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Original article

Association between *PPARγ rs1801282* polymorphism with diabetic nephropathy and type-2 diabetes mellitus susceptibility in south India and a meta-analysis

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ABSTRACT

Background: Diabetic Nephropathy (DN) is a major complication of Type 2 Diabetes Mellitus (T2DM) with high morbidity rates worldwide.
Objective: To determine the association of PPARγ rs1801282 polymorphism in T2DM and DN in south Indian population.
Methods: We have conducted a case–control study to test the association of rs1801282 polymorphism with T2DM and DN in 424 subjects (DN = 128; T2DM = 148 and controls = 148) belonging to the south Indian population using ARMS-PCR and Sanger sequencing method. Further, a meta-analysis was performed for rs1801282 polymorphism from the published literature retrieved from various electronic databases to determine the susceptibility among T2DM and DN across various ethnic populations under five genetic models.
Results: The genotyping of rs1801282 polymorphism showed significant (p-value < 0.05) association with DN and T2DM compared to controls. In the meta-analysis, no significant association (p-value > 0.05) was noticed for rs1801282 with DN vs. controls in homozygote, heterozygote, allelic, recessive and dominant genetic models. However, a significant association was observed between rs1801282 SNP and T2DM under heterozygote (Jj vs JJ) genetic model with OR = 0.56, (95%CI [0.43–0.74]), $p \leq 0.0001$ of Asian and Caucasian populations.
Conclusion: Overall analysis suggests that the rs1801282 polymorphism might be associated with DN and T2DM. More case–control studies on the PPARγ gene with a larger sample size including all the confounding factors are required to corroborate the findings from this meta-analysis.

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Asociación entre el polimorfismo *rs1801282* de PPARγ con nefropatía diabética y sensibilidad a la diabetes mellitus de tipo 2 en el sur de India y un metaanálisis

Palabras clave:
Nefropatía diabética
PNU
PPARγ
RCP-ARMS
Modelos genéticos

RESUMEN

Antecedentes: La nefropatía diabética (ND) es una complicación importante de la diabetes mellitus de tipo 2 (DMT2) con altas tasas de morbilidad mundial.
Objetivo: Determinar la asociación del polimorfismo *rs1801282* de PPARγ en la DMT2 y la ND en la población del sur de India.
Métodos: Hemos llevado a cabo un estudio de casos y controles para analizar la asociación del polimorfismo *rs1801282* con la DMT2 y la ND en 424 sujetos (ND = 128; DMT2 = 148 y controles = 148) pertenecientes a la población del sur de India mediante RCP-ARMS y método de secuenciación de Sanger. Además, se realizó un metaanálisis para el polimorfismo de *rs1801282* a partir de la literatura publicada en varias bases de datos electrónicas para determinar la sensibilidad entre la DMT2 y la ND en varias poblaciones étnicas con 5 modelos genéticos.
Resultados: El genotipado de polimorfismo *rs1801282* demostró una asociación significativa (valor de $p < 0,05$) con la ND y la DMT2 en comparación con los controles. En el metaanálisis no se observó asociación significativa (valor de $p > 0,05$) de *rs1801282* con la ND frente a los controles en modelos genéticos homocigóticos, heterocigóticos, alélicos, recesivos y dominantes. Sin embargo, se observó una asociación significativa entre el polimorfismo de nucleótido único (PNU) *rs1801282* SNP y la DMT2 en el modelo genético heterocigótico (*Jj* frente a *JJ*) con OR = 0,56, (IC del 95%: 0,43-0,74; $p \leq 0,0001$ de poblaciones asiáticas y caucásicas.
Conclusión: El análisis general sugiere que el polimorfismo *rs1801282* puede asociarse a ND y a DMT2. Se precisan más estudios de casos y controles sobre el gen PPARγ con un tamaño de la muestra mayor que incluya todos los factores de confusión para corroborar los resultados de este metaanálisis.

Introduction

Diabetes Mellitus (T2DM) is a complex metabolic disorder characterized by abnormal lipid, protein metabolism, and hyperglycemia. T2DM is considered as serious health problem globally and ranks as the world's sixth-leading factor causing death.[1] Diabetic Nephropathy is one of the major complication of T2DM and the primary cause of the end-stage renal disease (ESRD), with a high morbidity rate from 25 to 40% globally.[2] Abnormal glycaemic control and diabetes duration are considered as the major risk factors for DN. The pathogenesis and advancement of T2DM to DN is not well established, previous epidemiological and clinical studies have demonstrated the role of hyperlipidemia in the glomerular injury through the initiation and activation of multiple signaling pathways.[3] A meta-analysis was performed in the year 2011 to evaluate the genetic associations with DN from various ethnic populations which revealed 21 significantly associated genes such as APOC1, ACE, AKR1B1, APOE, CHN2, EPO, 'GREM1, NOS3, HSPG2, FRMD3, CPVL, VEGFA, CARS, and UNC13B.[4] In addition, several Genome-Wide Association studies have documented 56 genetic loci associated with T2DM susceptibility. Also, few single nucleotide polymorphisms (SNPs) were reported in candidate genes such as FTO, MC4R, PPARγ, and ADIPOQ with T2DM.[5,6]

Peroxisome Proliferator-Activated Receptor-Gamma (PPARγ) gene belongs to the nuclear hormone receptor subfamily which controls the expression of genes involved in insulin sensitivity, lipogenesis, adipocyte differentiation, inflammation, and metabolic syndrome.[7] PPARγ gene is localized at 3p25.2 spanning 9 exons encodes 505 amino acids. The PPARs mainly comprise nuclear fatty acid receptors, which have a hydrophobic ligand-binding pocket and a zinc finger DNA binding motif (type-II).[8] To date, numerous case–control studies have been conducted to identify the possible relationship between PPARγ gene polymorphisms with the risk of T2DM and DN in various ethnic populations.[9] The most common variant located in exon-2 of PPARγ rs1801282 (Proline-12-Alanine), leading to the substitution of cytosine to guanosine at 34th nucleotide position. This substitution leads to a change in the structure of PPARG protein, which in turn decreases the binding effect of target genes and thereby reducing transcriptional activity.[10] In addition, studies have indicated that rs1801282 polymorphism is found involved in reducing the albuminuria risk and insulin resistance among DM patients.[11] Among worldwide population, Indians belong to the Asian ethnic background, considered as a high-risk

Table 1 – Designed primers for *PPARγ (rs1801282)* genotyping.

Primer-ID[a]	Primer sequence (5′-3′)	Allele	No of base pairs	Tm (°C)	Total length (Bp)
SNP-1 OF-	AAACTGATGTCTTGACTCATGGGTGTATT		29	65	361
SNP-1 OR-	GCAACGAGCTAAGCATTAAAATACTGGA		28	65	
SNP-1 IF-	GAAACTCTGGGAGATTCTCCTATTGTCC	**C**	28	65	221
SNP-1 IR-	GTATCAGTGAAGGAATCGCTTTCAGC	**G**	26	65	194

[a] IF-inner forward, IR-inner reverse, OF-outer forward, OR- outer reverse.

population for T2DM. It has been well documented that Asian Indians have a high waist-hip ratio, greater insulin resistance, increased susceptibility to diabetes and posses the risk of coronary heart disease (CHD) compared with Europeans or the Caucasoids.[12] We conducted a case–control study among the south Indian population belong to Asian ethnic background to determine the rs1801282 association in DN, T2DM, compared to healthy controls, Further, the resulting genotypic frequencies of rs1801282 polymorphism were analyzed with the previously published literature based on the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) criteria to confirm its significance across various populations.

