorphisms and DN in south Indian population.

Patients and Methods

A total of 261 individuals with persistent urine albuminuria (>300 mg/L) in 2 consecutive measurements or without renal failure were included in this study. All DN cases were recruited through the Department Nephrology of Sri Ramachandra University, Chennai, during November 2013 to February 2014. The subjects were divided into 5 CKD stages based on their glomerular filtration rate (GFR). The stage 5 of CKD is further divided into 2 groups based on hemodialysis. Finally, the study subjects were grouped into 3, group 1 (early; CKD stages 1-3), group 2 (advanced; CKD stage 4 and stage 5 without dialysis) and group 3 (CKD stage 5 on dialysis). Following the clinical assessments by nephrologists, the peripheral blood samples from all subjects were collected for DNA isolation. Genomic DNA was isolated by standard techniques and the single nucleotide polymorphisms (SNPs) of insulin signalling pathway encoding genes such as *INSR* (rs1799817, rs2963, rs2059807 and rs8108622), *IRS1* (Gly-972-Arg: rs1801278), *IRS2* (Gly-1057-Asp; rs1805097) and *PPARG* (-681 C>G: rs10865710; Pro-12-Ala: rs1801282; 1431 C>T: rs3856806) were analysed. The fluorescence resonance energy transfer (FRET)-based KASPar SNP genotyping assay design was chosen for genotyping. Briefly, the assays were performed in a final reaction volume of 5 µL containing 1× KASP reaction mix (KBioscience, Hoddesdon, UK), 0.07 µL of assay mix (12 µM each allele-specific forward primer and 30 µM reverse primer) and 10-20 ng of genomic DNA. The fluorescent endpoint readings were carried out using the 7900 SDS software (ABI Prism 7900, Foster City, CA, USA).

Ethical issues

The institutional ethics committee of Sri Ramachandra University, Chennai has approved the study. All study participants gave written informed consent before participating in the study. This study followed the tenets of the Declaration of Helsinki.

Statistical analysis

Allele frequencies were determined by direct gene counting method. The genotype distribution for each SNP was evaluated for Hardy-Weinberg equilibrium by using the chi-square goodness-of-fit test. The association of between candidate gene polymorphisms and the disease status was assessed via univariate logistic regression analysis. The relationship between different CKD stages and glycemic control was assessed for each genotype by means of Mantel-Haenszel stratified analysis. All the statistical analysis was carried out using SPSS statistical software. Linkage disequilibrium (LD) and haplotype-phenotype analysis were performed using Haploview 3.12 (14).

Results

The mean age of different CKD groups such as early, advanced and ESRD on dialysis is 57.3 ± 11.6 years, 54.8 ± 13.3 years and 52.1 ± 8.6 years respectively. The differences in the age is statistically significant among the groups ($P = 0.010$). The other baseline data among 3 study groups were documented in Table 1. Only serum creatinine and family history of diabetes are statistically significant among the groups. Occurrence of coronary artery disease, stroke, hypoglycemic episodes and family history of CKD are not statistically significant (Table 1).

Genotype and allele frequencies

The genotypic frequencies, minor allele frequencies and Hardy-Weinberg equilibrium for all gene polymorphisms were depicted in Figure 1. The genotype frequencies were in Hardy-Weinberg equilibrium for all SNPs, but only *IRS1* rs1801278 genotypes deviated from HWE proportions (Figure 1).

Association with CKD and ESRD

The comparison of genotype frequencies between early and advanced CKD groups as well as between early and ESRD group on dialysis did not support the association with CKD progression for majority of the SNPs studied (Table 2). The distribution of *INSR* rs2059807 genotypes were significantly different between early and advanced CKD groups ($P = 0.027$). The genotype relative risk that is calculated using the presumed wild-type genotype as reference showed the odds ratios (ORs) are very near to 1 for all SNPs (Table 2), indicating that these SNPs are not contributing to increased or decreased risk of CKD progression for majority of the SNPs studied. However homozygous mutant genotype of *INSR* rs2059807 genotype reduced the risk developing advanced CKD (OR: 0.219; 95% CI: 0.060-0.797; $P = 0.021$).

