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ORIGINAL ARTICLE

Association between the T869C polymorphism of transforming growth factor-beta 1 and diabetic nephropathy: a meta-analysis

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Abstract Accumulating evidence has suggested that transforming growth factor-beta 1 (*TGF-β1*) is a functional candidate for diabetic nephropathy (DN). However, association studies investigating the relationship of *TGF-β1* gene T869C polymorphism and DN generate inconsistent results. To comprehensively clarify this issue, we performed a meta-analysis to evaluate the impact of the polymorphism on DN. We searched studies from PubMed and China National Knowledge Infrastructure (CNKI) through March 2011. Pooled ORs were calculated under allelic/additive/dominant/recessive/over-dominant genetic models. Nine studies with 1776 cases and 1740 controls were included. Our results indicated that C allele of T869C conferred a significantly increased risk of DN compared with T allele (OR = 1.25, 95% CI: 1.05–1.48) for allelic contrast. Similar results were also found under additive (OR = 1.57, 95% CI: 1.10–2.23) and dominant (OR = 1.40, 95% CI: 1.06–1.85) genetic models. However, subgroup analyses stratified by types of diabetes showed that significantly increased risks were only observed in type 2 diabetic patients, and the association persistently existed in further analysis for Asian populations. As for type 1 diabetic subjects, no significant association was detected under all the genetic models ($P > 0.05$). Our meta-analysis suggested that the *TGF-β1* T869C polymorphism conferred an elevated risk of DN. However, significant associations were only observed in type 2 diabetic patients.

Keywords Diabetic nephropathy · Meta-analysis · *TGF-β1* · Polymorphism

Introduction

Diabetic nephropathy (DN) is one of the most serious microvascular complications of diabetes mellitus (DM) and is the most common cause of end-stage renal disease (ESRD) worldwide [1, 2]. Structurally, DN is characterized by renal hypertrophy, mesangial matrix expansion, glomerulosclerosis and tubulointerstitial fibrosis [3]. Although the exact mechanism underlying the development of DN is not well elucidated, accruing evidences implicated that *TGF-β1*, a fibrogenic cytokine with strong regulatory effects on renal cell hypertrophy and extracellular matrix accumulation, play a pivotal role in the initiation and progression of DN [4]. *TGF-β1* was massively up-regulated in the renal glomeruli and tubulointerstitium in experimental and human diabetes [5]. Animal researches showed that mice over-expressing *TGF-β1* were affected by progressive renal failure [6], while administration of anti-*TGF-β1* antibody could inhibit glomerular hypertrophy and excessive extracellular matrix gene expression, and thus prevent the development of DN [7, 8]. Therefore, *TGF-β1* is considered as a functional candidate for DN.

The *TGF-β1* gene is located at chromosome 19q13, including 7 exons and 6 introns [9]. Recently, several polymorphisms of *TGF-β1* have been investigated as potential genetic risk factors for DN. Among them, T869C

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(rs1800470; rs1982073; T29C; Leu10Pro) polymorphism, with a transition from T to C in exon 1 resulting in a substitution of leucine to proline, attracted the most attention. This single-nucleotide polymorphism (SNP) was suggested to be associated with elevated serum concentration of *TGF-β1* [10]. To date, a number of studies have investigated the association between the *TGF-β1* T869C polymorphism and DN risk, but results of these studies were conflicting. The absence of concordance among these studies may be due to small sample size, diversity of ethnicity, and other limited study design. In order to derive a more precise estimation of the association of *TGF-β1* T869C polymorphism with DN, and to find the source of the discrepancies, we conducted this meta-analysis.

Materials and methods

Publication search

A systematic search was conducted for eligible studies from PubMed and China National Knowledge Infrastructure (CNKI) through March 2011. Languages were limited to English and Chinese. The search keywords were used with different combinations: "diabetic nephropathy or diabetic renal disease" and "*TGF-β1* or transforming growth factor-β1" and "polymorphism or variant". All the reference lists of relevant articles were manually searched for additional eligible studies.

Studies meeting all the following criteria were included: (1) was a case–control study evaluating the association between the *TGF-β1* T869C polymorphism and DN; (2) chose diabetic patients without nephropathy as controls; (3) provided genotype distribution information both in cases and controls or odds ratio (OR) with its 95% confidence interval (CI) or sufficient data that could allow us to calculate the corresponding estimate effect [OR, (95% CI)]. For overlapped studies, the more complete or the more recent study was included. Cases were defined as type 1 or type 2 diabetic subjects with microalbuminuria or various other advanced diabetic nephropathies, including macroalbuminuria or proteinuria, chronic renal failure and ESRD. Controls were predominantly confined to diabetic subjects with normoalbuminuria. Since macroalbuminuria is an indication of obvious DN, studies with subjects representing macroalbuminuria as controls were excluded.