Methods

Sample characteristics

The samples (diabetic nephropathy, type-2 diabetes mellitus, and healthy controls) for this study were recruited from the subjects attending Department of General Medicine, Chettinad Hospital and Research Institute (CHRI), Tamil Nadu, India, from January to June 2016. The selection criteria for DN subjects by clinical examination including the routine biochemical assessment such as excreting urine albuminuria (>300 mg/L) and serum creatinine (>1 mg/dL) were considered and histopathological investigation. The T2DM subjects were diagnosed based on the criteria defined by the World Health Organization (WHO).[13] The controls were selected by following the similar clinical and biochemical examination as done for DN and T2DM. All study participant were age-matched, belonging to South India, Asian ethnicity. The current study protocols were reviewed and approved by the Institutional Human Ethics Committee (03/IHEC/3-16) of the Chettinad Academy of Research and Education. Informed consent was obtained from the participants before the sampling process. Likewise, HIV positive patients, Juvenile diabetic subjects, physically challenged and subjects with cardiac problems were excluded. The demographic data and baseline clinical characteristics from each study subject including gender, age, Body Mass Index (BMI), the onset of disease, symptoms of the disease, family history, diet, and physical activity were recorded at the time sample collection.

Genetic analysis of rs1801282 polymorphism

Venous blood (3 ml) was collected in EDTA coated tube (BD, USA) from the participants to isolate Genomic DNA using the phenol-chloroform method with minor modifications.[14] The genetic analysis of rs1801282 was performed by Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) using allele-specific primers (Table 1).[15] For each PCR reaction, a 10 μL reaction mix was setup containing 25 ng DNA, 1 U TAQ Brazilian Origin, 0.3 mmol of each dNTP, 12 pmol/μL of each primer. The ARMS-PCR program was performed using an ABI Veriti® Thermal Cycler (California, USA). The reaction condition for ARMS-PCR was set as initial denaturation (94 °C, 5 min), denaturation (94 °C, 45 s), annealing (68 °C, 45 s), elongation (72 °C, 45 s) and final elongation at 72 °C for 5 min. The amplicons were visualized on 1.6% agarose gel together with 100 bp DNA Ladder (Dye Plus, Cat no: 3422A, Takara Bio). Finally, the identified genotypes were further confirmed in the randomly selected subset of overall samples (DN = 12; T2DM = 10; controls = 08) using Sanger based sequencing (Applied Biosystems 3130, USA). Additionally, to determine the chromosomal interactions among the SNP variants, we used 3DSNP (http://www.cbportal.org/3dsnp/index.html) software package to generate Circos (circular) plots based on r^2 values for visualizing the genomic data.

Statistical analyses

The statistical analysis for case–control study was executed by SPSS V.21 (IBM-Analytics, USA) software. The allelic and genotypic frequencies of rs1801282 polymorphism in DN, T2DM and healthy controls were calculated using the Pearson Chi-square test. To determine the distribution of SNP in controls, Hardy-Weinberg Equilibrium (HWE value > 0.05) was used. Additionally, their association of polymorphism was analyzed by determining the odds ratio (OR), confidence intervals (95% CIs) in dominant (Jj + jj vs. Jj) (J-major, j-minor allele) and recessive (jj vs. JJ + Jj) genetic models.

Literature search strategy for Meta-analysis

An extensive literature to select the studies published between January 1991 to December 2017, that discuss the association between rs1801282 polymorphism with DN and T2DM from the database of MEDLINE, PubMed, Cochrane Library, EMBASE, and Google Scholar. The multiple key terms such as “Diabetic Nephropathy” “Type 2 Diabetes Mellitus”, “DN”, “T2DM”, “Peroxisome proliferator-activated receptor gamma gene”, “PPARγ gene”, “Polymorphism”, “rs1801282” were used to retrieve the relevant articles published in English Language for the meta-analysis with Diabetic Nephropathy vs. Type 2 Diabetes Mellitus. Similarly, multiple keywords such as “Type 2 Diabetes Mellitus”, “T2DM”, “Peroxisome proliferator-activated receptor gamma gene”, “PPARγ gene”, “Genetic association studies”,

Table 2 – Demographic and base-line clinical characteristics of DN, T2DMand Controls.

Factors	Diabetic nephropathy (N = 128)	Type 2 diabetes mellitus(N = 148)	Controls (N = 148)
Men:women	106:22	96:52	110:38
Mean Age (years)	56.26 ± 8.9	54.75 ± 6.37	54.15 ± 7.50
Body mass index (kg/m²)	21.09 ± 3.3	26.23 ± 3.68	23.08 ± 2.37
Duration of diabetes (years)	11.4 ± 5.97	6.65 ± 4.61	Nil
Family history of diabetes (%)	77	48	33
Controlling insulin levels			
Oral hypoglycaemic drugs	54	108	Nil
Insulin	74	40	Nil
Food habits			
Vegetarian	20	44	30
Non-vegetarian	108	104	118
Physical activity (30 min/day)	18	46	32

Data are presented as mean ± SD.

"SNP", "rs1801282" were used to perform the similar meta-analysis between T2DM vs. controls.

Data extraction for meta-analysis

Studies included in this meta-analysis (Diabetic Nephropathy vs. Type 2 Diabetes Mellitus; Type 2 Diabetes Mellitus vs. controls) were essential to meet the following criteria's: first, the association of PPARγ gene and rs1801282 polymorphism with DN and T2DM, T2DM and controls should be assessed. Second, study should be cases–controls (two groups). Third, the articles should provide adequate data to calculate allele and genotype frequencies for the meta-analysis. The information from the collected articles was extracted by two authors [AH and PA], any discrepancy between the authors was resolved by a group discussion (IR, SSJ, and RK). The study characteristics such as first author name, year of publication, country, ethnicity, the source of DNA, the sample size for cases-controls, genotypic frequency and genotyping method were extracted.

Quality assessment and meta-analysis for rs1801282 polymorphism

The methodological quality assessment of each study was evaluated by two independent variables such as the Hardy-Weinberg Equilibrium (HWE)[16] and the Newcastle Ottawa Scale (NOS).[17] The genotype distribution of included studies among the controls should follow HWE *p*-value above 0.05. The NOS grading system relies on three main factors such as selection, comparability, and exposure. The studies score up to a maximum of nine points, six or above were considered in this meta-analysis. In the meta-analysis, rs1801282 SNP with risk of DN vs. T2DM and T2DM vs. controls, were performed to calculate the pooled odds ratios (OR) and 95% confidence interval (CI) with (p-value < 0.05) under allelic (j vs. J) (J-major, j-minor allele), homozygote (jj vs. JJ), heterozygote (Jj vs. JJ), dominant (Jj + jj vs. JJ) and recessive (jj vs. JJ + Jj) genetic models. The heterogeneity between the included studies in this meta-analysis was assessed by the I^2 test[18] and Q-statistics. Based on the results obtained from heterogeneity (I^2 < 50), fixed effect (Mantel-Haenszel's)[19] method was used, if not, the random-effect method (DerSimonian and Laird's) was applied. The publication bias for meta-analysis was assessed by Begg's funnel plot and Egger's test.[20] Leave-one-out method was executed to validate the consistency of our results.[21] The meta-analysis was performed using Revman V.5.0 (Cochrane Collaboration, UK) and STATA V.12.0 (Stata Corp, USA).

