Meta-analysis of variant rs 7903146 of TCF7L2 gene with secondary complications of Type 2 Diabetes Mellitus

Kanhaiya Singh[1], NK Agrawal[2], SK Gupta[3], Kiran Singh[1]*

[1]Department of Molecular & Human Genetics, Banaras Hindu University, Varanasi-221005, India.
[2]Department of Endocrinology and Metabolism, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, India.
[3]Department of Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, India.

ARTICLE INFO	ABSTRACT
Article history: Received on: 15/09/2014 Revised on: 24/09/2014 Accepted on: 14/10/2014 Available online: 30/10/2014 *Key words*: Transcription factor 7-like 2 (TCF7L2), Polymorphism, T2DM, secondary complications, Meta analysis	Persistent hyperglycemia in patients with Type 2 diabetes mellitus is driving force for developing secondary complications like neuropathy, retinopathy, nephropathy, cardiomyopathy and wound healing impairment in them. Other genetic risk factor includes variants of transcription factor 7-like 2 (TCF7L2) gene, which have been shown to be associated with these several secondary complications individually. The present study represents a systematic review and Meta analysis using electronic databases like PubMed, OMIM, ISI web of science and Embase to see the role of rs7903146 C>T variant of the TCF7L2 gene with secondary complications of T2DM. Data was collected using literature-based searching to perform a Meta analysis to pool the odds ratio (OR). This Meta analysis comprised of a total of 2060 cases with secondary complication of T2DM and 3049 age matched controls. Publication bias and study-between heterogeneity were also evaluated. The present Meta analysis of included studies showed a highly significant association of risk genotype TT with development of secondary complications in T2DM.

1.   INTRODUCTION

Type 2 diabetes Mellitus (T2DM) is a chronic non communicable disease which is proving to be a leading cause of morbidity and mortality, especially in low income developing countries. In India alone, the prevalence of T2DM has increased about two fold from 32 million in 2000 to 62 million in the last decade [1]. World Health Organization and International diabetes foundation claim T2DM as the most challenging problem of 21st century due to secondary complications like cardiovascular disease, neuropathy, nephropathy and retinopathy associated with it [2]. The etiology of T2DM is generally polygenic but recent researchers have identified certain variants like *PPARG* Pro12Ala and *KCNJ11* E23K which lead to monogenic form of diabetes [3]. Apart from candidate gene approaches, some hypothesis free genome wide analyses are also being done in T2DM cases to screen the common variants present in the population. One such genome wide study revealed the possible involvement of APC/C-catenin/TCF pathway, commonly called Wnt pathway, in the patho-physiology of T2DM [4]. Wnt is a gene related to developmental pathway and it play important roles in embryonic induction, cell polarity generation and in the specification of cell fate. They have also been shown to work in co-ordination with matrix metalloproteinases which are also involved in regulation of wound healing [5].

Recent reports suggest that Wnt signaling brings about the movement and differentiation of cardiac fibroblasts following myocardial infarction [6]. Downstream agents of wnt signaling like β-catenin and c myc help in the movement of epidermal stem cells to the wound site during wound healing [7]. The transcription factor 7 like 2 gene (TCF7L2) product is a High mobility group box transcription factor and it is a part of β-catenin signaling cascade [8]. This transcription factor is guided by β-catenin and helps in the transcription of downstream genes like PPAR-γ, Myc, Cyclin D1 etc. and probably affect β-cell function either by modulating β-cell response to glucose or by modulating the incretin action or secretion [9].

Multiple intronic single nucleotide polymorphisms (SNPs) in TCF7L2 gene have shown to be associated in several European, African, American and Asian cohorts of world including Indians, although a marked variation in allele frequencies is seen due to difference in ethnic descend. Re-sequencing and comparative association studies in search of putative element revealed that rs7903146 is a most likely casual variant SNP located in intron 4 of TCF7L2 gene. Being situated in intronic region this variant likely