Table 1. Baseline characters of the study subjects

Variable	Early stage (110)	Advanced stage (77)	ESRD on dialysis (74)	*P* value
Age	57.3 ± 11.6	54.8 ± 13.3	52.1 ± 8.6	0.010
Onset of diabetes (years)	12.1 ± 7.1	12.7 ± 6.6	13.3 ± 7.3	0.545
RBS	182.8 ± 91.3	185.8 ± 103.3	191.6 ± 97.2	0.831
Serum creatinine	1.6 ± 1.0	5.5 ± 1.1	8.5 ± 1.7	<0.001
Smoking				0.050
No	61	40	52	
Yes	49	37	22	
Alcohol				0.001
No	61	42	62	
Yes	48	35	12	
CAD				0.868
No	75	51	52	
Yes	35	26	22	
Stroke				0.479
No	108	76	71	
Yes	2	1	3	
Hypoglycemic episodes				0.695
No	93	64	59	
Yes	17	13	15	
Family history of DM				0.036
No	51	42	25	
Yes	59	35	49	
Family history of CKD				0.951
No	98	69	67	
Yes	12	8	7	

Abbreviations: CAD, coronary artery disease; RBS, random blood sugar; DM: diabetes mellitus; CKD, chronic kidney disease.

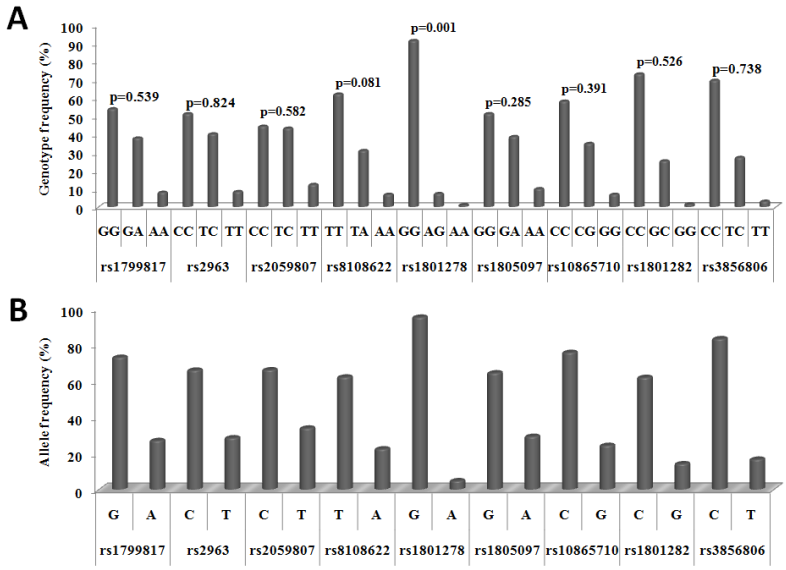

Figure 1. Distribution of genotype (A) and allele frequencies (B) of various gene polymorphisms in the study subjects. *P* values presented above each genotype are of Hardy-Weinberg equilibrium for the respective polymorphism.

Glycemic control and CKD progression

Distribution of gene polymorphisms in controlled (HbA1c <6.5) and uncontrolled glycemic status (HbA1c >6.5) groups is not statistically different among the study groups (Table 3), indicating that these polymorphisms are not influencing the variation in the glycemic control of CKD patients. To know the interaction of uncontrolled diabetes with CKD progression we performed Mantel–Haenszel (M-H) heterogeneity test independently. The results of M-H analysis for uncontrolled diabetes