Results

Characteristics of study subjects in case–control study

The baseline clinical characteristics of the study participants (DN = 128; T2DM = 148 and controls = 148) were illustrated in Table 2. Among the samples enrolled in the study, their average ± SD of age in DN, T2DM, and control groups were 56.26 ± 8.9, 54.75 ± 6.37 and 54.15 ± 7.50 years respectively. Similarly, the body mass index (BMI) were (21.09 ± 3.3), (26.23 ± 3.68) and (23.08 ± 2.37) for DN, T2DM and control.

Frequencies rs1801282 polymorphism between DN vs. T2DM

The genotyping of PPARγ gene polymorphism (rs1801282) was performed using ARMS-PCR and Sanger dideoxy sequencing (Fig. 1). The genotype frequency distributions in controls (T2DM) were in concurrence with Hardy–Weinberg Equilibrium (p > 0.05). The allele and genotype frequencies of rs1801282 polymorphisms were assessed to determine the odds ratio (OR), confidence intervals (95%CIs), χ^2 and p-value (Table 3). The genotype distribution of rs1801282 polymorphism was 73.43% for JJ, 17.18% for Jj, 09.37% for jj in DN cases and 67.56%, 27.02%, 5.40% in T2DM, respectively. The analysis of rs1801282 polymorphism illustrated a positive association with DN compared with T2DM (p-value ≤ 0.05) in south India. In heterozygous (Jj) and homozygous recessive genotype (jj) model of rs1801282 showed significant risk association in DN patients compared with T2DM with OR = 1.19 (95%CI [0.75–1.98]) p-value = 0.049, OR = 2.72 (95%CI [0.96–7.67]) p-value = 0.047, respectively. Further, the analysis of dominant and recessive genetic models showed an insignificant difference between DN and T2DM for rs1801282 polymorphism.

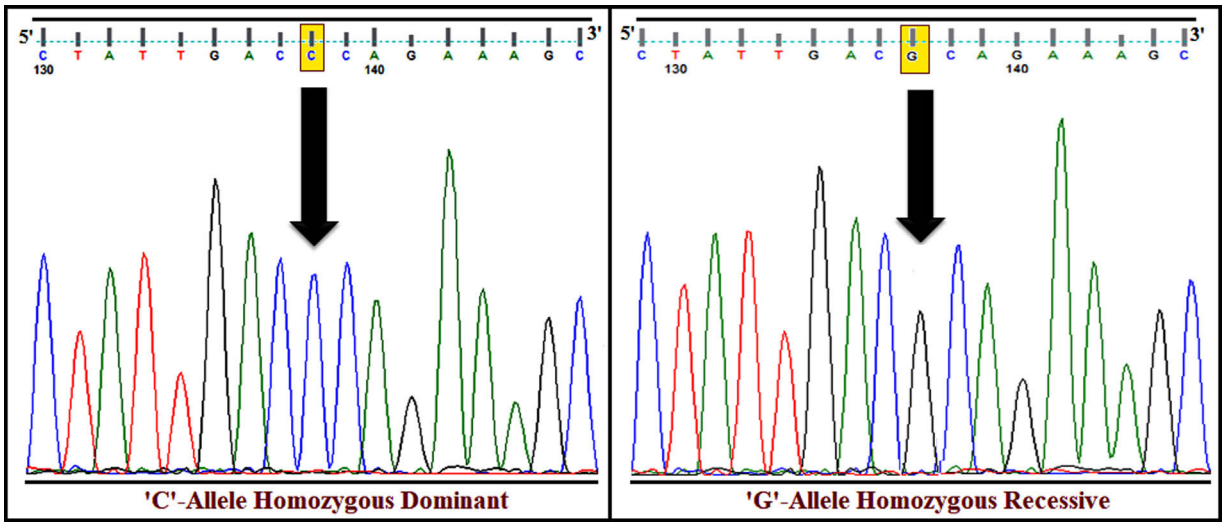

Fig. 1 – DNA sequence electropherograms of *rs1801282* polymorphism in the *PPARγ* gene. Examples of homozygous dominant (JJ genotype) and homozygous recessive (jj genotype) condition of the studied SNP.

Table 3 – Association of the *PPARγ* Gene polymorphism with DN and T2DM.

Polymorphism	Frequencies	Diabetic nephropathy $n=128$	Type 2 diabetes mellitus $n=148$	HWE	OR	95% CI	χ^2	*p*-Value
rs1801282	*Allele*							
	C	210 (82.03)	240 (81.08)	–		Reference	0.08	0.431
	G	46 (17.96)	56 (18.91)	–	0.93	0.60–1.44		
	Genotype							
	CC	94 (73.43)	100 (67.56)	0.147		Reference	3.19	0.049*
	CG	22 (17.18)	40 (27.02)		1.19	0.75–1.98		
	GG	12 (09.37)	08 (05.40)		2.72	0.96–7.67	3.74	0.047*
Genetic model								
Dominant	CG + GG vs CC	–	–	–	1.32	0.78–2.23	1.13	0.175
Recessive	GG vs CC + CG	–	–	–	1.81	0.71–4.57	1.61	0.150

HWE, Hardy–Weinberg equilibrium, OR, Odd's ratio; χ^2, Chi-square; *p* value-one tailed test.
* Significant *p*-value.

Frequencies of rs1801282 polymorphism with T2DM vs. controls

The susceptibility analysis of rs1801282 polymorphism with T2DM and controls was performed. The genotypic frequency distribution in healthy controls was in agreement with HWE ($p>0.05$). The allele and genotype frequencies of rs1801282 polymorphisms were used to estimate the odds ratio (OR), confidence intervals (95% CIs), χ^2 and *p*-value (Table 4). The genotypic distribution of rs1801282 polymorphism was 67.56% for JJ, 27.02% for Jj, 05.40% for jj in T2DM and 81.08%, 16.21%, 02.70% in controls, respectively. The analysis of rs1801282 polymorphism showed a positive association with T2DM compared with controls (*p*-value ≤ 0.05). The minor 'G' allele showed a positive association among T2DM and controls with OR = 1.92 (95%CI [1.20–3.07]) *p*-value = 0.003. In heterozygous (Jj) genotype of rs1801282 showed significant risk association among T2DM and controls with OR = 2.00 (95%CI [1.12–3.54]) *p*-value = 0.011, respectively. Likewise, the analysis of dominant genetic model showed significant association among T2DM and controls with OR = 1.48 (95%CI [0.28–3.83]) *p*-value = 0.005 for rs1801282 polymorphism.

Frequencies of rs1801282 polymorphism between DN and controls

The susceptibility analysis of rs1801282 variant with DN and controls was performed (Table 5). The genotype frequency distributions in controls were in agreement with HWE ($p>0.05$). The genotype distribution of rs1801282 polymorphism was 73.43% for JJ, 17.18% for Jj, 09.37% for jj in DN cases and 81.08%, 16.21%, 02.70% in control, respectively. The minor 'G' allele showed a positive association among T2DM and control with OR = 1.80 (95%CI [1.13–2.93]) *p*-value = 0.011. However, the analysis of rs1801282 polymorphism showed a negative association between DN and control with *p*-value ≥ 0.05 in the heterozygote, homozygous recessive, dominant and recessive genetic models, respectively. The nucleotide sequences of PPARγ genotypes were deposited (Accession numbers:

Table 4 – Association of the PPARγ Gene polymorphism with T2DM and controls.

