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Methodology of Biomedical Research, Biostatistics and Clinical Bioinformatics

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"The association of TNF-a 308G/A polymorphism and Diabetic Nephropathy. A meta-analysis"

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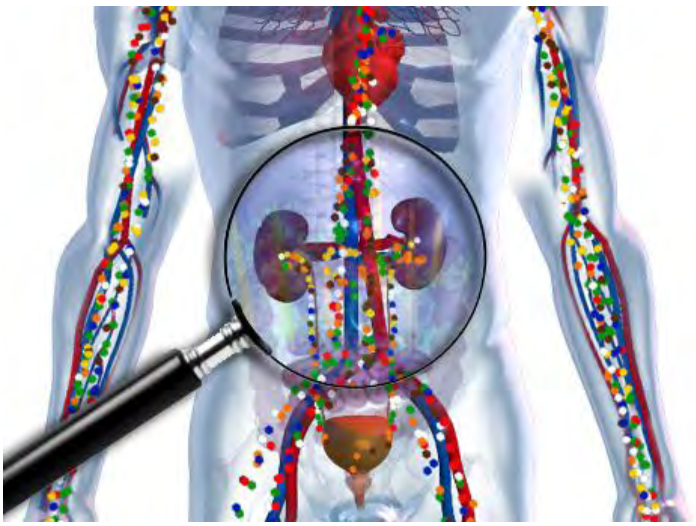

- Eleni Paschou -

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1. Abstract

Introduction

Diabetic nephropathy, an irreversible progressive kidney disease, represents the most common cause of end-stage renal disease in adults globally. The pathophysiology of the disease is multifactorial and remains unidentified, although extensive research in this field has revealed various underlying mechanisms. Inflammatory cytokines such as TNF-a have been suggested that participate fundamentally in the pathogenesis of renal insufficiency.

Purpose of the study

Several genetic variations of the promoter region of TNF-a such as 308 G/A in patients with diabetes mellitus and nephropathy have been studied. In this meta-analysis we aim to examine the association between this polymorphism and the risk of diabetic nephropathy.

Methods

The electronic search of the Pubmed database literature that conducted until August 2015 revealed nine candidate-genome associated studies that examine the distribution of AA, AG and GG genotypes of the current polymorphism in cases and controls subjects. Due to the complexity of the pathophysiology observed in diabetic nephropathy, studies were divided into two subgroups: one with diseased controls and one with healthy controls. Thus, two different meta-analyses were performed.

Results

In studies with healthy controls, dominant model showed increased risk of diabetic nephropathy in A carriers (OR=1.45, 95% CI: 0.797-2.635). Heterogeneity was significant (P_Q<0.01, I^2=86.78%) and sensitivity analysis for studies in HWE showed further significant association (OR= 2.701, 95% CI: 1.089 – 6.701). Subgroup analysis produced increased risk of diabetic nephropathy for Asians but not for Caucasians. Meta-analysis of studies with diseased controls showed no statistically significant association between TNF-a 308 G/A polymorphism and the risk of diabetic nephropathy.

Conclusions

The results suggest that the TNF-a 308 G/A polymorphism may be associated with an increased risk of nephropathy in Asians with diabetes mellitus compared to healthy individuals but not in Caucasians. Since the pathogenesis of the disease implicates several mechanisms, further large-scale genome associated studies are required in order to minimize potential bias and interpret those results correctly.

2. Introduction

Diabetic nephropathy is a progressive kidney disease associated with diabetes mellitus type 1 and type 2, affecting kidney glomeruli, arterioles, tubules and interstitium. It is the most common cause leading to chronic kidney disease in adults, consisting a worldwide unsolved problem with unprecedented social and economic repercussions [1].

Gradually, diabetic nephropathy is characterized by the clinical triad of albuminuria, hypertension and reduction in glomerular filtration rate. The presence of microalbuminuria in a person with diabetes mellitus may progressively lead to end-stage renal disease, requiring dialysis or kidney transplantation. In this context, microalbuminuria heralds the onset of a dramatically increased risk of death correlated with accelerated atherosclerosis and cardiovascular events [2].

2.1 Epidemiology of Diabetic Nephropathy

Nephropathy affects around 30% of patients with type 1 diabetes and 20% of patients with type 2 diabetes [1]. Duration of diabetes is the major risk factor for the development of kidney disease. Men appear to be at a higher risk of developing nephropathy [3]. Ethnicity seems to be an important factor since there are studies supporting that South-Asians and African-Caribbeans are at higher risk [4, 5].

Early studies showed a 10-year survival after the onset of persistent albuminuria of 28% [6], but more recent statistics suggest dramatically improved prognosis in diabetic nephropathy (82% 10-year survival) due to advances in blood pressure therapy and renal replacement therapy.

2.2 Pathophysiology of Diabetic Nephropathy

In diabetic nephropathy, as in most diabetes-associated complications, the pathophysiology is multifactorial mostly because the involved molecular pathways constitute a wide and complex network of regulators.

Pathophysiological events in diabetic nephropathy evolve a much more complex scenario with both genetic and environmental factors [7, 8]. In the classical view, renal damage results due to metabolic and hemodynamic alterations that increase systemic and intraglomerular pressure. In these conditions, modification of molecules under hyperglycemic conditions – such as AGEs - play significant role in renal endothelial dysfunction. It seems that the key mechanism in the etiology of diabetic nephropathy is subclinical inflammation observed in cellular and molecular level. In this direction, new potential targets are identified leading to the design of innovative anti-inflammatory therapeutic strategies.

2.3 The role of Inflammatory Cytokines in Diabetic Nephropathy

A potential participation of inflammatory cytokines in the pathogenesis of diabetic nephropathy was suggested for the first time in 1991 when Hasegawa et al. [9] demonstrated that peritoneal macrophages cultured with glomerular basement membranes from diabetic rats produced significantly higher amounts of tumor necrosis factor-alpha (TNF-*α*) and interleukin- (IL-) 1 than those cultured with glomerular basement membranes from normal rats.

In general, renal cells are capable of synthesizing proinflammatory cytokines such as TNF-*α*, IL-1, and IL-6. These cytokines may induce a variety of effects on different renal structures [10], acting in a paracrine or autocrine way, that play a significant role in the development and progression of renal impairment. Mainly, cytokine effects in renal function and structure provoke the expression of different molecules, intraglomerular hemodynamic abnormalities, endothelial permeability and oxidative stress, alteration of extracellular matrix and glomerular basement membranes, apoptosis and necrosis [11] resulting to the development of diabetic nephropathy.