* Corresponding Author
Kiran Singh, Department of Molecular & Human Genetics Banaras Hindu University, Varanasi-221005, India
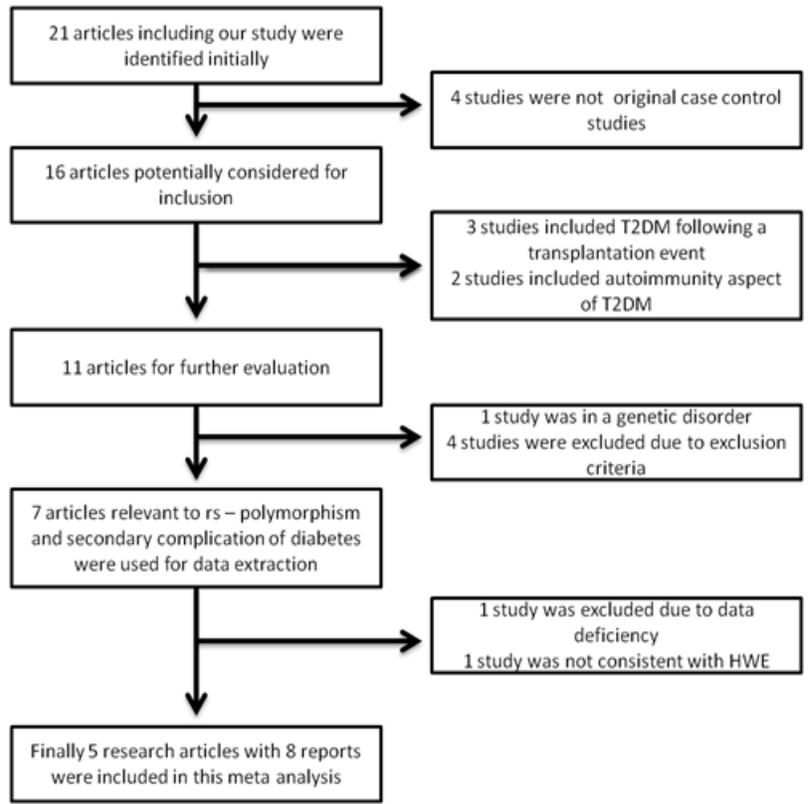*E-mail: skiran@bhu.ac.in, singhk4@rediffmail.com*

Fig. 1: Data sheet for Meta analysis- genotype data of studies in secondary complications of T2DM. Genotype data for SNP rs 7903146 polymorphism were extracted according to standard extraction form as shown in the figure. A total of 5 research articles comprised of 8 studies including our previous study were found to be eligible on our search criteria.
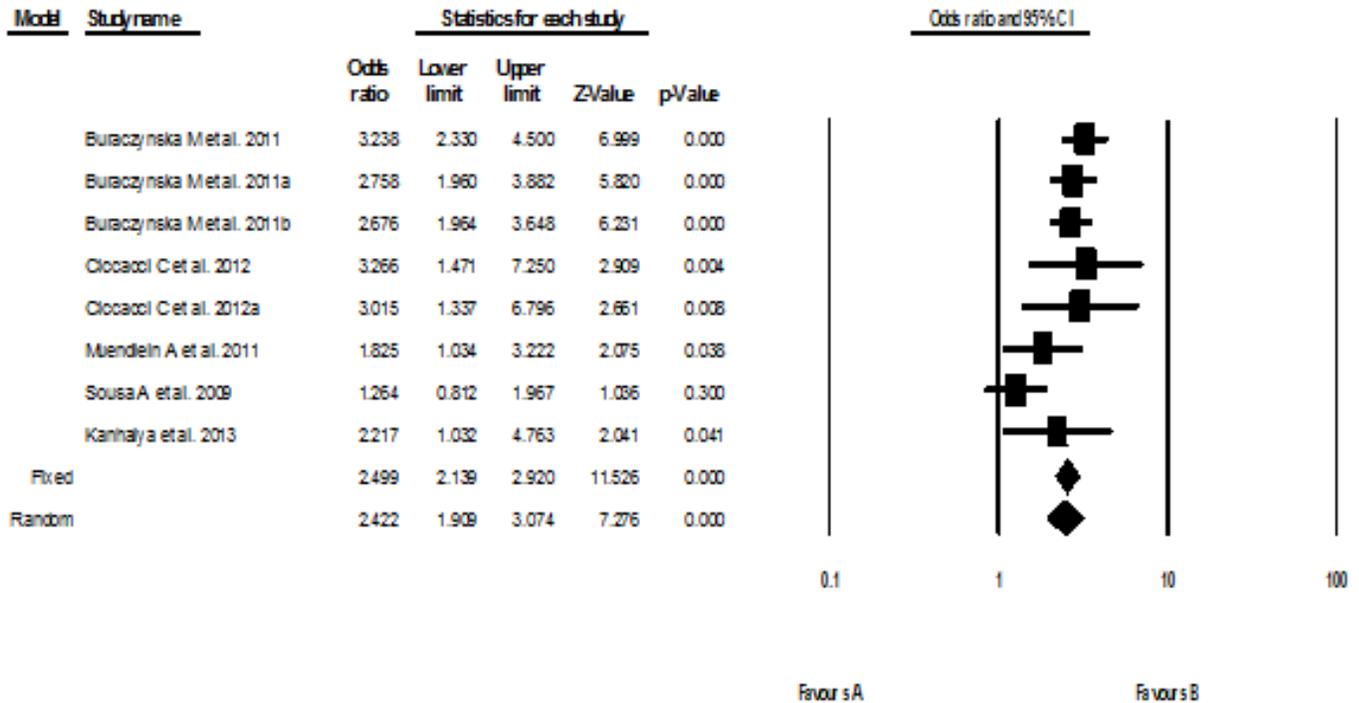