Polymorphism	Frequencies	Type 2 diabetes mellitus $n=148$	Controls $n=148$	HWE	OR	95% CI	χ^2	p-Value
rs1801282	*Allele*							
	C	240 (81.08)	264 (89.18)	–		Reference	4.69	0.003*
	G	56 (18.91)	32 (10.81)	–	1.92	1.20–3.07		
	Genotype							
	CC	100 (67.56)	120 (81.08)	0.052		Reference	5.76	0.011*
	CG	40 (27.02)	24 (16.21)		2.00	1.12–3.54		
	GG	08 (05.40)	04 (02.70)		1.20	0.32–4.41	0.0	0.528
Genetic model								
Dominant	CG + GG vs CC	–	–	–	1.48	0.28–3.83	7.08	0.005*
Recessive	GG vs CC + CG	–	–	–	2.05	0.60–6.98	1.39	0.188

HWE, Hardy–Weinberg equilibrium; OR, Odd's ratio; χ^2, Chi-square; p-value-one tailed test.
* Significant p-value.

Table 5 – Association of the PPARγ gene polymorphism with DN and controls.

Polymorphism	Frequencies	Diabetic nephropathy $n=128$	Controls $n=148$	HWE	OR	95% CI	χ^2	p-Value
rs1801282	*Allele*							
	C	210 (82.03)	264 (89.18)	–		Reference	5.80	0.011*
	G	46 (17.96)	32 (10.81)	–	1.80	1.13–2.93		
	Genotype							
	CC	94 (73.43)	120 (81.08)	0.052		Reference	0.63	0.373
	CG	22 (17.18)	24 (16.21)		1.17	0.61–2.21		
	GG	12 (09.37)	04 (02.70)		3.27	0.91–4.61	3.54	0.061
Genetic model								
Dominant	CG + GG vs CC	–	–	–	0.64	0.36–1.13	2.30	0.085
Recessive	GG vs CC + CG	–	–	–	3.72	1.17–6.34	5.60	0.169

HWE, Hardy–Weinberg equilibrium; OR, Odd's ratio; χ^2, Chi-square; p-value-one tailed test.
* Significant p-value.

KY547832, KY547833, KY547834, KY547835, and KY547836) in the NCBI Genbank [https://www.ncbi.nlm.nih.gov/genbank/] database. The Circos plot (outer circle to inner) illustrates the chromatin states, annotated genes, histone modifications, transcription factors, currently studied variant (rs1801282) with related SNPs (r^2) and 3D chromatin interactions (Fig. 2).

Studies characteristics of rs1801282 polymorphism with DN vs. TD2 M for meta-analysis

In meta-analysis, a total of 678 papers published before December 2017 was retrieved from the initial search in literature databases. The editorials, review articles, duplicates, and case reports were removed after a screening of the abstracts. Further, the studies were assessed for quality control methods such as Hardy-Weinberg Equilibrium and Newcastle Ottawa Scale. Thus, four studies[22–25] were added to the meta-analysis of rs1801282 polymorphism and the characteristics of included studies in this meta-analysis were explained in supplementary Table 1. The genotypic, allelic frequencies and HWE/Chi-square values for the included studies were presented in Table 6.

Studies characteristics of rs1801282 polymorphism with T2DM vs. control for meta-analysis

The literature search published from 1991 up to December 2017 retrieved 846 relevant studies from several electronic databases. After removing the duplicate studies, case reports, studies on cell lines, 236 records remained. According to the quality assessment criteria, followed by HWE analysis, nine studies T2DM = 6365, controls = 5322 participants[26–33] were eligible for this meta-analysis. The characteristics (author name, year of publication, country, ethnicity, DNA source, a sample size of cases-controls and genotyping methods) of each study included in the meta-analysis were defined in supplementary Table 2. The genotype, allele frequencies, and HWE/Chi-square values for the studies included in this meta-analysis were presented in Table 7.

Meta-analysis of rs1801282 polymorphism with DN vs. TD2 M susceptibility

The analysis of rs1801282 polymorphism among DN and T2DM revealed no heterogeneity in allelic ($I^2 = 22\%$) and dominant ($I^2 = 18\%$). Whereas, moderate heterogeneity was noticed in homozygote ($I^2 = 49\%$), heterozygote ($I^2 = 52\%$) and recessive ($I^2 = 44\%$) genetic models. Based on values of heterogeneity (I^2) the fixed effects (Mantel–Haenszel's) model was implemented, which showed insignificant association with DN risk in allelic (j vs J) with (Q test $p = 0.27$), OR = 0.89, (95%CI [0.71–1.10]), $p = 0.26$, homozygote (jj vs JJ) (Q test $p = 0.12$), OR = 0.87, (95% CI [0.66–1.12]), $p = 0.27$, dominant (Jj + jj vs JJ) (Q test $p = 0.30$), OR = 0.86, (95% CI [0.66–1.12]), $p = 0.27$ and recessive (jj vs JJ + Jj) (Q test $p = 0.15$), OR = 0.88, (95% CI