2.4 TNF-a and Diabetic Nephropathy

Many clinical studies in patients with diabetic nephropathy have reported that serum and urinary concentrations of TNF-*α* are elevated compared to nondiabetic individuals or with diabetic subjects without renal failure. These concentrations increase concomitantly with the progression of diabetic nephropathy. Such findings indicate a potential relationship between the elevated levels of this inflammatory cytokine and the development and progression of chronic kidney disease in diabetes mellitus [12, 13].

TNF-*α* codes by TNF gene located in chromosome 6q21 in the human leukocyte antigen (HLA) region class III [14]. It is mainly produced by monocytes, macrophages, and T cells but also intrinsic kidney cells [15-17] and it increases the release of other cytokines, chemokines, growth factors, and acute phase proteins [18]. Experimental studies in animal models of diabetes have showed that TNF-*α* protein and expression levels are enhanced in renal glomeruli and tubules [19-22].

TNF-*α is a* pleiotropic cytokine that exerts multiple effects. It contributes to the development of diabetic nephropathy through several mechanisms. The most important are reduction of the glomerular blood flow and glomerular filtration rate. As a consequence, the disequilibrium between factors promoting vasoconstriction and vasodilation [23] such as endothelin- 1 and disruption of the glomerular filtration barrier lead to proteinuria [18]. Increased production of TNF-*α* can also arouse oxidative stress, through the activation of nicotinamide adenine dinucleotide phosphate (NADPH) in mesangial cells. Finally, TNF-*α* appears to have a direct apoptotic and cytotoxic effect on glomerular cells [11, 18, 24, 25].

Genetic variations in the promoter region may regulate TNF-a production. The most well studied TNF-a promoter variants are guanine (G) to adenine (A) substitutions in positions -238 and -308 [26]. It has been reported that TNF-a 308 G/A polymorphism is associated with the onset of obesity [27] and insulin resistance in

diabetes mellitus [28]. In vitro researches showed that these polymorphisms lead to higher rate of TNF gene transcription and thus higher level of TNF production.

Despite extensive research performed the last decades, there are still unexplored areas and unanswered questions. Thus, the implication of TNF-α promoter polymorphisms in the initiation and progression of inflammatory cascade in diabetic nephropathy [12, 21, 25, 29, 30] remains controversial.

In order to achieve integrated and reliable conclusions, meta-analyses are required. In the present meta-analysis we aim to review candidate-genome associated studies for TNF-a 308 G/A polymorphism in correlation with diabetic nephropathy risk.

3. Methods

3.1 Identification and Eligibility of relevant studies

An electronic search of the Pubmed literature was conducted until August 2015 in order to investigate the association between TNF-a 308 G/A polymorphism and diabetic nephropathy risk. The search terms that were used in multiple combinations included: “polymorphism” or “mutation” or “allele” or “SNP”, “TNF” or “tumor necrosis factor” and “diabetic nephropathy” or “nephropathy” or “diabetes”. Only articles in English language were recorded.

Studies that were eligible for inclusion in the meta-analysis were candidate-gene association studies (GAS) that determined the distribution of TNF-a 308 G/A polymorphism in subjects with diabetic nephropathy and in a. diseased controls (subjects with diabetes mellitus but not nephropathy or subjects with other primary cause or nephropathy and no diabetes mellitus) or in b. healthy controls.

Cases with diabetes mellitus were considered as suffering from diabetic nephropathy on the basis of persistent albuminuria or microalbuminuria with or without chronic renal insufficiency and in the absence of nondiabetic renal disease or patients with end-stage renal disease.

The diseased control group consisted of subjects with diabetes and free of diabetic kidney disease.

Only studies in human subjects that used validated genotyping methods were considered. Case reports, editorials and review articles were also excluded. The retrieved studies were read in their entirety to assess their appropriateness for inclusion in the meta-analysis. All references cited in the studies were also reviewed in order to identify additional published work that was not indexed by the Pubmed database.

3.2 Data extraction

The following information was extracted from each study: First author’s last name, Journal, Year of Publication, Country of origin, Ethnicity, demographics, clinical characteristics, Sample Size, Type of DM, Genotype number in cases and controls.

3.3 Statistical Analysis

The TNF 308G/A genotypes include GA, GG and AA. The above genetic models were performed: (1) additive: AA versus GG, (2) co-dominant: GG+AA versus GA, (3) dominant: GA+AA versus GG and (4) recessive: AA versus AG+GG respectively [31].

The strength of the association between TNF-a 308 G/A polymorphism and diabetic nephropathy was indicated as a pooled Odds Ratio (OR) with the corresponding 95% Confidence Interval (95% CI). The statistical significance of

summary OR was determined using a Z-test. The heterogeneity between studies was assessed by a $\chi 2$-based Q statistic and a p value of <0.10 was considered statistically significant. Heterogeneity was quantified with the I^2 metric [32]. Pooled OR was analyzed by a fixed-effects (FE) model (Mantel-Haenszel method) or a random-effects (RE) model (DerSimonian and Laird method) according to heterogeneity [33, 34]. Finally the generalized OR was calculated [35].

The meta-analysis consisted of the main (overall) analysis, which included all available data. Further efforts were conducted on the subgroup analyses according to ethnicity defined as Asian and Caucasian and the type of DM (DM1 or DM2).

Sensitivity analysis examined the effect of excluding specific studies that did not follow the Hardy-Weinberg equilibrium (HWE) test [36].