Fig. 2: Odds ratio of each study and the pooled estimate is shown by the forest plot. Cochran's χ^2 test (Q test) was used to evaluate heterogeneity between studies; a threshold P value of 0.1 was considered statistically significant, and the inconsistency value (I^2) test was also conducted to evaluate heterogeneity of studies. The 'I^2' value of 49.70% for between studies comparison showed low level of true heterogeneity. The fixed model test for the genotype data for the studies yielded a statistically significant result (Q = 13.91, p value <0.001, I^2 = 49.70, tau squared = 0.053)

act by affecting the expression of TCF7L2. T allele of variant rs7903146 of TCF7L2 gene is less prone to be bound by protein factors like GATA3 in hepatic cell lines and hence there is aberrant TCF7L2 expression in patients with TT genotype [10]. Similar effect was seen in other cell lines like HepG2 (liver), 3T3-L1 (adipocytes), and β-TC3 (β cells) where rs7903146 T allele bound with lesser efficiency to protein factors compared to alternative C allele. Our group has recently shown the genetic association of transcription factor 7 like 2 (TCF7L2) gene, a part of β-catenin signaling cascade, to be associated with impairment of wound healing in T2DM individuals [11]. In the present study, a Meta analysis was done to systematically review the association of this SNP with common secondary complication associated with T2DM by combining our data with data from previous studies.

2. MATERIALS AND METHODS

2.1 Literature search

Different databases like PubMed, OMIM, ISI web of science and Embase were comprehensively searched for the association of SNP rs 7903146 polymorphism with secondary complication of T2DM published after January 2009 till August 2014. The search strategy was based on combination of keywords like "transcription factor 7 – like 2" or "TCF7L2" or "rs 7903146" polymorphism with "secondary complication of diabetes" or "retinopathy" or "neuropathy" or "cardiomyopathy" or "wound healing impairment". All original articles which reported data from original studies were included in this Meta analysis.

### 2.2 Inclusion and exclusion criteria

Thorough reviewing of full text of each short listed article was done to fetch necessary information for Meta analysis. The inclusion criteria of the study eligible for Meta analysis was chosen so that trails being (i) independent case control study, (ii) with sufficient information required for calculation of Odds ratio, (iii) good resolution SNP genotyped (iv) similar in statistical and purpose methodology were included for Meta analysis. The exclusion criteria of the study was (i) T2DM occurrence after any transplantation event, (ii) any metabolic associated circumstance like obesity or inflammation, (iii) evidence of auto immunity like that in type 1 diabetes or LADA (latent autoimmune diabetes in adults), (iv) involvement of one or other Genetic disorder.

2.3 Raw Data Extraction

Genotype data for SNP rs 7903146 polymorphism were extracted according to standard extraction form (Fig. 1). Data for secondary complications of T2DM were collected. Tabulation of the arranged data was then done with particular details regarding the first author, year of publication, sample size, ethnicity of the study population, and genotype frequency.

2.4 Quantitative data synthesis

For Meta analysis all statistical test were performed using software comprehensive Meta Analysis V2. Odds ratio (ORs) was chosen as effect size to find secondary complications of T2DM associated with mutant genotype (TT) of SNP rs 7903146 polymorphism.