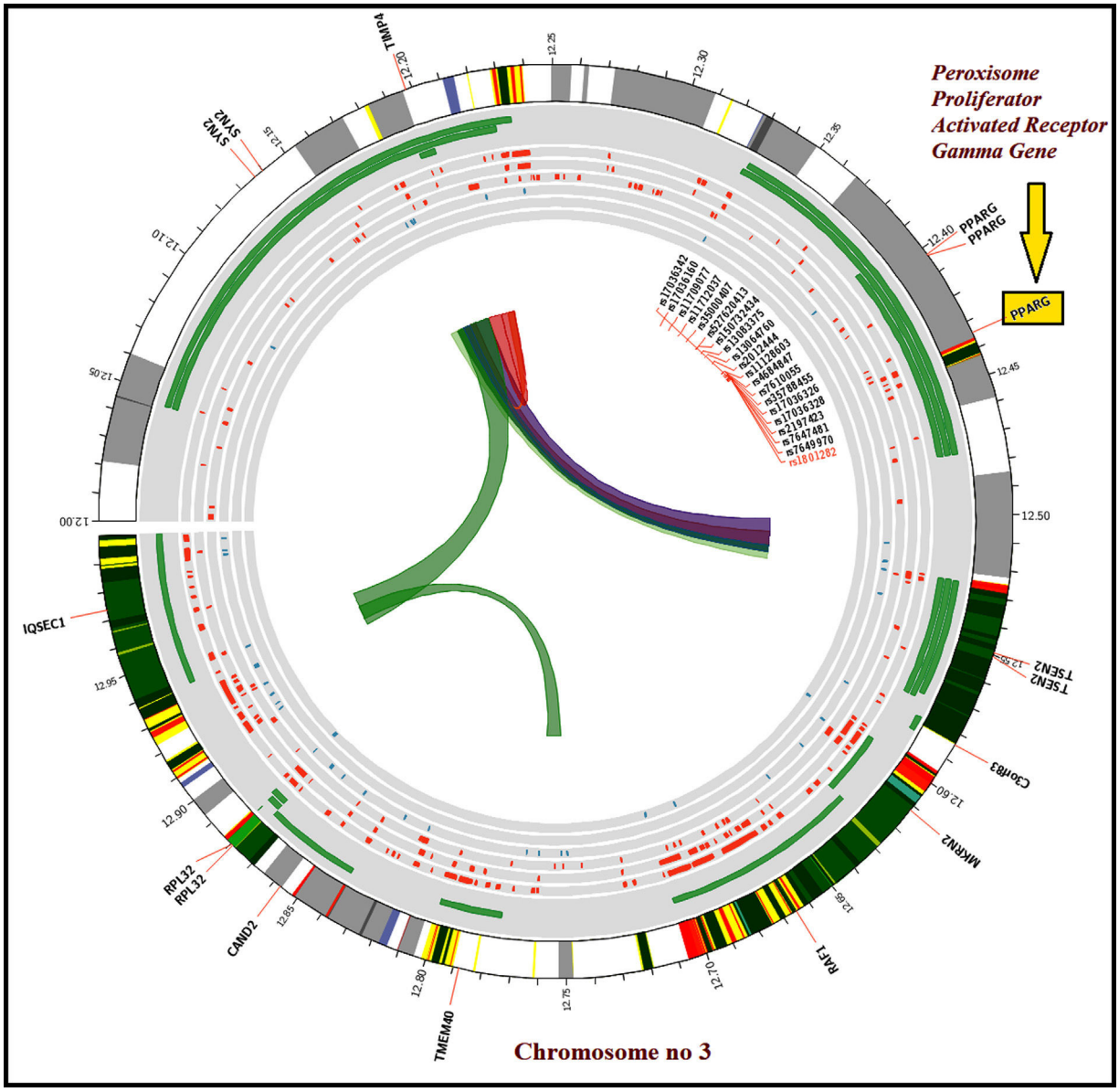

Fig. 2 – Circos plot illustrating the chromosomal interactions among the current SNP (*rs1801282*) and its associated SNPs. The Circos plot (outer to inner) denotes chromatin states, annotated genes, histone modifications, transcription factors, rs1801282 variant with associated SNPs (r2).

Table 6 – Genotype and allele frequencies of *PPARγ* gene polymorphism with DN and T2DM.

Study name	Cases (CC/CG/GG)	Controls (CC/CG/GG)	Cases (C/G-Allele)	Controls (C/G-Allele)	No of cases	No of controls	HWE[a]	Chi-square
Bhaskar et al.	14/29/11	24/30/13	57/51	78/56	54	67	0.5143	0.425
Erdogan et al.	37/6/0	39/7/2	80/6	85/11	43	48	0.0513	3.797
Maeda et al.	55/24/0	46/15/0	134/24	107/15	79	61	0.273	1.198
This study et al.	94/22/12	100/40/8	210/46	240/56	128	148	0.147	2.097
Wu et al.	130/76/9	93/70/15	336/94	256/100	215	178	0.723	0.125

[a] HWE, Hardy–Weinberg equilibrium.

[0.54–1.44]), $p = 0.61$ genetic models, respectively. Whereas, the random effects model was used which showed insignificant association with DN risk in the heterozygote (Jj vs JJ) (Q test $p = 0.10$), OR = 1.03, (95% CI [0.44–2.41]), $p = 0.94$. The results of the meta-analysis were shown as forest plots (Fig. 3). Further, funnel plot (Fig. 5) and Egger's test was executed which revealed no publication bias in the studied genetic models. Since a low number of studies were from

Table 7 – Genotype and allele frequencies of *PPARγ* gene polymorphism with T2DM and controls.

Study Name	Cases (CC/CG/GG)	Controls (CC/CG/GG)	Cases (C/G-Allele)	Controls (C/G-Allele)	No of cases	No of controls	HWE[a]	Chi-square
Deeb et al.	87/4/0	45/8/1	178/4	98/10	91	54	0.384	0.756
Hara et al.	400/15/0	496/45/0	815/15	1037/45	415	541	0.312	1.368
Lin et al.	1348/178/3	1328/107/4	2874/184	2763/115	1529	1439	0.242	1.368
Mori et al.	2097/103/3	1114/96/2	4297/109	2324/100	2203	1212	0.214	1.539
Pattanayak et al.	158/37/5	167/30/3	353/47	364/36	200	200	0.233	1.419
Phani et al.	155/253/181	181/254/83	563/615	616/420	589	518	0.361	0.831
Tripathi et al.	156/33/1	154/48/8	345/35	356/64	190	210	0.095	2.785
This study et al.	100/40/8	120/24/4	240/56	264/32	148	148	0.005	3.74
Vimaleswaran et al.	860/136/4	840/157/3	1856/144	1837/163	1000	1000	0.129	2.301

[a] HWE, Hardy–Weinberg equilibrium.

Caucasian and Asian ethnicity, sub-group meta-analysis was not performed.

Meta-analysis of rs1801282 polymorphism with T2DM vs. controls

The analysis of rs1801282 polymorphism various T2DM vs. controls revealed moderate heterogeneity in the heterozygote ($I^2 = 31\%$) and high heterogeneity was noticed in allelic ($I^2 = 90\%$), homozygote ($I^2 = 51\%$), dominant ($I^2 = 88\%$) and recessive ($I^2 = 45\%$) genetic models. Based on values of heterogeneity (I^2), the random-effect method (DerSimonian and Laird's) was adopted which showed insignificant association with T2DM risk in allelic (j vs J) with (Q test $p = 0.00001$), OR = 0.91, (95%CI [0.64–1.30]), $p = 0.61$, homozygote (jj vs JJ) (Q test $p = 0.05$), OR = 1.22, (95% CI [0.62–2.41]), $p = 0.57$, dominant (Jj +jj vs JJ) (Q test $p = 0.00001$), OR = 0.91, (95% CI [0.64–1.31]), $p = 0.62$ and recessive (jj vs JJ+ Jj) (Q test $p = 0.08$), OR = 1.23, (95% CI [0.65–2.30]), $p = 0.52$ genetic models respectively. The fixed effects (Mantel-Haenszel's) model was used, which showed significant association with DN risk in heterozygote (Jj vs JJ) (Q test $p = 0.18$), OR = 0.56, (95% CI [0.43–0.74]), $p \leq 0.0001$. The results of meta-analysis were illustrated as forest plot (Fig. 4). Further, Begg's funnel plot (Fig. 5) and Egger's regression test were performed which suggested no publication bias in the analyzed genetic models. Since a low number of studies were from Caucasian ethnicity, sub-group analysis was not performed.