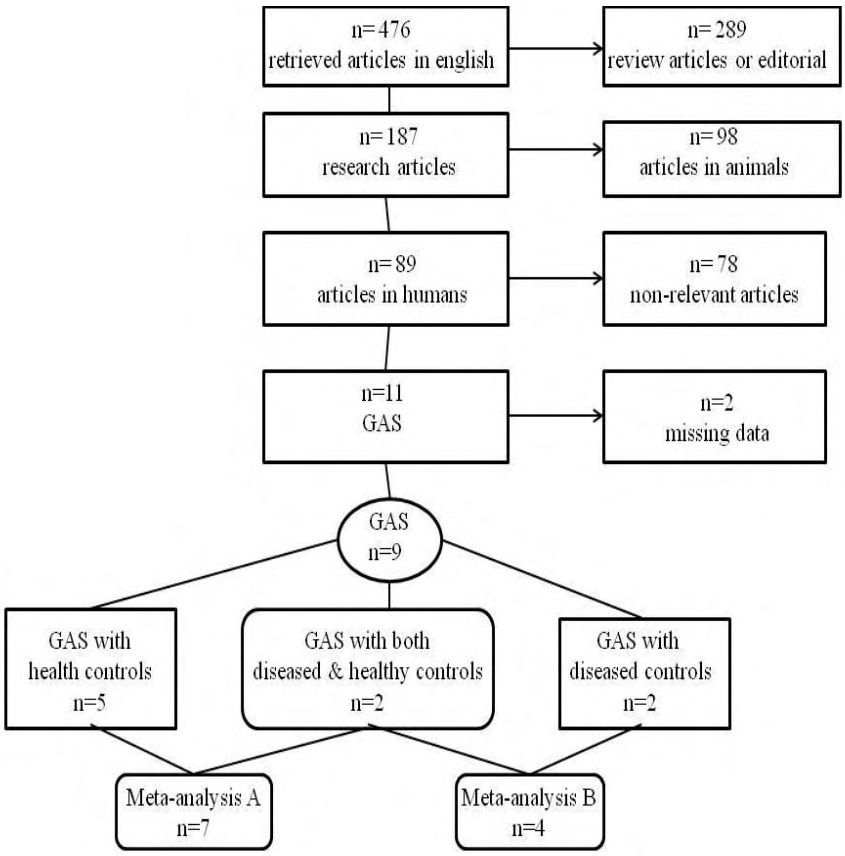
All Statistical Test were performed using Comprehension Meta Analysis V3, MedCal 15.8 and ORGGASMA [35] (available at http://biomath.med.uth.gr) software.

4. Results

4.1 Eligible Studies and study characteristics

The literature review identified 11 candidate-genome associated studies in Pubmed. The full articles of the retrieved studies were read to assess their appropriateness for meta-analysis according to the inclusion criteria. Data from 9 articles that investigated the association between TNF-a 308G/A polymorphism and the risk of DN met the inclusion criteria [37-45]. One study was excluded due to incomplete data on genotype frequencies [46]. Finally, one study investigated the association of TNF-a 308 G/A polymorphism between subjects with DM that presented with acute kidney injury while been hospitalized in an ICU. In those subjects DN could lurk but the study was also excluded because it did not met the criteria of a safe diagnosis of DN [47]. Figure 1 represents a flowchart of retrieved studies and studies excluded. The studies were published between 2004 and 2015.

Figure 1. Flow-chart of the retrieved studies



A list of details abstracted from the studies included in the meta-analysis and are provides in Table 1, 2 and 3.

Table 1. Characteristics of Cases of the Studies considered in the meta-analysis

Authors	Ethnicity	Selection Criteria	DM type	n of M (%)	Age (yr)	DM duration
Babel et al	Caucasian	renal failure or HD, diagnosis by renal biopsy	2	27(61%)	47,5±7,8	ND
Bucan et al	Caucasian	microalbuminuria or proteinuria	1			21,4±6
Buraczynska	Caucasian	DN on peritoneal dialysis	1+2	19(51.4%)	55,7(22-±78)	ND
Lindholm et al	Caucasian	DN due to DM1	1+2	ND	ND	>20 years
Manchanda et al	Asian	DN with s.creat >4mg/dL, under HD for at least 3 months	ND	ND	ND	ND
Peng et al	Asian	DN	ND	ND	ND	ND
Prasad et al	Asian	DM2 with s.creat > 3mg/dL, AER> 200mg/L and DR	2	ND	ND	>2 years
Sikka et al.	Asian	DN in HD	2	ND	ND	13,15±7,65
Singh et al	Asian	CRF/ ESRD due to DM	ND	ND	ND	ND

Table 2. Characteristics of the Controls of Studies considered in the meta-analysis

Authors	Ethnicity	Status	DM type	n of M (%)	Age (yr)	DM duration
Babel et al	Caucasian	healthy	2	52(43%)	41±8,4	NA
Bucan et al	Caucasian	diseased	1			12,8±8,9
Buraczynska	Caucasian	diseased (patients in HD with other primary renal diseases)	1+2	32(55.2%)	52,8(23-78)	ND
		or healthy		69(60%)	46,64(21-61)	ND
Lindholm et al	Caucasian	healthy	1+2	ND	ND	NA
Manchanda et al	Asian	healthy	ND	102)56,6%)	34,96±11,3	NA
Peng et al	Asian	healthy	ND	ND	ND	NA
Prasad et al	Asian	diseased (DM2 > 10 years, normoalbuminuria, AER< 20mg/L)	2	ND	ND	17,07 ±6,69
Sikka et al.	Asian	healthy	2	ND	ND	NA
		diseased		ND	ND	7,27±6,75
Singh et al	Asian	healthy	ND	ND	ND	NA

Table 3 Characteristic of Studies and distributions of genotypes and alleles

Authors	Year	Country	Ethnisity	DM type	Controls	Cases	Cases			Controls	Controls			A allele (%)	
					Status	(n)				(n)					
							GG	GA	AA		GG	GA	AA	Cases	Controls
Babe let al	2006	German	Caucasian	2	H	44	34	7	3	113	76	33	4	14,8	18,1
Bucan et al	2008	Croatia	Caucasian	1	D	14	9	4	1	33	22	8	3	27,2	26,9
Buraczynsla	2004	Poland	Caucasian	1+2	H	37	22	13	2	115	86	24	5	23	14,8
					D	37	22	13	2	58	40	15	3	23	22,1
Krayenbuehl et al*	2007	Switzerland	Caucasian	2		39	30	9	ND	37	28	9	ND	ND	ND
Lindholm et al	2008	Sweden	Caucasian	1+2	H	527	254	252	21	528	191	292	45	38,6	56,6
Manchanda et al	2006	China	Asian	ND	H	23	2	7	14	180	49	126	5	318,1	60,7
Peng et al	2015	China	Asian	ND	H	86	52	28	6	94	72	18	4	30,3	15,1
Prasad et al	2007	India	Asian	2	D	196	178	16	2	224	195	27	2	5,1	5,9
Sikka et al.	2014	India	Asian	2	H	137	124	21	1	203	176	27	0	6,5	6,6
					D	137	124	21	1	196	139	15	1	6,5	5,8
Singh et al	2015	India	Asian	ND	H	49	5	14	30	66	21	14	31	308,3	135,7
Wang et al*	2005	China	Asian	2		388	288	110		323	730	159		ND	ND
Wang et al*	2008	china	Asian	2		388	326	62	ND	323	261	62	ND	ND	ND