Data extraction

Two investigators independently conducted the search, extracted and tabulated all the relevant data. Discrepancies were settled by discussion and consensus with all the authors. The following information was extracted from each study: first author's name, publication year, ethnicity, country, distribution of genotypes both in cases and controls, genotyping methods, types of DM, definitions, number and clinical characteristics [gender distribution, age, body mass index (BMI), duration of diabetes, matching information] of cases and controls.

Statistical analysis

We firstly calculated the OR and corresponding 95% CI for individual studies. Then, we assessed the overall effect of T869C polymorphism allele C on the risk of DN compared with that of allele T for allelic contrast. Secondarily, three genotypes contrasts were used as well: an additive (CC vs. TT), dominant [(CC + CT) vs. TT] and recessive [CC vs. (CT + TT)] genetic models. Since a study conducted by Jahromi et al. [11] suggested that CT was the risk genotype for DN, we then compared the heterozygote CT with a combination of CC and TT homozygotes [CT vs. (CC + TT)] under over-dominant genetic model. Furthermore, we conducted subgroup analyses stratified by types of DM. In type 1 diabetic subgroup, the subjects were all Caucasians. As for type 2 diabetic subgroup, we further evaluated the association in Asian population. For limitation of study numbers, we did not perform further analysis for Caucasians or Mexicans with type 2 diabetes. The strength of association between T869C polymorphism of *TGF-β1* and DN was assessed by the pooled OR with 95% CI. The significance of the pooled OR was determined using the Z test, and P value < 0.05 was considered statistically significant.

Heterogeneity was assessed with Cochran's Q statistic test, and $P < 0.10$ was considered evidence of significant heterogeneity [12]. Heterogeneity was quantified by I^2 test ($I^2 = 0$–25%: no heterogeneity; $I^2 = 25$–50%: moderate heterogeneity; $I^2 = 50$–75%: large heterogeneity and $I^2 = 75$–100%: extreme heterogeneity) [13]. Considering that a random effects model takes into account both between-study and inter-study heterogeneity, and it produces a more conservative estimation than that based on a fixed effects model, the random effects model was adopted in our meta-analysis [14]. Deviation from Hardy–Weinberg equilibrium (HWE) ($P < 0.05$) among the case and control groups within each study was checked by exact test using an online HWE calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Studies not in HWE were subjected to sensitivity analyses in overall and subgroup analyses to assess the stability of the results. Publication bias was checked by the Egger's regression test for allele contrast (C allele versus T allele). P value < 0.1 indicated evidence of potential publication bias. All the statistical analyses were performed using Stata 11.0 software (Stata Corporation, College Station, TX, USA). All P-value were two-sided.

Results

Data summary

With our search criteria, we primarily identified 29 papers. After reviewing the titles and abstracts, 14 potential relevant papers evaluating the relationship between *TGF-β1* and DN were identified. Of these papers, 5 papers were excluded for the following reasons: 1 paper focused on other polymorphisms [15]; 2 papers did not provide genotype distribution information both in cases and controls or other sufficient data that allowed us to calculate the OR [16, 17]; 1 paper was not case–control design [18]; 1 paper took some participants with macroalbuminuria as controls [19]. Finally, a total of 9 papers (2 papers written in Chinese, 7 papers written in English), including 1,776 cases and 1,740 controls, were included in our study [11, 20–27]. Of these studies, 3 studies were conducted in type 1 diabetic subjects, and the participants were all of Caucasian descendents [11, 22, 24]. 6 studies were dealing with type 2 diabetic patients, with 1 study on Caucasian population, 1 on a mixed population and 4 on Asian population (3 Chinese, 1 Indian) [20, 21, 23, 25–27]. The distribution of genotypes in controls and cases satisfied HWE in all studies, except 1 study in cases, 1 in controls, 1 in both cases and controls [11, 20, 21]. One study mentioned that there were matching for blood pressure, glycemic control, duration of diabetes, age and gender distribution between cases and controls [20], and one study was matching for age and ethnicity [21]. The detailed characteristics of the selected studies in our meta-analysis were shown in Table 1.