Table 2. Association of various gene polymorphisms and CKD development

Gene	Genotype	Early stage n (%)	Advance stage n (%)	ESRD on dialysis n (%)	Early vs. Advanced		Early vs. ESRD on dialysis	
					OR (95% CI)	*P* value	OR (95% CI)	*P* value
				INSR				
rs1799817	GG	59 (53.6)	42 (54.5)	40 (54.1)	0.630*		0.326*	
	GA	43 (39.1)	32 (41.6)	24 (32.4)	1.045 (0.571-1.914)	0.886	0.823 (0.434-1.562)	0.552
	AA	8 (7.3)	3 (3.9)	10 (13.5)	0.527 (0.132-2.104)	0.364	1.844 (0.67-5.076)	0.236
rs2963	CC	60 (54.5)	34 (44.2)	40 (54.1)	0.339*		0.823*	
	TC	40 (36.4)	36 (46.8)	29 (39.2)	1.588 (0.858-2.941)	0.141	1.087 (0.583-2.028)	0.792
	TT	10 (9.1)	7 (9.1)	5 (6.8)	1.235 (0.431-543)	0.694	0.750 (0.239-2.358)	0.623
rs2059807	CC	50 (45.5)	36 (46.8)	30 (40.5)	0.027*		0.484*	
	TC	41 (37.3)	38 (49.4)	34 (45.9)	0.287 (0.696-2.381)	0.421	1.382 (0.728-2.625)	0.323
	TT	19 (17.3)	3 (3.9)	10 (13.5)	0.219 (0.060-0.797)	0.021	0.877 (0.360-2.135)	0.773
rs8108622	TT	72 (65.5)	47 (61)	43 (58.1)	0.058*		0.564*	
	TA	28 (25.5)	29 (37.7)	24 (32.4)	1.587 (0.840-2.998)	0.155	1.435 (0.739-2.786)	0.286
	AA	10 (9.1)	1 (1.3)	7 (9.5)	0.153 (0.019-1.236)	0.078	1.172 (0.415-3.307)	0.764
				IRS1				
rs1801278	GG	101 (91.8)	69 (89.6)	69 (93.2)	0.761*		0.997*	
	AG	7 (6.4)	7 (9.1)	5 (6.8)	1.464 (0.491-4.360)	0.494	1.046 (0.319-3.429)	0.941
	AA	2 (1.8)	1 (1.3)	0 (0)	0.732 (0.065-8.23)	0.800	-	-
				IRS2				
rs1805097	GG	57 (51.8)	39 (50.6)	38 (51.4)	0.313*		0.748*	
	GA	39 (35.5)	33 (42.9)	29 (39.2)	1.237 (0.667-2.292)	0.500	1.115 (0.593-2.098)	0.735
	AA	14 (12.7)	5 (6.5)	7 (9.5)	0.522 (0.174-1.567)	0.246	0.750 (0.277-2.030)	0.571
				PPARG				
rs10865710	CC	62 (56.4)	44 (57.1)	46 (62.2)	0.958*		0.324*	
	CG	39 (35.5)	26 (33.8)	26 (35.1)	0.939 (0.501-1.762)	0.846	0.899 (0.481-1.680)	0.738
	GG	9 (8.2)	7 (9.1)	2 (2.7)	1.096 (0.379-3.165)	0.866	0.30 (0.062-1.453)	0.135
rs1801282	CC	80 (72.7)	56 (72.7)	55 (74.3)	0.961*		0.953*	
	GC	28 (25.5)	20 (26)	18 (24.3)	1.020 (0.523-1.99)	0.953	0.935 (0.472-1.854)	0.848
	GG	2 (1.8)	1 (1.3)	1 (1.4)	0.714 (0.063-0.070	0.786	0.727 (0.064-8.219)	0.797
rs3856806	CC	75 (68.2)	53 (68.8)	54 (73)	0.925*		0.775*	
	TC	31 (28.2)	22 (28.6)	18 (24.3)	1.004 (0.524-1.923)	0.990	0.806 (0.409-1.589)	0.534
	TT	4 (3.6)	2 (2.6)	2 (2.7)	0.708 (0.125-4.005)	0.696	0.694 (0.123-3.929)	0.680

Abbreviations: ESRD, end-stage renal disease; OR, Odds ratio; CKD, chronic kidney disease.

were presented respectively in Table 3, indicating no confounding effects on the relationship between CKD progression and uncontrolled glycemic status except for rs1799817 (M-H test $P=0.048$). Although the rs1799817 mutant homozygous genotypes showed increased risk for uncontrolled glycemic status (OR: 14.0; 95% CI: 0.579-338.7), evidence of confounding effect was found with the M-H combined OR for uncontrolled diabetes as 0.845 and 95% CI as 0.467-1.528 (Table 3). This indicates that the rs1799817 showed heterogeneity in causing CKD progression in the absence of good glycemic control.

Linkage disequilibrium and haplotypes

Paired LD of *PPARG* and *INSR* gene polymorphisms along with their LD measures (D' and r^2) were respectively presented in Figure 2A and 2B. As indicated by the lower r^2, these polymorphisms were completely not in LD and form no haplotype block, suggesting that the haplotypes of these polymorphisms may not contribute to phenotype.

Discussion

Analysis of important polymorphisms of 4 important genes involved in insulin signaling and resistance such as *INSR* (Insulin receptor), *IRS1* (Insulin receptor substrate 1), *IRS2* (Insulin receptor substrate 2) and *PPARG* (peroxisome proliferator-activated receptor-γ) in 261 DN patients revealed that these SNPs are polymorphic in the study subjects. The *IRS1*, *IRS2* and *PPARG* polymorphisms are not associated with the CKD progression. Only *INSR* rs2059807 showed decreased risk of CKD progression in non-dialysis patients. Out of 9 analyzed polymorphic variants only *INSR* rs1799817 showed heterogeneity in causing CKD progression in the absence of glycemic control. Remaining polymorphisms failed to show association with uncontrolled glycemic status. Pair-wise LD between SNPs of *INSR* or *PPARG* did not reveal strong LD.