**Studies not included in meta-analysis, ND: not defined, H: healthy, D: disease*

Four studies involved cases with T2DM and two with both T1DM and T2DM. The remaining studies did not specify the type of DM. In five studies the controls were healthy subjects while only in two studies the controls were diseased. In the remaining studies there were two control groups, one healthy and one diseased. Studies were conducted in two populations: Asians and Caucasians.

4.2 Summary statistics

Since the control groups in the nine studies were of two categories, two meta-analyses were performed. The first consisted of healthy controls subjects and included seven studies and the second consisted of diseased subjects and included four studies. Data from the two studies with both healthy and diseased controls were extracted and those studies participated in both meta-analyses.

In four studies [38-40, 44], the distribution of genotypes in control groups was not in HWE ($P<0.05$), indicating genotyping errors and/ or population stratification, therefore, a sensitivity analysis was preformed excluding those studies.

4.2.1 Meta-analysis A: Healthy Controls

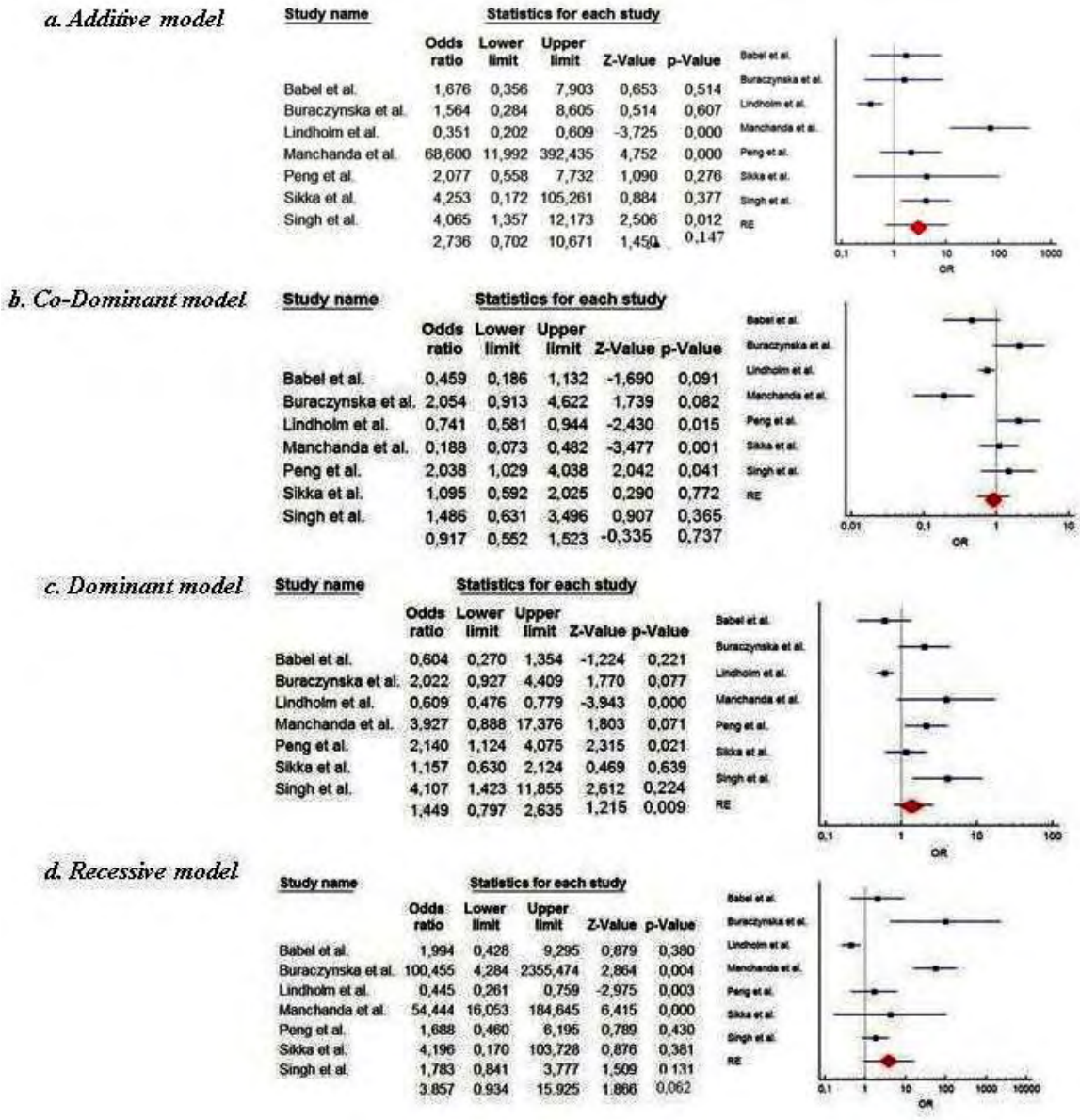
In this meta-analysis, seven studies were included (Table 3).