Funnel Plot of Standard Error by Log odds ratio
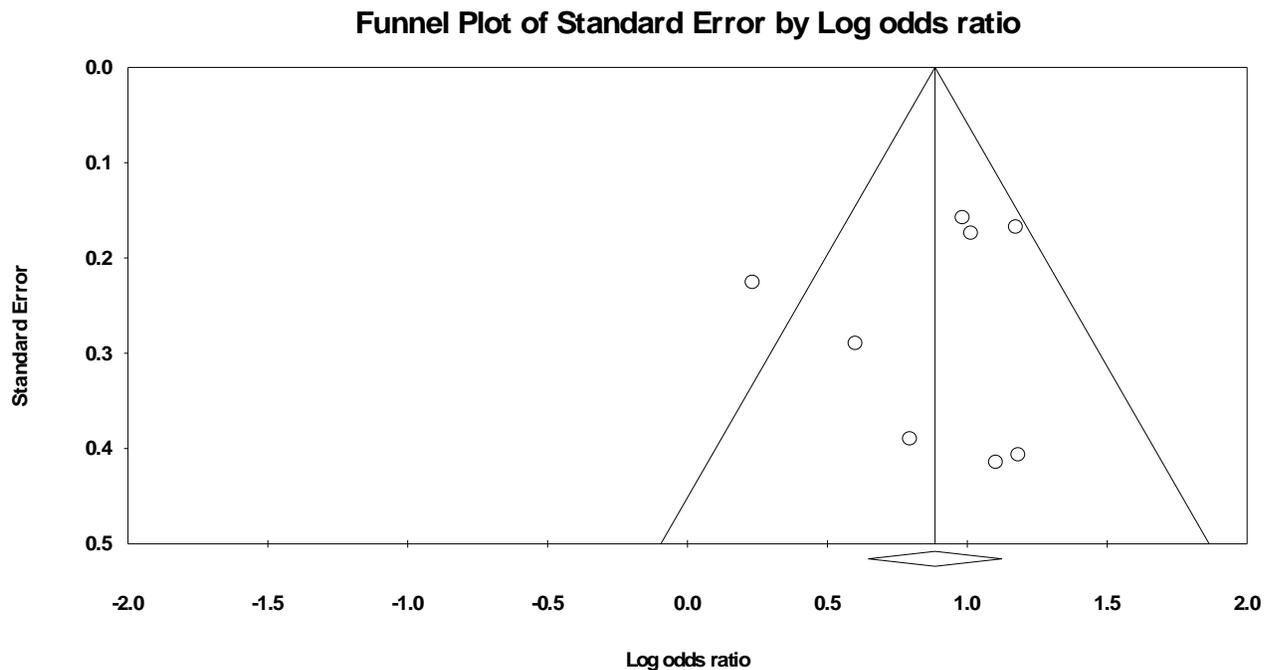
Fig. 3: Publication bias of studies was calculated using funnel plot of standard error calculated by using log odds ratio. Funnel plot of precision based on log odds ratio was created using various statistical tools such as Egger's regression intercept, Duvall and Tweedie trim and fill procedure Classic fail safe 'N' to nullify the effect of publication bias owing to small studies. The possibility of publication bias was rejected due to the evidence of symmetrical distribution of studies on funnel plot.

Cochran's χ^2 test (Q test) was used to evaluate heterogeneity between studies; a threshold P value of 0.1 was considered statistically significant, and the inconsistency value (I^2) test was also conducted to evaluate heterogeneity of studies. Since no evidence of heterogeneity was found in the studies, fixed model was utilized to calculate the p value and effect size of the study. High resolution plots were generated equivalent to ORs and CI at 95% for fixed as well as random effects models. Sensitivity analysis was performed to check the validation and robustness of method utilized in the analysis. Funnel plot of precision based on log odds ratio was created using various statistical tools such as Egger's regression intercept, Duvall and Tweedie trim and fill procedure Classic fail safe 'N' to nullify the effect of publication bias owing to small studies. Classic Fail safe 'N' suggests the number of null studies which have to be included to subsidize the effect to non-significant. Egger's test for intercept provides the overall estimate of the effect size.

3. RESULTS

A total of 5 research articles comprised of 8 studies including our previous study were found to be eligible on our search criteria. Buraczynska *et al*. [12] reported association of rs 7903146 with nephropathy, diabetic retinopathy and Cardiomyopathy, while Ciccacci *et al*. [13] associated rs 7903146 with diabetic retinopathy and CAD. Hence there were total 8 eligible studies including our study comprised of a total of 2060 cases with secondary complication of T2DM and 3049 age matched controls without T2DM. The genotypes were classified into high risk (TT) and low risk (CC+CT) for statistical calculations. The 'I^2' value of 49.70% for between studies comparison showed low level of true heterogeneity. The fixed model test for the genotype data for the studies yielded a statistically significant result (Q = 13.91, p value <0.001, I^2 = 49.70, tau squared = 0.053).