Diabetes and DN are the most common causes of CKD. Few studies have explored the relationship between T2DM genes and etiology of DN. In Taiwanese T2DM patients, SNPs from the T2DM-related genes increased the risk of DN independently or in an interactive manner (15). Insulin receptor gene (*INSR*) plays an important role in insulin metabolism. The insulin receptor is also

Table 3. Association between CKD stages and diabetes control stratified by genotypes

Gene	Genotype	Early vs. advanced	
		OR (95% CI) for HbA1c >6.5	*P* value*
		INSR	
rs1799817	GG	1.114 (0.491-2.527)	
	GA	0.434 (0.167-1.130)	
	AA	14.0 (0.579-338.7)	0.048
M-H-Combined		0.845 (0.467-1.528)	
rs2963	CC	0.540 (0.215-1.356)	
	TC	1.5 (0.604-3.724)	
	TT	0.250 (0.021-2.945)	0.178
M-H-Combined		0.819 (0.446-1.503)	
rs2059807	CC	0.922 (0.379-2.241)	
	TC	0.771 (0.318-1.869)	
	TT	0.556 (0.043-7.214)	0.932
M-H-Combined		0.889 (0.480-1.647)	
rs8108622	TT	1.010 (0.482-2.115)	
	TA	0.672 (0.210-2.149)	
	AA	0.875 (0.673-1.137)	0.703
M-H-Combined		0.875 (0.471-1.624)	
		IRS1	
rs1801278	GG	1.002 (0.533-1.886)	
	GA	0.160 (0.016-1.627)	
	AA	0.500 (0.125-1.999)	0.219
M-H-Combined		0.846 (0.465-1.538)	
		IRS2	
rs1805097	CC	1.071 (0.462-2.482)	
	TC	0.914 (0.351-2.385)	
	TT	0.188 (0.016-2.137)	0.381
M-H-Combined		0.879 (0.481-1.606)	
		PPARG	
rs10865710	CC	0.766 (0.343-1.710)	
	CG	0.711 (0.248-2.039)	
	GG	3.125 (0.382-25.57)	0.425
M-H-Combined		0.848 (0.464-1.552)	
rs1801282	CC	0.770 (0.378-1.569)	
	GC	1.030 (0.319-3.329)	
	GG	2.00 (0.50-7.997)	0.599
M-H-Combined		0.862 (0.472-1.573)	
rs3856806	CC	0.479 (0.357-1.571)	
	TC	0.154 (0.384-3.471)	
	TT	1.00 (0.034-29.81)	0.812
M-H-Combined		0.861 (0.471-1.573)	

Abbreviations: M-H, Mantel-Haenszel; CKD, chronic kidney disease.
*Homogeneity test *P* value.

expressed throughout the nephron (16), but a few pieces of animal data suggested about its precise role in glomerular function (17). The *INSR* gene is located on chromosome 19 and composed of 22 exons (18). Although *INSR* polymorphisms were studied well with regard to insulin sensitivity in polycystic ovary syndrome (PCOS) (19), studies relating to *INSR* gene with DN or CKD are very less (20). *INSR* rs2059806 plays a role in the predisposition of the Han Chinese population to T2DM and DN (21).

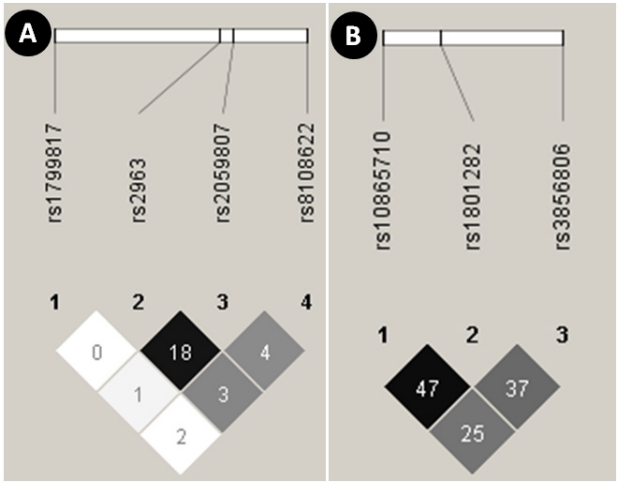

Figure 2. Linkage disequilibrium profiles in *INSR* (A) and *PPARG* (B) gene single-nucleotide polymorphisms.
Colour coding represents the D'/LOD values and the values in cells are r^2 multiplied by 100.