Main meta results

We firstly evaluated the overall association between the *TGF-β1* T869C polymorphism and DN risk. The pooled OR suggested that the risk C allele was associated with increased risk of DN compared with T allele: OR = 1.25 [95% CI (1.05–1.48)], $P = 0.01$, $P_{\text{heterogenity}} = 0.005$ under allelic genetic model (Table 2; Fig. 1). Significant associations were also found under additive, dominant models: OR = 1.57 [95% CI (1.10–2.23)], $P = 0.01$, $P_{\text{heterogenity}} = 0.005$; OR = 1.40 [95% CI (1.06–1.85)], $P = 0.02$, $P_{\text{heterogenity}} = 0.001$, respectively (Table 2). No significant association was detected under recessive and over-dominant models ($P > 0.05$) (Table 2). Sensitivity analyses were performed by excluding the studies with cases and/or controls not in HWE from the overall analysis. And the results showed that the overall association detected

Table 1 General characteristics of the studies included in the meta-analysis

Author (reference)	Year	Ethnicity (country)	Genotyping methods	Diabetes types	Distribution of T869C genotypes						Frequency of C allele (%)		P_{HWE}	
					Cases			Controls						
					CC	CT	TT	CC	CT	TT	Cases	Controls	Cases	Controls
Wong et al. [20]	2003	Asian (China)	PCR-RFLP	Type 2	27	26	5	24	24	17	69	55	1.00	0.05
Ahluwalia et al. [21]	2009	Asian (India)	PCR-RFLP	Type 2	55	41	144	37	74	144	31	29	<0.001	<0.001
Mcknight et al. [22]	2007	Caucasian (UK/Ireland)	Taqman	Type 1	20	101	151	31	132	204	26	26	0.64	0.18
Jahromi et al. [11]	2010	Caucasian (Kingdom of Bahrain/UK)	ARMS-PCR	Type 1	9	41	14	17	23	20	46	48	0.04	0.28
Buraczynska et al. [23]	2007	Caucasian (Poland)	PCR-RFLP	Type 2	69	114	62	25	72	71	51	36	0.31	0.40
Wei et al. [26]	2005	Asian (China)	PCR-SSP	Type 2	31	48	12	21	46	25	60	48	0.39	1.00
Wei et al. [27]	2008	Asian (China)	PCR-SSP	Type 2	94	128	58	72	142	66	56	51	0.27	0.90
Ng et al. [24]	2003	Caucasian (USA)	ASO-PCR	Type 1	34	126	138	36	107	118	33	34	0.51	0.17
Valladares-Salgado et al. [25]	2010	Mixed (Mexico)	PCR–RFLP	Type 2	64	127	37	47	95	50	56	49	0.06	0.89

PCR–RFLP polymerase chain reaction-restriction fragment length polymorphism, *ARMS-PCR* amplification refractory mutation system-polymerase chain reaction, *ASO-PCR* allele-specific oligonucleotide-polymerase chain reaction, *PCR-SSP* polymerase chain reaction-sequence-specific primers, *HWE* Hardy–Weinberg equilibrium

under all the genetic models were not significantly altered (Table 2).

Subgroup analyses

We further refined our analyses to two subgroups by diabetes types. No significant association was observed in Caucasian type 1 diabetic subgroup under allelic (Table 2; Fig. 1) and other genetic models ($P > 0.05$) (Table 2). However, in type 2 diabetic subgroup, significant increased risks were observed under allelic, additive, dominant and recessive genetic models: OR = 1.42 [95% CI (1.19–1.68)], $P < 0.001$, $P_{\text{heterogenity}} = 0.11$ (Table 2; Fig. 1); OR = 2.04 [95% CI (1.49–2.78)], $P < 0.001$, $P_{\text{heterogenity}} = 0.17$; OR = 1.64 [95% CI (1.10–2.44)], $P = 0.01$, $P_{\text{heterogenity}} = 0.003$ and OR = 1.58 [95% CI (1.29–1.92)], $P < 0.001$, $P_{\text{heterogenity}} = 0.70$, respectively (Table 2). When we confined type 2 diabetic patients to Asians, significant association was also detected under allelic model: OR = 1.32 [95% CI (1.09–1.60)], $P = 0.004$, $P_{\text{heterogenity}} = 0.25$, additive model: OR = 1.83 [95% CI (1.24–2.70)], $P = 0.002$, $P_{\text{heterogenity}} = 0.23$, and recessive model: OR = 1.58 [95% CI (1.24–2.02)], $P < 0.001$, $P_{\text{heterogenity}} = 0.92$ (Table 2). Sensitive analyses showed that the significant association detected in Asian type 2 diabetic subgroup under additive genetic model disappeared, while the other results did not significantly altered (Table 2).