Table 3. Meta-analysis A (healthy controls): Studies participating

Authors	Ethnicity	Cases	Controls	HWE *p value*
Babel et al.	Caucasian	44	113	0,858
Buraczynska et al.	Caucasian	37	115	0,065
Lindholm et al.	Caucasian	527	528	0
Manchanda et al.	Asian	23	180	0
Peng et al.	Asian	86	94	0,056
Sikka et al.	Asian	137	203	0,31
Singh et al	Asian	49	66	0

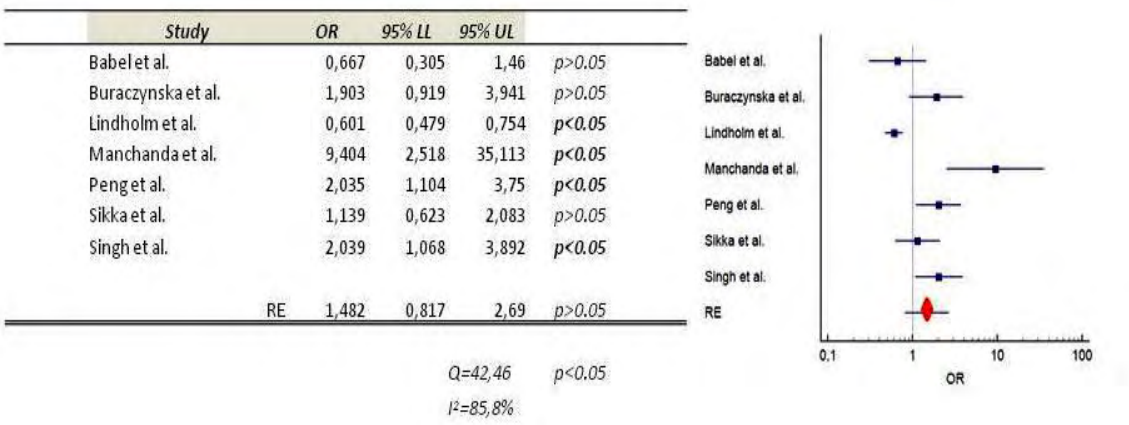
Overall, for the TNF-a 308 G/A polymorphism and its relationship to DN, the dominant model showed a marginally significant association (OR= 1.449, 95% CI: 0.797 – 2.635), indicating that A carriers have 45% more chance of developing diabetic nephropathy. The heterogeneity between studies was significant (P_Q<0.01, I^2=86.78%) as it is shown in Figure 2.

Figure 2. Meta-analysis A (healthy controls): overall analysis



The generalized OR is 1.482 (95% CI: 0.817 – 2.69) but it is not significant (Figure 3).

Figure 3. Meta-analysis A (healthy controls): ORG

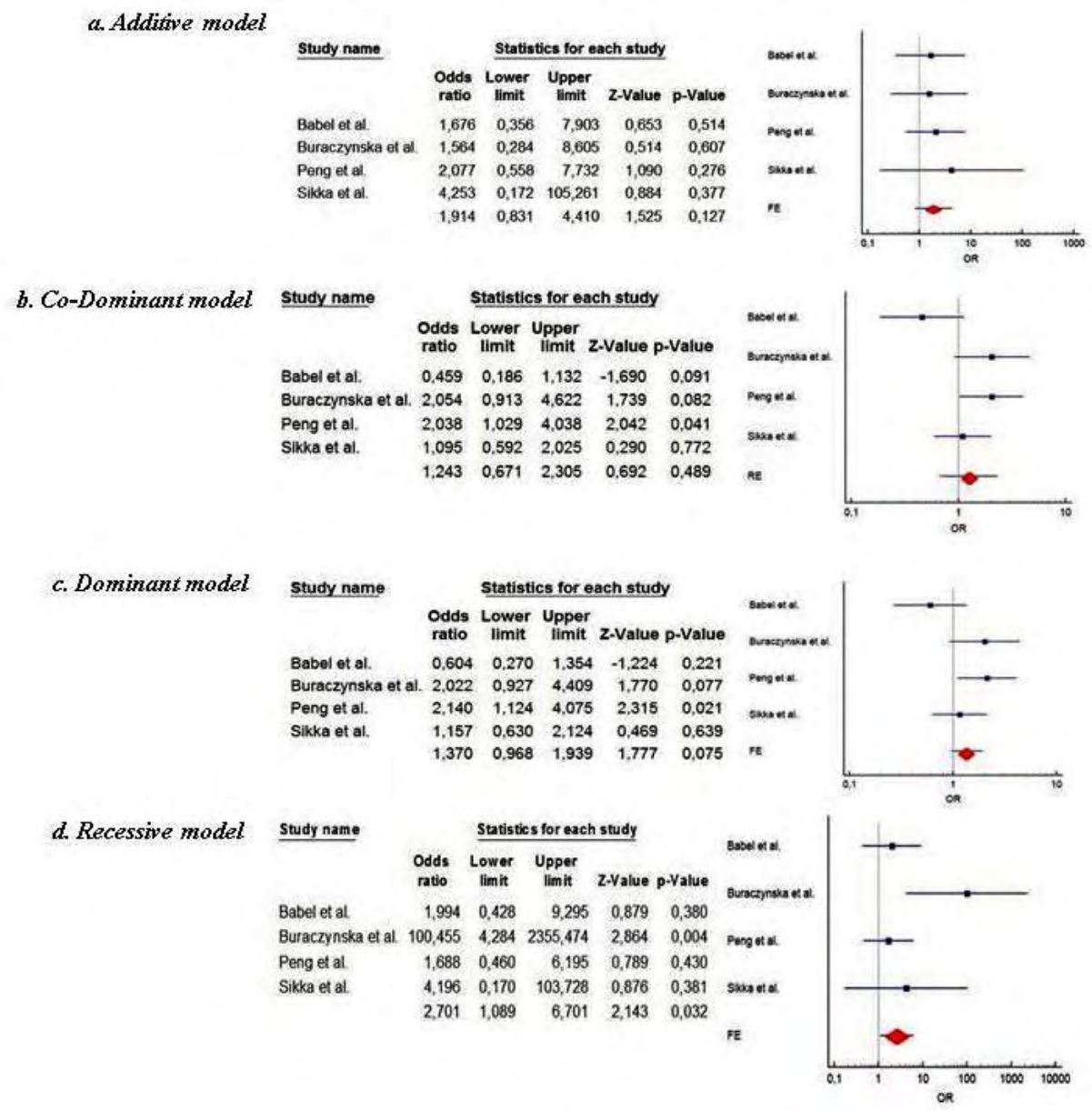


Study	OR	95% LL	95% UL	
Babel et al.	0,667	0,305	1,46	p>0.05
Buraczynska et al.	1,903	0,919	3,941	p>0.05
Lindholm et al.	0,601	0,479	0,754	p<0.05
Manchanda et al.	9,404	2,518	35,113	p<0.05
Peng et al.	2,035	1,104	3,75	p<0.05
Sikka et al.	1,139	0,623	2,083	p>0.05
Singh et al.	2,039	1,068	3,892	p<0.05
RE	1,482	0,817	2,69	p>0.05
			Q=42,46	p<0.05
			I²=85,8%	

4.2.1.1. Sensitivity Analysis

Sensitivity analysis performed after the exclusion of the studies that were not into HWE (Figure 4). According to the results, the recessive model showed significant association (OR= 2.701, 95% CI: 1.089 – 6.701), indicating that AA genotype has better chance of developing diabetic nephropathy, while the heterogeneity between studies was not statistically significant (P_Q=0.123, I^2=48%). Thus, data from publications not in the HWE may have a significant influence on the overall result.