In the present study *INSR* rs2059807 showed decreased risk of CKD progression in non-dialysis patients and *INSR* rs1799817 showed heterogeneity in causing CKD progression in the absence of glycemic control. Although the functional importance of these polymorphisms is not known, the rs1799817 influenced insulin resistance in PCOS in north Indian women (22).

Insulin receptor substrates (IRS1 and IRS2) are well-characterized adapter proteins that relay signals from receptor tyrosine kinases to downstream components of signalling pathways. Mutations in the *IRS1* and *IRS2* genes have been reported to have a role in determining susceptibility to traits related to type 2 diabetes (23). Our study results revealed no significant association of CKD risk with the polymorphisms in the *IRS1* (rs1801278) and *IRS2* (rs1805097) genes. The rs1801278 deviated from Hardy-Weinberg equilibrium in the study subjects. This deviation from Hardy-Weinberg equilibrium in the study group was possible due to these being diabetic patients, not genotyping error. However, the chromosomal regions 2q35-37 and 13q33.3 that were respectively harboring *IRS1* and *IRS2* genes were highlighted to have strongest linkage with GFR in diabetes patients (24,25). An intergenic region between *MYO16* and *IRS2* is associated with the susceptibility to kidney disease in type 1 and 2 diabetes (26). A comprehensive analysis of *IRS1* has suggested that *IRS1* G972R polymorphism (rs1801278) has been associated to such a marked reduction in GFR in Mexican-Americans (24). Subsequent replication study in diabetic patients of European ancestry failed to support the role of *IRS1* G972R in determining GFR (27). In a cohort of DN patients with a range of CKD severity, *IRS2* mRNA levels were elevated approximately 9-fold, with the majority of *IRS2* staining evident in the kidney tubules in DN patients (28). The "T" allele of the His1085His polymorphism in the *INSR* gene shows significant protection against diabetes (29). Further studies are certainly needed to deeper address the exact role of *IRS1* G972R and *IRS2* rs1805097 variants on kidney

function in diabetic patients.

Peroxisome proliferator-activated receptor γ (PPARγ) is a critical factor for adipogenesis and glucose metabolism. A functional study has shown that the Ala12 allele decreases the DNA-binding affinity of the PPARγ2, thus reducing its transcriptional activity (30). This successively implies that non-Ala allele carriers show increased insulin resistance, which contributes to the development of DN or higher rate of decline in GFR (31-33). In contrast to this several association studies could not detect the association between the Ala12 allele and the DN (34-37). Another polymorphism rs10865710 (-681C>G) is located in a signal transducer and activator of transcription5B (STAT5B) consensus binding site, and 681G is known to abolishSTAT5B binding to the cognate promoter element and affecting the transactivation of PPARγ3 promoter. The*PPARG*-681C>G polymorphism showed significant association with the risk of CKD among Japanese subjects with hypertension, indicating the biological roles of PPARs in the genesis of CKD also in Asian populations (38).

Conclusion

Our study results revealed that the *INSR* gene polymorphisms modify the progression of kidney failure in DN patients. Further, no significant association of CKD risk with the polymorphisms in the *IRS1*, *IRS2* and *PPARG* genes was observed. While insulin signalling pathway is not sufficient to cause DN, several potentially modifiable mechanisms including circulating hormones, neuroendocrine pathways and chronic inflammation, are said to be involved in the worsening of IR. Replication studies must be conducted by utilising the new high-throughput technologies in order to better understand multiple biochemical pathways by which hyperglycemia induces DN complications.

Limitations of the study

This study has some limitations which have to be pointed out. First, it was a nested study, hence there was a possibility of selection bias. Second, there was the limited sample size in each sub-group of the study. Despite these limitations, analysis of *INSR*, *IRS1*, *IRS2* and *PPARG* gene polymorphisms revealed that the *INSR* polymorphisms had a significant modifier effect on the progression of CKD in DN patients.

Acknowledgements

The authors would like to thank Sri Ramachandra University for providing necessary facilities and the subjects.

Conflicts of interest

There are no conflicts of interests.

Authors' contribution

PS, ER and LVKS defined the research theme. RVM designed methods and experiments, carried out 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.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Funding/Support

No funding from any source.

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