Egger's test for intercept showed no evidence of publication bias (intercept = - 0.76, SE = 1.50, 95% CI = -4.44 to 2.90, p 2 tailed = 0.62). Classic Fail safe 'N' = 223, missing from the analysis for every published study included, which further supports absence of publication bias. Funnel plot of standard error by log odds ratio was also generated (Fig. 2). The possibility of publication bias was rejected due to the evidence of symmetrical distribution of studies on funnel plot (Fig. 3).

4. CONCLUSION

Wnt signaling works via disheveled receptors and transcription factors like β-catenin and TCF7L2 to modulate the expression of downstream target genes like IL1, TNF-α, FGF, KGF and VEGF [14]. The transcription factor 7 like 2 gene product, a High mobility group box transcription factor, is a part of β-catenin signaling cascade [15]. Duggirala *et al*. [16] described about a region on chromosome 10q that was linked to T2DM. Further Grant *et al*. [17] showed a strong association between a

microsatellite marker located within intron 3 of TCF7L2 and following that study an another study revealed that SNP rs 7903146 allele T within the gene TCF7L2 is in linkage disequilibrium with this microsatellite marker [18]. The association of this variant with T2DM has already been established in different cohorts of world including Asian populations like India and Pakistan [19-22]. This TCF7L2 gene polymorphism has been already shown to be involved in secondary complications of diabetes like nephropathy, neuropathy, retinopathy and cardiovascular disease [12, 13, 23]. TCF7L2 may affect β- cell function either by modulating β-cell response to glucose or by modulating the incretin action or secretion [24]. The minor T allele of this SNP is strongly associated with HOMA-B level and hence results in diabetic condition either by affecting function of pancreatic β-cell or by impaired GLP-1 induced insulin secretion [25, 26]. Functional studies show that T allele of SNP rs 7903146 is less prone to be bound by protein factors like GATA3 in hepatic cell lines and hence there is aberrant TCF7L2 expression in patients with TT genotype [27]. Cauchi *et al*. showed that this polymorphism have no impact on expression of specific TCF7L2 isoforms in Non Glucose Tolerant (NGT) subjects [28]. Further he added that in T allele NGT carriers of this SNP has low TCF7L2 expression in their adipose tissue and anergetic nutrition i.e. high calorie diet may help in increasing TCF7L2 expression. Interestingly the minor T allele of SNP is neither associated with higher BMI nor it has been associated with risk lipid profile i.e. the patients with this T allele has lesser chance to develop metabolic syndrome [29]. To the best of our knowledge only 5 studies including our study have evaluated the association of SNP rs 7903146 of TCF7L2 gene with secondary complications in diabetes. Sousa *et al*. in 2009 [30] reported the association of TCF7L2 polymorphism rs7903146 and coronary artery disease in diabetic and non-diabetic subjects in two independent population of Brazil, but the association was non significant in case of diabetic patients. In another study Buraczynska *et al*. in 2011 [12] showed the association of this variant with diabetic nephropathy, diabetic retinopathy and cardiovascular disease among Caucasians of Polish origin. They also concluded that T allele of this polymorphism was strongly associated with nephropathy, particularly in early onset of diabetes. Muendlein *et al*. in 2011 [22] investigated the association between this polymorphism and angiographically determined CAD in diabetic and non-diabetic patients in whites of Austria. They also found that this variant was significantly associated with angiographically diagnosed CAD, specifically in patients with T2DM. In a study conducted by Ciccacci *et al*. in 2012 [13], the authors studied this gene polymorphism in the pathogenesis of diabetic retinopathy and neuropathy. They concluded that a strong correlation was present between the rs7903146 variant of TCF7L2 and the presence of cardiovascular autonomic neuropathy and diabetic retinopathy. Our group has analyzed the association of TT genotype of SNP rs 7903146 with another secondary complication of T2DM which is impairment in healing of wounds in T2DM patients and found a significant association of TT genotype with wound healing

impairment [11]. Based on above previous studies and combining our own study we conducted a Meta analysis for analyzing the association of this polymorphism with secondary complication of T2DM. Meta analysis of all five studies also showed highly significant association of risk genotype TT with development of secondary complication in T2DM.

5. CONFLICT OF INTEREST

The authors declare no conflict of interest.

6. ACKNOWLEDGEMENT

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