Figure 4. Meta-analysis A (healthy controls): Sensitivity analysis



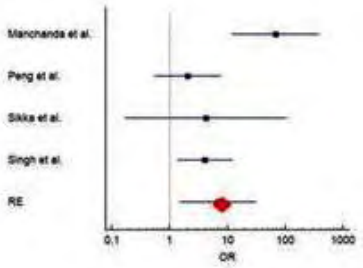
4.2.1.2. Subgroup analysis

Subgroup analysis showed significant association between the polymorphism and diabetic nephropathy in both additive and recessive models (OR=6.838, 95% CI: 1.494 – 31.298 and OR=1.888, 95% CI: 1.274 – 2.798 respectively) for Asians (Figure 5) but no significance observed in Caucasians (Figure 6).

Figure 5. Meta-analysis A (healthy controls): Subgroup analysis - Asians

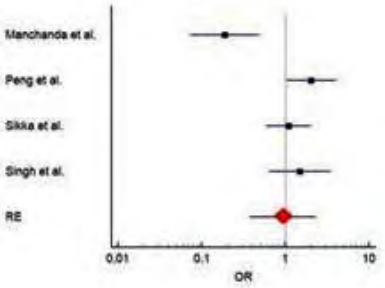
a. Additive model

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Manchanda et al.	68,600	11,992	392,435	4,752	0,000
Peng et al.	2,077	0,558	7,732	1,090	0,276
Sikka et al.	4,253	0,172	105,261	0,884	0,377
Singh et al.	4,065	1,357	12,173	2,506	0,012
	6.838	1.494	31.298	2.477	0.013



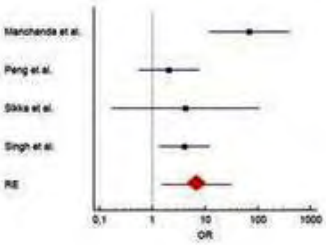
b. Co-Dominant model

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Manchanda et al.	0,188	0,073	0,482	-3,477	0,001
Peng et al.	2,038	1,029	4,038	2,042	0,041
Sikka et al.	1,095	0,592	2,025	0,290	0,772
Singh et al.	1,486	0,631	3,496	0,907	0,365
	0,924	0,373	2,285	-0,172	0,863



c. Dominant model

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Manchanda et al.	3,927	0,888	17,376	1,803	0,071
Peng et al.	2,140	1,124	4,075	2,315	0,021
Sikka et al.	1,157	0,630	2,124	0,469	0,639
Singh et al.	4,107	1,423	11,855	2,612	0,009
	1,888	1,274	2,798	3,164	0,002



d. Recessive model

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Manchanda et al.	54,444	16,053	184,645	6,415	0,000
Peng et al.	1,688	0,460	6,195	0,789	0,430
Sikka et al.	4,196	0,170	103,728	0,876	0,381
Singh et al.	1,783	0,841	3,777	1,509	0,131
	5,141	0,806	32,791	1,732	0,083

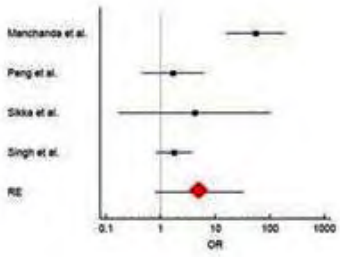
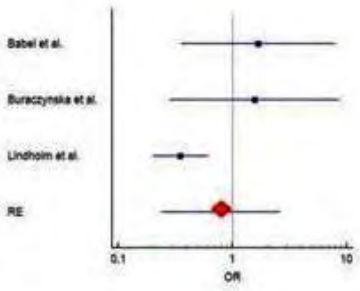


Figure 6. Meta-analysis A (healthy controls): Subgroup analysis - Caucasians

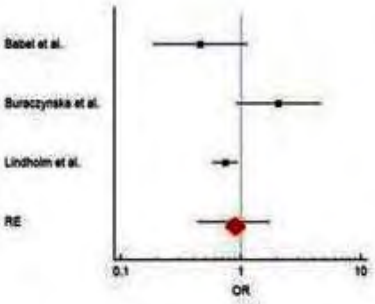
a. Additive model

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Babel et al.	1,676	0,356	7,903	0,653	0,514
Buraczynska et al.	1,564	0,284	8,605	0,514	0,607
Lindholm et al.	0,351	0,202	0,609	-3,725	0,000
	0,468	0,285	0,768	-3,000	0,003
	0,79	0.239	2,611	-5,782	0,7



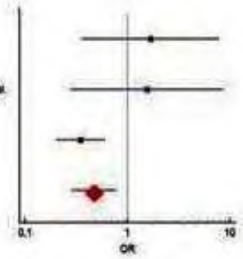
b. Co-Dominant model

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Babel et al.	0,459	0,186	1,132	-1,690	0,091
Buraczynska et al.	2,054	0,913	4,622	1,739	0,082
Lindholm et al.	0,741	0,581	0,944	-2,430	0,015
	0,876	0,438	1,752	-0,375	0,708



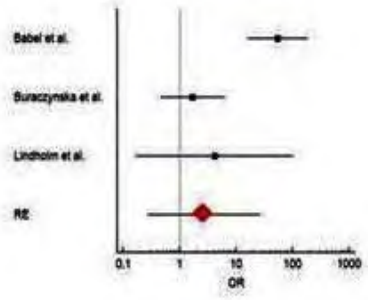
c. Dominant model

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Babel et al.	0,604	0,270	1,354	-1,224	0,221
Buraczynska et al.	2,022	0,927	4,409	1,770	0,077
Lindholm et al.	0,609	0,476	0,779	-3,943	0,000
	0,862	0,423	1,758	-0,409	0,683



d. Recessive model

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Babel et al.	1,994	0,428	9,295	0,879	0,380
Buraczynska et al.	100,455	4,284	2355,474	2,864	0,004
Lindholm et al.	0,445	0,261	0,759	-2,975	0,003
	2,698	0,272	26,796	0,847	0,397



4.2.2. *Meta-analysis B: Diseased Controls*

In this meta-analysis, four studies were included (Table 4).