Heterogeneity and publication bias

Significant heterogeneity was detected among all the studies (Table 2). When we stratified by diabetes types into subgroup analyses, the heterogeneity was removed in type 1 diabetes and decreased in type 2 diabetes (Table 2). Publication bias was checked by the Egger's regression test for allele contrast (C allele vs. T allele), and no significant publication bias was revealed ($P = 0.74$).

Discussion

TGF-β1 as an important regulator of tissue fibrosis plays a pivotal role in the pathogenesis of DN [4]. Involvement of *TGF-β1* in DN has been indicated by prior findings that protein and mRNA production of *TGF-β1* were significantly enhanced in the renal tissues of patients with DN [5, 28]. Elevated concentration of *TGF-β1* could induce renal hypertrophy and promote excessive accumulation of extracellular matrix [6]. And exactly these pathological changes contribute to the initiation and progression of DN. Evidences from in vivo and in vitro studies have indicated that increased concentration of glucose could stimulate *TGF-β1* expression both in cultured renal cells and in the kidney [29, 30], which suggested that *TGF-β1* might play an important role in the etiology of DN both in type 1 and type 2 diabetes.

Our meta-analysis based on 9 studies with 1,776 cases and 1,740 controls evaluated the association between the *TGF-β1* T869C polymorphism and DN. The results suggested that the variant conferred increased risk of DN. However, significant heterogeneity was identified among all the studies. The existing heterogeneity was mainly due to diabetes types. When we conducted subgroup analyses stratified by types of diabetes, the heterogeneity was removed in type 1 diabetes and decreased in type 2 diabetes. The results of subgroup analyses indicated that significant association of *TGF-β1* T869C polymorphism with DN only existed in type 2 diabetic subjects, but not in type 1 diabetic patients. Sensitivity analyses did not significantly alter all the overall and subgroup results except the subgroup analysis result involving Asian type 2 diabetes under additive genetic model, suggesting that the association was predominantly reliable and stable.

Although the exact mechanism underlying the effect of *TGF-β1* T869C polymorphism on DN susceptibility was not well known, multiple studies conducted in various populations suggested that the T869C polymorphism was associated with altered *TGF-β1* protein expression. Yamada et al. [10] firstly suggested that the number of allele C was positively correlated with serum *TGF-β1* concentration in Japanese. Similar results were also found by Suthanthiran et al. [31] in African-Americans and white Europeans. In addition, it has been reported that the C allele carriers of T869C polymorphism were associated with increased risks of various organ fibrosis, such as liver cirrhosis and lung fibrosis [32, 33]. Our meta-analysis also provided the association with the same trend between *TGF-β1* T869C polymorphism and DN susceptibility in type 2 diabetes. While in type 1 diabetic patients, no significant association was detected under all the genetic models.

The conflicting results of the association between type 1 diabetes and type 2 diabetes may be explained by the following reasons. First, Selection criteria might play an important role in affecting the results of genetic association studies. In studies involving type 1 diabetes, the basic clinical characteristics, including gender distribution and glycemic control, did not match well between cases and controls as that in type 2 diabetes. Second, it is well known that the etiology, metabolic status and disease-specific genetic background vary quite a lot between these two types of diabetes. Type 1 diabetes is an autoimmune disease characterized by destruction of pancreatic beta-cells. However, type 2 diabetes is a complex metabolic disease characterized by insulin resistance and/or pancreatic beta-cell dysfunction, and is always related to metabolic abnormalities, including obesity, hypertension and

Table 2 Summary risk estimates for the association of *TGF-β1* T869C polymorphism with DN