Discussion

T2DM is a complex metabolic disorder where environmental, genetic factors and lifestyle contributing to disease pathogenesis.[34] The DN susceptibility has been related to various genes localized at different chromosomes. Of various genes, the PPARγ nuclear receptor expressed in adipose tissue and renal glomeruli play a major role in the developmental process of DN.[35] The rs1801282 polymorphism has been found to be linked with the reduced ability of trans-activate responsive promoters and thereby lowering the PPAR-γ transcriptional activity.[36] On the other hand, PPARs is a potential target for the Glitazones or Thiazolidinediones (TZDs) for the management of T2DM.[37]

In this study, we have validated the association between PPARγ (rs1801282) polymorphism with the risk of developing DN, T2DM in the south Indian population by comparing 1) DN vs. T2DM, 2) DN vs. controls and 3) T2DM vs. controls. The analysis revealed a positive association with p-value < 0.05 between DN and T2DM subjects suggesting that rs1801282 polymorphism might be a risk factor for DN in our study population. These results deviate from a previously published study from the DN subjects of Turkey.[23] The analysis of rs1801282 polymorphism among T2DM and controls showed significant association (p-value ≤ 0.05) in derived'G' allele and heterozygous genotype. These results were in accordance with a previously published study on Japanese subjects belonging to East Asian ethnicity.[29]

In addition, we have performed a meta-analysis for rs1801282 polymorphism to investigate the effect of the PPARγ gene on susceptibility with DN and T2DM in mixed population. For, which the articles related on DN and T2DM were retrieved from several electronic databases based on PRISMA guidelines and NOS scaling was used for quality assessment. The results of our meta-analysis showed insignificant association with DN in the allelic OR = 0.89, (95%CI [0.71–1.10]), homozygote OR = 0.87, (95% CI [0.66–1.12]), heterozygote OR = 1.03, (95% CI [0.44–2.41]), dominant OR = 0.86, (95% CI [0.66–1.12]), and recessive OR = 0.88, (95% CI [0.54–1.44]) genetic models respectively. Similar to the results of this meta-analysis, rs1801282 polymorphism shows an insignificant association in Turkey 23 and Taiwan 25 populations.

The meta-analysis of Type 2 Diabetes Mellitus and controls showed significant association among T2DM and controls in the heterozygote genetic model with OR = 0.56, (95% CI [0.43–0.74]) and insignificant association allelic OR = 0.91, (95%CI [0.64–1.30]), homozygote OR = 1.22, (95%CI [0.62–2.41]), dominant OR = 0.91, (95%CI [0.64–1.31]), and recessive OR = 1.23, (95%CI [0.65–2.30]) genetic models respectively. The results of meta- were in agreement with Japan 29 and Central India 32 whereas disagreement was noticed with Han Chinese 28 population. Overall, the analysis provides significant knowledge on rs1801282 with DN and T2DM in both south Indian and mixed population. However, the results of our analysis can be considered with certain limitations. First, we studied the association between PPARγ genotypes (rs1801282) among DN, T2DM and their phenotypes were

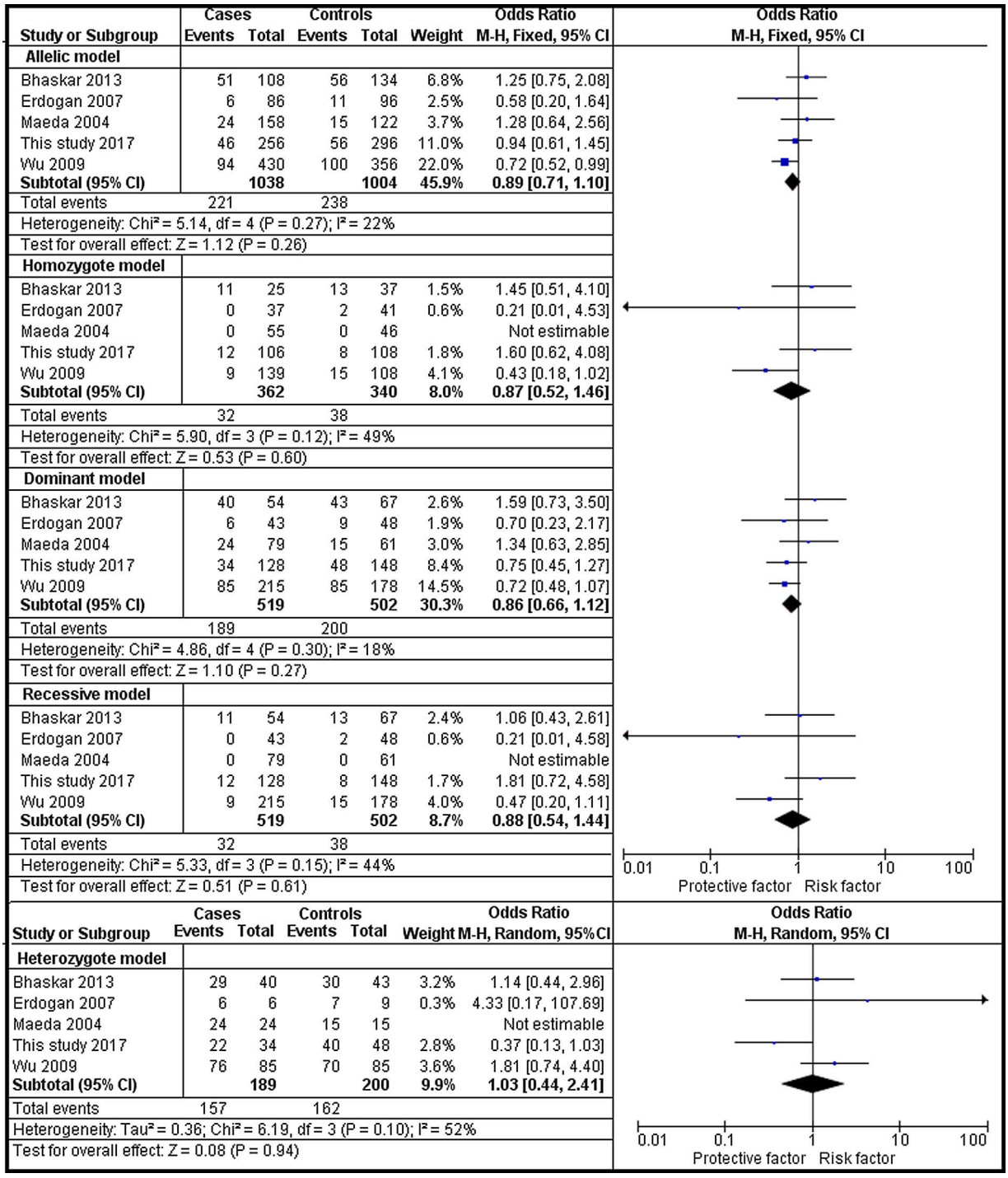

Fig. 3 – Forest plots showing individual and pooled ORs (95% CI) of the risk for *rs1801282* polymorphism with Diabetic Nephropathy and Type 2 Diabetes Mellitus. The Forest Plots provides an insignificant association between the PPARγ polymorphism with DN and T2DM under five genetic models. The error bars indicate 95% CIs. Squares represent individual studies in the meta-analysis. Diamonds represent the pooled OR.

not studied extensively. Second, T2DM is a multi-factorial metabolic disorder, other confounding factors including age, gender, obesity, lifestyle habits, and other environmental factors were not considered in our analysis. Third, the studies included in our meta-analysis were restricted to articles published in the English language. Fourth, the genotyping methods were not the same in the included studies. Fifth, we have studied only one SNP (rs1801282) in the PPARγ gene other polymorphic variants were not considered.