Table 4. Meta-analysis B (diseased controls) : Studies participating

Authors	Ethnicity	Patient	Controls	HWE *p-value*
Bucan et al.	Caucasian	14	33	0,007
Buraczynska et al.	Caucasian	37	58	0,33
Prasad et al.	Asian	196	224	0,336
Sikka et al.	Asian	137	196	0,408

Overall, for the TNF-a 308 G/A polymorphism and its relationship to DN no significant association was observed (Figure 7). The generalized OR is 0.213 (95% CI: 0.103 – 4.38) but it is not statistically significant (Figure 8).

Sensitivity analysis performed after the exclusion of the studies that were not into HWE. According to the results, no significant association between TNF-a 308 G/A polymorphism and DN was observed (Figure 9).

Subgroup analysis was not performed because of the small number of studies thus, definite conclusions cannot be drawn.

Figure 7. Meta-analysis B (diseased controls): overall analysis

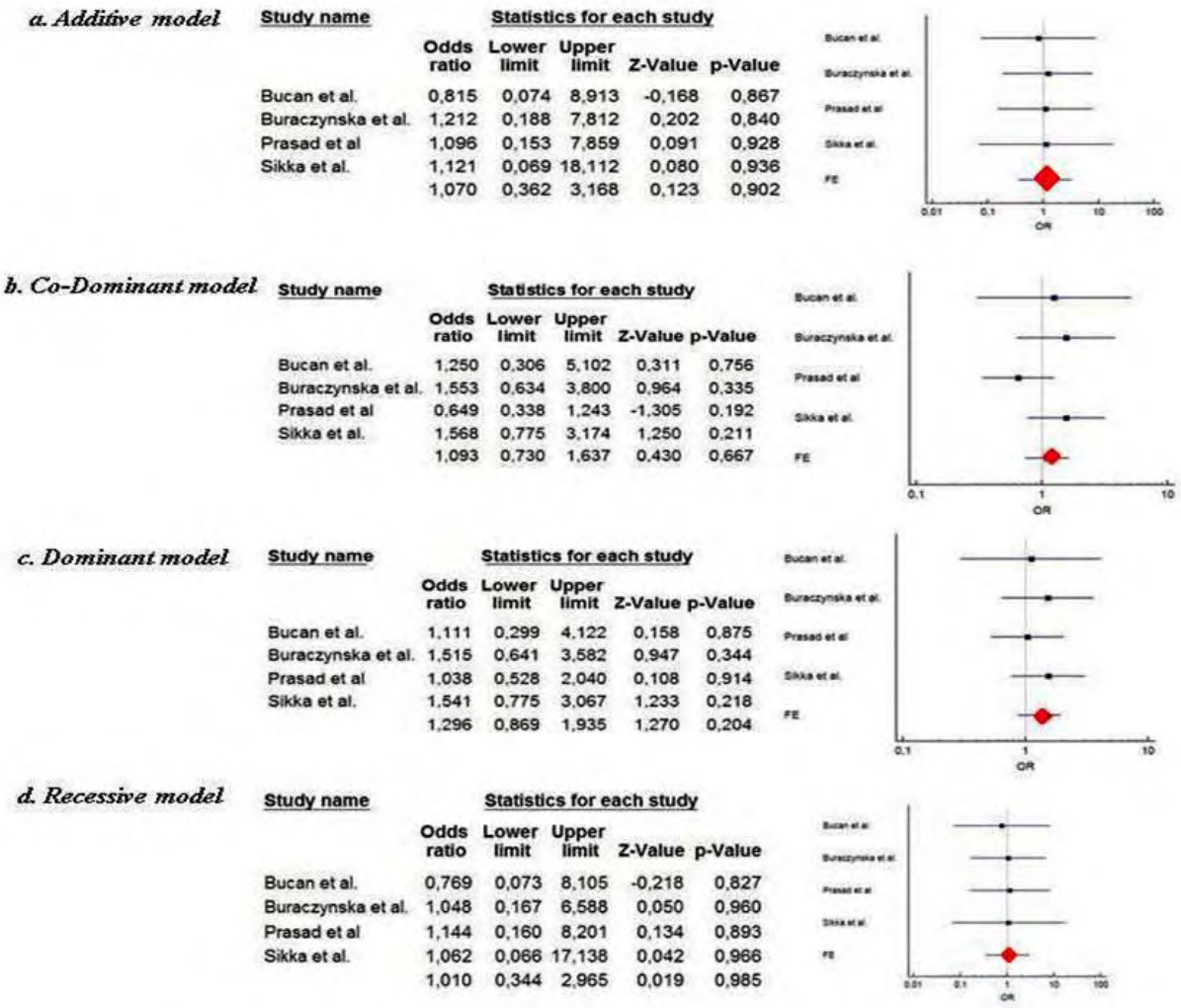


Figure 8. Meta-analysis B (diseased controls): ORG

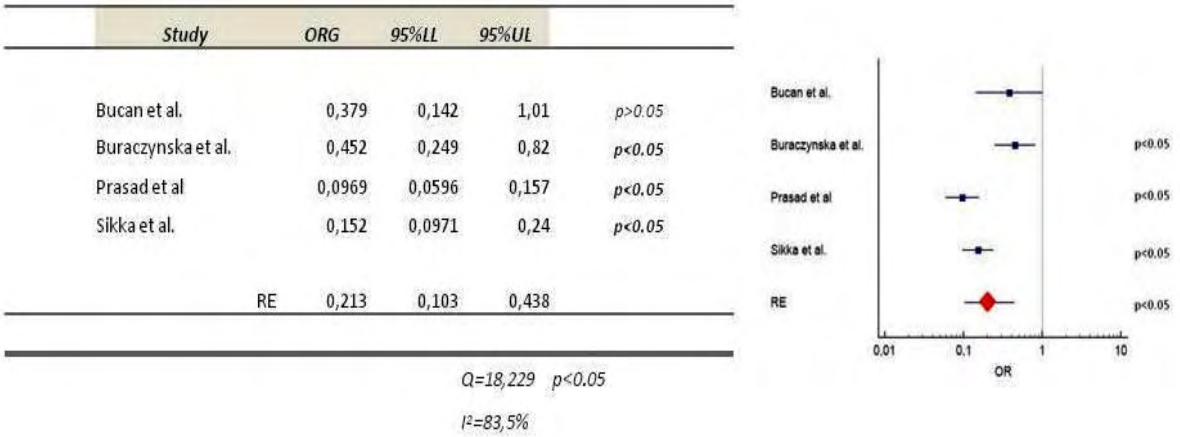
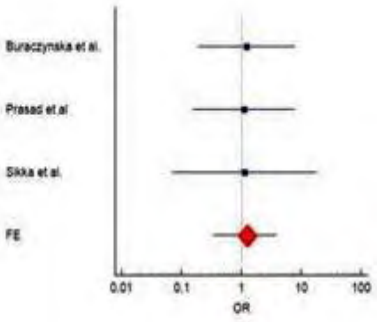


Figure 9. Meta-analysis B (diseased controls): Sensitivity analysis

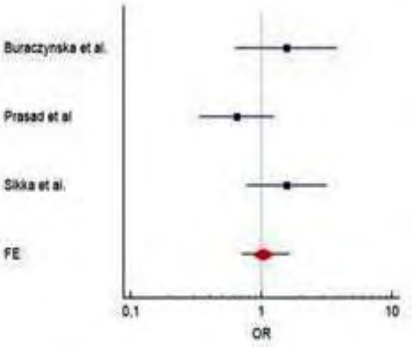
a. Additive model

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Buraczynska et al.	1,212	0,188	7,812	0,202	0,840
Prasad et al	1,096	0,153	7,859	0,091	0,928
Sikka et al.	1,121	0,069	18,112	0,080	0,936
	1,149	0,340	3,881	0,223	0,823



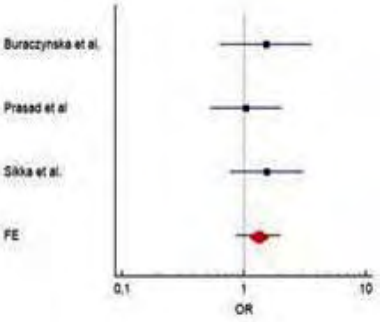
b. Co-Dominant model

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Buraczynska et al.	1,553	0,634	3,800	0,964	0,335
Prasad et al	0,649	0,338	1,243	-1,305	0,192
Sikka et al.	1,568	0,775	3,174	1,250	0,211
	1,080	0,708	1,646	0,356	0,722



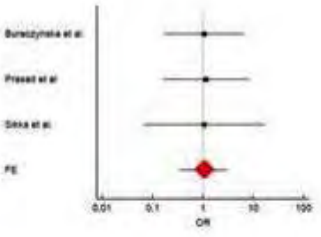
c. Dominant model

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Buraczynska et al.	1,515	0,641	3,582	0,947	0,344
Prasad et al	1,038	0,528	2,040	0,108	0,914
Sikka et al.	1,541	0,775	3,067	1,233	0,218
	1,317	0,865	2,006	1,283	0,199



d. Recessive model

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Buraczynska et al.	1,048	0,167	6,588	0,050	0,960
Prasad et al	1,144	0,160	8,201	0,134	0,893
Sikka et al.	1,062	0,066	17,138	0,042	0,966
	1,086	0,324	3,642	0,134	0,894



5. Conclusions

It has been observed that some diabetic patients that experience long-term hyperglycemia develop nephropathy while others do not [48] and this phenomenon is inexplicable. Environmental exposure is only one side of the problem and this is why genetic background is under the microscope. Several valid explanations for this situation have been proposed but the most striking cause lies in the multifactorial and highly redundant nature of the molecular pathways of this disease.

During the past years, the role and association of TNF-a variation has raised attention. While several studies have been reported [49, 50], the results remain inconclusive due to parameters such as difference in ethnicity, age, sex and lifestyle. These studies were not powerful enough to provide us with reliable results. Therefore, a meta-analysis is needed in order to give a more conclusive answer.