Genetic contrasts	Population and subgroups under analysis	Studies (n)	Cases/ controls	OR (95% CI)	*P*	*Q* test, *P* value	I^2 (%)
C versus T	All	9	1776/1740	1.25 (1.05–1.48)	0.01	0.005	63.8
	All in HWE	6	1414/1360	1.26 (1.02–1.57)	0.04	0.002	73.2
	Type 1 diabetes	3	634/688	0.95 (0.80–1.12)	0.53	0.96	0
	Type 1 diabetes in HWE	2	570/628	0.95 (0.80–1.13)	0.56	0.78	0
	Type 2 diabetes	6	1142/1052	1.42 (1.19–1.68)	<0.001	0.11	44.9
	Type 2 diabetes in HWE	4	844/732	1.47 (1.20–1.79)	<0.001	0.14	46.1
	Asians	4	669/692	1.32 (1.09–1.60)	0.004	0.25	26.4
	Asians in HWE	2	371/372	1.37 (1.04–1.79)	0.02	0.23	32.1
CC versus TT	All	9	1776/1740	1.57 (1.10–2.23)	0.01	0.005	64.0
	All in HWE	6	1414/1360	1.58 (1.00–2.48)	0.05	0.003	72.2
	Type 1 diabetes	3	634/688	0.83 (0.57–1.20)	0.31	0.97	0
	Type 1 diabetes in HWE	2	570/628	0.84 (0.56–1.24)	0.37	0.85	0
	Type 2 diabetes	6	1142/1052	2.04 (1.49–2.78)	<0.001	0.17	35.1
	Type 2 diabetes in HWE	4	844/732	2.13 (1.45–3.13)	<0.001	0.17	39.8
	Asians	4	669/692	1.83 (1.24–2.70)	0.002	0.23	30.1
	Asians in HWE	2	371/372	1.93 (0.98–3.83)	0.06	0.15	50.8
(CC + CT) versus TT	All	9	1776/1740	1.40 (1.06–1.85)	0.02	0.001	68.2
	All in HWE	6	1414/1360	1.40 (1.03–1.91)	0.03	0.006	69.1
	Type 1 diabetes	3	634/688	1.03 (0.82–1.29)	0.81	0.36	1.6
	Type 1 diabetes in HWE	2	570/628	0.98 (0.78–1.23)	0.87	0.84	0
	Type 2 diabetes	6	1142/1052	1.64 (1.10–2.44)	0.01	0.003	72.5
	Type 2 diabetes in HWE	4	844/732	1.75(1.26–2.42)	0.001	0.14	44.8
	Asians	4	669/692	1.49(0.87–2.54)	0.15	0.01	72.6
	Asians in HWE	2	371/372	1.58 (0.78–3.19)	0.20	0.10	64.2
CC versus (CT + TT)	All	9	1776/1740	1.27 (0.96–1.67)	0.09	0.01	58.0
	All in HWE	6	1414/1360	1.30 (0.96–1.76)	0.09	0.05	55.1
	Type 1 diabetes	3	634/688	0.75 (0.53–1.06)	0.10	0.38	0
	Type 1 diabetes in HWE	2	570/628	0.83 (0.57–1.21)	0.33	0.87	0
	Type 2 diabetes	6	1142/1052	1.58 (1.29–1.92)	<0.001	0.70	0
	Type 2 diabetes in HWE	4	844/732	1.56 (1.21–2.01)	0.001	0.32	15.3
	Asians	4	669/692	1.58 (1.24–2.02)	<0.001	0.92	0
	Asians in HWE	2	371/372	1.52 (1.11–2.10)	0.01	0.64	0
CT versus (CC + TT)	All	9	1776/1740	1.06 (0.84–1.35)	0.62	0.004	64.1
	All in HWE	6	1414/1360	1.05 (0.90–1.22)	0.54	0.62	0
	Type 1 diabetes	3	634/688	1.30 (0.84–2.03)	0.24	0.04	69.6
	Type 1 diabetes in HWE	2	570/628	1.05 (0.83–1.33)	0.67	0.99	0
	Type 2 diabetes	6	1142/1052	0.96 (0.71–1.31)	0.81	0.02	63.6
	Type 2 diabetes in HWE	4	844/732	1.05 (0.85–1.31)	0.65	0.32	14.4
	Asians	4	669/692	0.84 (0.56–1.25)	0.39	0.05	62.0
	Asians in HWE	2	371/372	0.88 (0.66–1.18)	0.40	0.36	0

DN diabetic nephropathy, *OR* odds ratio, *CI* confidence interval, *HWE* Hardy–Weinberg equilibrium

hyperlipidemia. So, it is reasonable to hypothesize that different contributing genes and abnormal physiological milieu of the two different diabetes may interplay with *TGF-β1* or the related signal pathway molecules, and thus affect the susceptibility of DN. Third, the studied populations involving type 1 diabetes were limited to Caucasians, which might not preclude the possibility that the *TGF-β1* T869C polymorphism exerts effects on DN risk in a population-specific manner in type 1 diabetes. Last but not the least, the conclusion was based on only 3