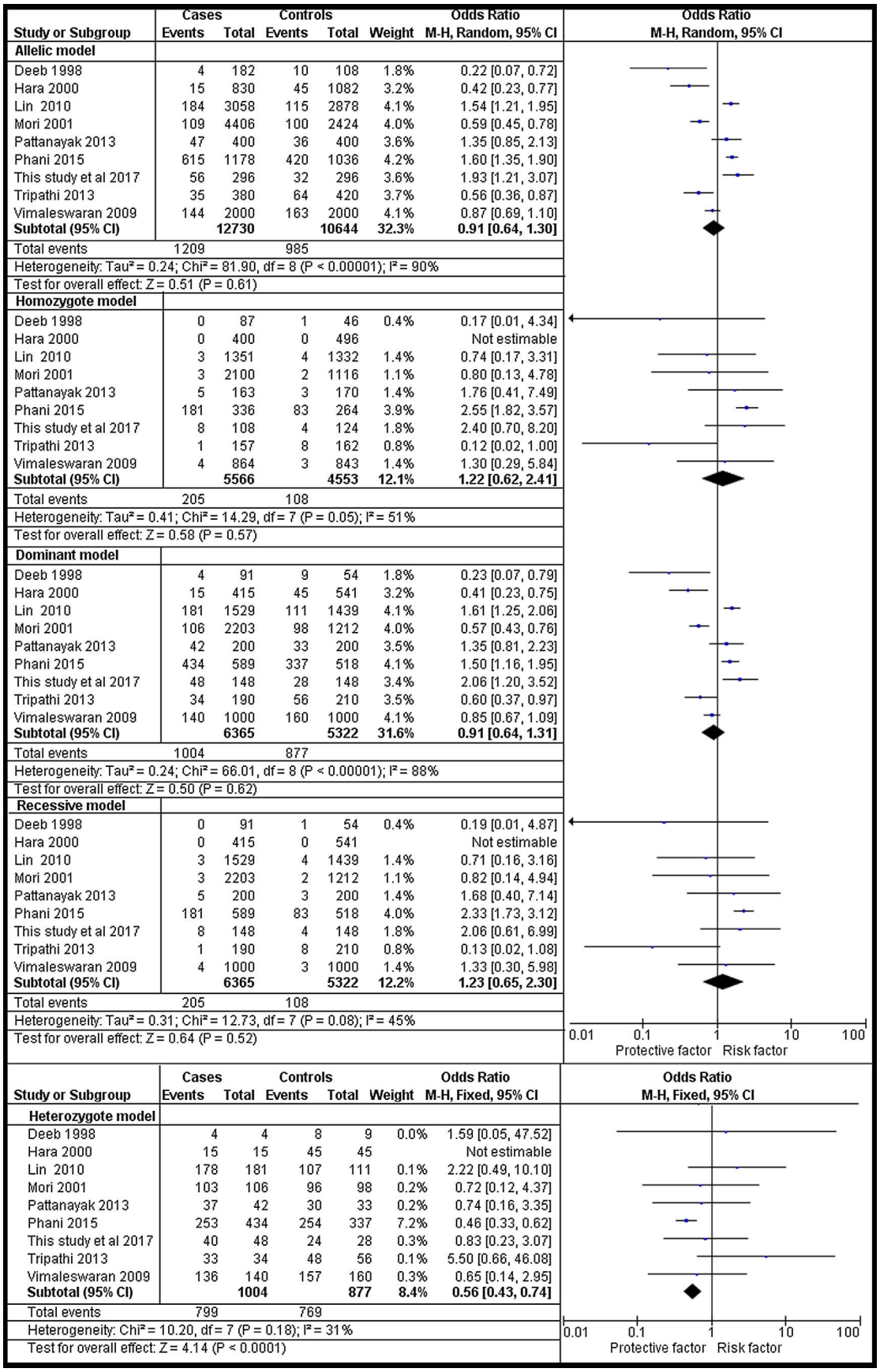

Fig. 4 – Forest plots showing individual and pooled ORs (95% CI) of the risk for *rs1801282* polymorphism with Type 2 Diabetes Mellitus and controls. The Forest Plots provides an insignificant association between the PPARγ polymorphism with T2DM and controls under five genetic models. The error bars indicate 95% CIs. Solid squares represent individual studies included in the meta-analysis. Solid diamonds represent the pooled OR.

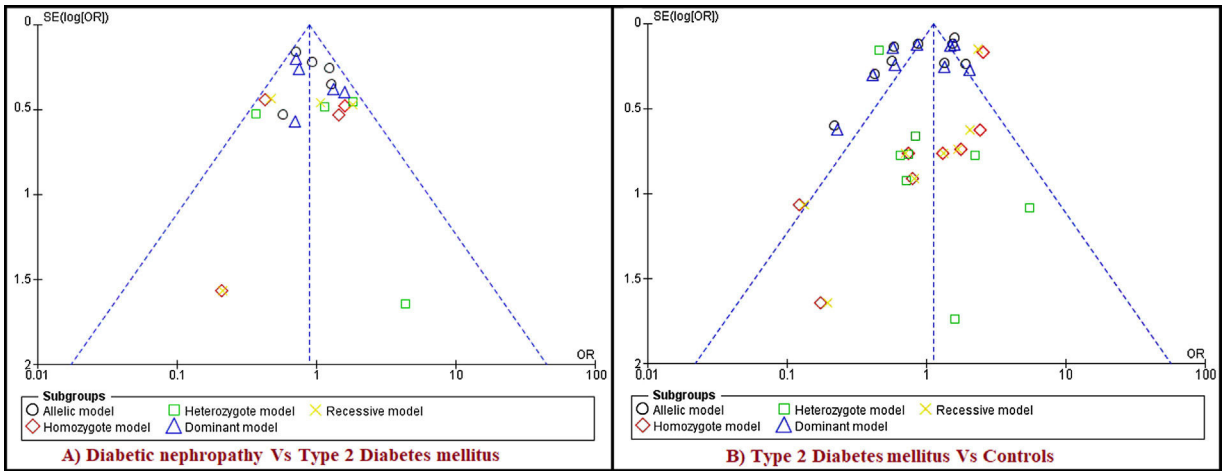

Fig. 5 – Funnel plots of publication biases on the relationship of *PPARγ (rs1801282)* polymorphism. Funnel plots for publication bias (five genetic models). Each point indicates the individual study included in this meta-analysis. A) Diabetic Nephropathy and Type 2 Diabetes Mellitus B) Type 2 Diabetes Mellitus and Controls.

Conclusion

In conclusion, our case–control study demonstrates the involvement of PPARγ gene (rs1801282) variant among DN and T2DM in South Indian population. Alternative results were noticed in other population may be due to genetic diversity. However, independent replication studies with larger sample size are required to confirm the associations that we observed in this study. Alternatively, the meta-analysis of DN vs. controls suggests that the PPARγ polymorphism is not a risk factor for developing DN. Whereas, a meta-analysis of T2DM vs. controls suggests that rs1801282 polymorphism result showed a positive association for developing T2DM. Further, well designed, large-scale studies combining with other multiple factors are required to validate the PPARγ gene associated with risk of developing DN and T2DM.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.nefro.2020.01.005