The two meta-analyses previously performed, examined the TNF-a 308 G/A polymorphism and its relationship to susceptibility for diabetic nephropathy. In total, the meta-analyses involved 9 candidate genome associated studies for diabetic nephropathy which provided 1113/1810 cases /controls. In this study, the effects of the additive, co-dominant, dominant and recessive models as well as the generalized odds ratio were estimated. In addition the consistency of genetic effects across populations from different ethnicities were investigated [51] through subgroup analysis by rage. No other subgroup analysis was able to be produced (i.e. subgroup analysis by type of diabetes mellitus or gender) due to missing data. Sensitivity analysis for studies not in HWE was also performed.

In overall analyses and subgroup analyses, the testing of associations was based on different amount of information. Therefore, any comparisons between the effect sizes should be interpreted with caution.

These meta-analyses had some limitations. First, the results may be applicable to two ethnic groups, as the case controlled studies used involved Caucasian and Asian populations. Further studies are required to investigate the association in other populations. Second, gender specific subgroup analysis should have been carried out since diabetic nephropathy is more frequent in men [3], but the original data were found to be insufficient. Third, the diagnostic criteria of diabetic nephropathy varied while, in some studies, were not precisely described. This may be a cause of potential bias. Fourth, in two articles [45, 46], accurate numbers on genotypes distributions were not accessible. As a result, related data were not included in analyses. Fifth, in one study [47], subjects participating may introduce themselves to diabetic nephropathy, but the study was excluded since it was not defined whether there was a diagnosis or not.

The fact that non-English, nonindexed and nonpublished studies literature were not reviewed may introduce some bias [52]. Subsequently studies that demonstrate statistically significant results are more likely to be published in contrast with studies presenting negative finding, especially in English language indexed journal.

The results suggest that the TNF-a 308 G/A polymorphism may be associated with an increased diabetic nephropathy risk in Asians but not in Caucasians. Large-scale genome associated studies are required to confirm these findings.

These outcomes indicate that the pathogenesis of DM and its related complications is rather complex. Correlations between genes single nucleotide polymorphisms, ethnicity, environmental and other factors may potentially influence such diseases.

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