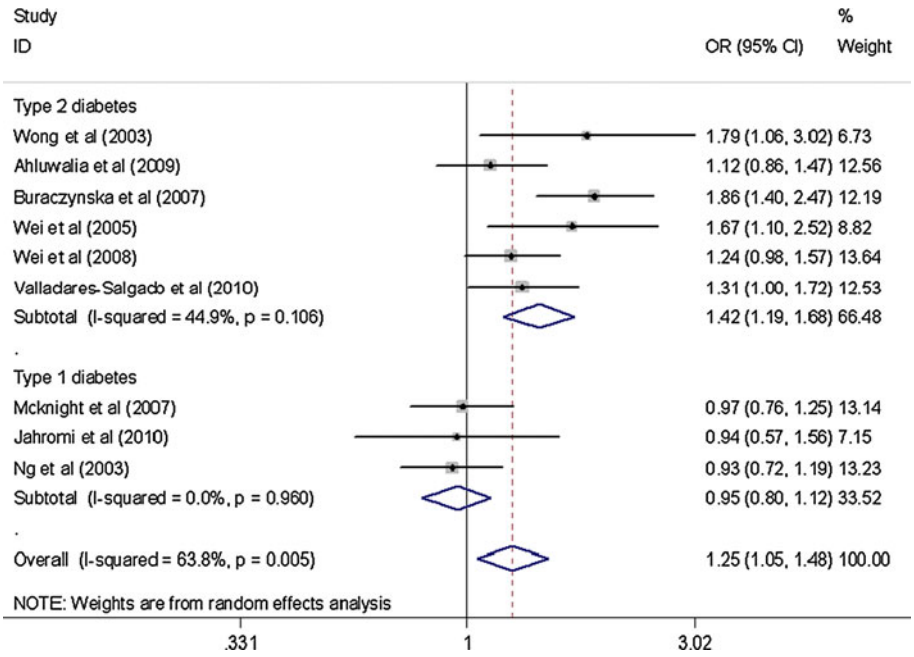

Fig. 1 Forest plot for the association of *TGF-β1* T869C polymorphism with DN stratified by types of diabetes (C vs. T). The diamond shows the pooled random-effects odds ratio

studies with 634 cases and 688 controls. The study samples were relatively small, which might not have sufficient power to detect a small effect size.

It should be noticed that there were several limitations in our meta-analysis. First, the conclusion was based on relatively small study samples. Larger studies, involving type 1 and/or type 2 diabetes, should be warranted in future to elucidate the role of the variant in DN susceptibility. Second, although microalbuminuria may be an early manifestation of DN, it is not inevitably equivalent to it. Patients representing microalbuminuria do not invariably progress to advanced nephropathy, but some may even return to normoalbuminuria [34, 35]. 4 out of 9 studies in our study included patients with microalbuminuria as their cases [20, 25–27]. In addition, 2 studies involving type 2 diabetes chose subjects with relatively small disease duration (no more than ten years) as their controls [26, 27]. Thus, there might be possible misclassification of cases and controls in some studies. Third, although all the genotyping methods applied in the selected studies were verified as validated, only one study mentioned quality control of genotyping [25]. Fourth, although no publication bias was detected for allele contrast in our meta-analysis, it should be noticed that possible publication bias might exist for other genotypes contrasts in overall or subgroup analyses especially when the included studies and study samples were relatively small. Fifth, the results were based on unadjusted ORs, while a more precise estimation should take into account the effect of multiple confounders such as age, sex distribution, glycemic control and blood pressure on the association. Undoubtedly, all the limitations will inevitably affect the summary result, but the strength of our meta-analysis was based on the accumulating studies, and thus had much better power to reach a more precise estimation.

In conclusion, our meta-analysis supported an association between the T869C polymorphism of *TGF-β1* gene and increased risk of DN. However, significant association only existed in type 2 diabetic subjects. As for type 1 diabetic subjects, no significant association was detected under all the genetic models. Considering that the conclusion was based on limited studies with relatively small samples, larger well-designed studies involving type 1 and/or type 2 diabetes subjects in different ethnic populations are required to confirm our findings.

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Conflict of interest None.

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