REFERENCES

1. McCarthy MI, Froguel P. Genetic approaches to the molecular understanding of type 2 diabetes. Am J Physiol-Endocrinol Metab. 2002;283:E25–17.
2. MacIsaac RJ, Ekinci EI, Jerums G. Markers of and risk factors for the development and progression of diabetic kidney disease. Am J Kidney Dis. 2014;63:S39–62.
3. Kanwar YS, Sun L, Xie P, Liu F-y, Chen S. A glimpse of various pathogenetic mechanisms of diabetic nephropathy. Annu Rev Pathol. 2011;6:395–423.
4. Mooyaart A, Valk E, Van Es L, Bruijn J, De Heer E, Freedman B, et al. Genetic associations in diabetic nephropathy: a meta-analysis. Diabetologia. 2011;54:544–53.
5. Kooner JS, Saleheen D, Sim X, Sehmi J, Zhang W, Frossard P, et al. Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. Nat Genet. 2011;43:984.
6. Scherag A, Dina C, Hinney A, Vatin V, Scherag S, Vogel CI, et al. Two new Loci for body-weight regulation identified in a joint analysis of genome-wide association studies for early-onset extreme obesity in French and german study groups. PLoS Genet. 2010;6:e1000916.
7. Cai G, Zhang X, Weng W, Shi G, Xue S, Zhang B. Associations between PPARG polymorphisms and the risk of essential hypertension. PLOS ONE. 2017;12:e0181644.
8. Fernández XM, Birney E. Ensembl genome browser. Vogel and Motulsky's Human Genetics. Springer; 2010. p. 923–39.
9. Zhang H, Zhu S, Chen J, Tang Y, Hu H, Mohan V, et al. Peroxisome proliferator-activated receptor γ polymorphism Pro12Ala is associated with nephropathy in type 2 diabetes: evidence from meta-analysis of 18 studies. Diabetes Care. 2012;2:1388–93.
10. Tamori Y, Masugi J, Nishino N, Kasuga M. Role of peroxisome proliferator-activated receptor-γ in maintenance of the

characteristics of mature 3T3-L1 adipocytes. Diabetes. 2002;51:2045–55.
11. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl M-C, Nemesh J, et al. The common PPARγ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet. 2000;26:76.
12. Abate N, Chandalia M. The impact of ethnicity on type 2 diabetes. J Diab Compl. 2003;17:39–58.
13. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part-1, Diagnosis and classification of diabetes mellitus. 1999.
14. Mathew C. The isolation of high molecular weight eukaryotic DNA. Nucleic Acids. Springer; 1984. p. 4–31.
15. Ye S, Dhillon S, Ke X, Collins AR, Day IN. An efficient procedure for genotyping single nucleotide polymorphisms. Nucleic Acids Res. 2001;29:e88–90.
16. Ahmed SS, Husain RA, Kumar S, Ramakrishnan V. Association between NOS1 gene polymorphisms and Schizophrenia in Asian and Caucasian populations: a meta-analysis. Neuromol Med. 2017;19:452–61.
17. Ramakrishnan V, Alphonsa TA, Husain RA, Ahmed SS, Subramaniyan K, Kumar S. Association of rs1801582 and rs1801334 PARK2 polymorphisms with risk of Parkinson's disease: a case–control study in South India and meta-analysis. Meta Gene. 2016;10:32–8.
18. Turner RM, Davey J, Clarke MJ, Thompson SG, Higgins JP. Predicting the extent of heterogeneity in meta-analysis, using empirical data from the Cochrane Database of Systematic Reviews. Int J Epidemiol. 2012;41:818–27.
19. DerSimonian R, Laird N. Meta-analysis in clinical trials revisited. Contemp Clin Trials. 2015;45:139–45.
20. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315:629–34.
21. Ramakrishnan V, Husain RA, Ahmed SS. PSEN1 gene polymorphisms in Caucasian Alzheimer's disease: a meta-analysis. Clin Chim Acta. 2017;473:65–70.
22. Bhaskar LV, Mahin S, Ginila RT, Soundararajan P. Role of the ACE ID and PPARG P12A polymorphisms in genetic susceptibility of diabetic nephropathy in a South Indian population. Nephro-urology. 2013;5:813.
23. Erdogan M, Karadeniz M, Eroglu Z, Tezcanlı B, Selvi N, Yilmaz C. The relationship of the peroxisome proliferator-activated receptor-gamma 2 exon 2 and exon 6 gene polymorphism in Turkish type 2 diabetic patients with and without nephropathy. Diab Res Clin Pract. 2007;78:355–9.
24. Maeda A, Gohda T, Funabiki K, Horikoshi S, Tomino Y. Peroxisome proliferator-activated receptor γ gene polymorphism is associated with serum triglyceride levels and body mass index in Japanese type 2 diabetic patients. J Clin Lab Anal. 2004;18:317–21.
25. Wu LS-H, Hsieh C-H, Pei D, Hung Y-J, Kuo S-W, Lin E. Association and interaction analyses of genetic variants in ADIPOQ, ENPP1, GHSR PPARγ and TCF7L2 genes for diabetic nephropathy in a Taiwanese population with type 2 diabetes. Nephrol Dial Transplant. 2009;24:3360–6.
26. Deeb SS, Fajas L, Nemoto M, Pihlajamäki J, Mykkänen L, Kuusisto J, et al. A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet. 1998;20:284.
27. Hara K, Okada T, Tobe K, Yasuda K, Mori Y, Kadowaki H, et al. The Pro12Ala polymorphism in PPAR γ2 may confer resistance to type 2 diabetes. Biochem Biophys Res Commun. 2000;271:212–6.
28. Lin Y, Li P, Cai L, Zhang B, Tang X, Zhang X, et al. Association study of genetic variants in eight genes/loci with type 2 diabetes in a Han Chinese population. BMC Med Genet. 2010;11:97.
29. Mori H, Ikegami H, Kawaguchi Y, Seino S, Yokoi N, Takeda J, et al. The Pro12→ Ala substitution in PPAR-γ 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;50:891–4.
30. Pattanayak AK, Bankura B, Balmiki N, Das TK, Chowdhury S, Das M. Role of peroxisome proliferator-activated receptor gamma gene polymorphisms in type 2 diabetes mellitus patients of West Bengal, India. J Diab Invest. 2014;5:188–91.
31. Phani NM, Vohra M, Rajesh S, Adhikari P, Nagri SK, D'Souza SC, et al. Implications of critical PPARγ2 ADIPOQ and FTO gene polymorphisms in type 2 diabetes and obesity-mediated susceptibility to type 2 diabetes in an Indian population. Mol Genet Genom. 2016;291:193–204.
32. Tripathi AK, Shukla S, Dwivedi MK, Tripathi JK, Chauhan UK, Indurkar M, et al. Type 2 diabetes in a central Indian population: association with PPARG2 P121A allele but not ENPP1 K121Q. Adv Genomics Genet. 2013;3:1–9.
33. Vimaleswaran KS, Radha V, Jayapriya MG, Ghosh S, Majumder PP, Rao MS, et al. Evidence for an association with type 2 diabetes mellitus at the PPARG locus in a South Indian population. Metab-Clin Exp. 2010;59:457–62.
34. Gayathri SB, Radha V, Vimaleswaran KS, Mohan V. Association of the PPARGC1A gene polymorphism with diabetic nephropathy in an Asian Indian population (CURES-41). Metab Syndrome Relat Disord. 2010;8:119–26.
35. Petr M, Stastny P, Zajac A, Tufano JJ, Maciejewska-Skrendo A. The role of peroxisome proliferator-activated receptors and their transcriptional coactivators gene variations in human trainability: a systematic review. Int J Mol Sci. 2018;19:1472.
36. Jia Z, Sun Y, Yang G, Zhang A, Huang S, Heiney KM, et al. New insights into the PPARγ agonists for the treatment of diabetic nephropathy. PPAR Res. 2014.
37. Thomas MC, Jandeleit-Dahm KA, Tikellis C. The renoprotective actions of peroxisome proliferator-activated receptors agonists in diabetes. PPAR Res. 2012, 456529.