Journal of Preventive Epidemiology

The M235T polymorphism in the angiotensinogen gene is not a major risk factor for diabetic nephropathy; a meta-analysis

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Abstract

Introduction: Diabetic nephropathy (DN) is the leading cause of chronic kidney disease in diabetes patients. The angiotensin AGT M235T gene polymorphism, which is linked to the renin-angiotensin-aldosterone system (RAAS), has been extensively studied in DN patients, but the results are still conflicting. The current study's goal is to conduct a meta-analysis to assess the relationship between AGT M235T gene polymorphism and DN susceptibility.

Methods: Fourteen case-control studies related to AGT M235T polymorphism and DN were searched using PubMed, Web of Science and Google Scholar databases. Genotype data from the T2DM and T2DN groups were collected from all papers. The pooled odds ratio (OR) and 95 percent confidence interval (95% CI) were calculated employing a random-effects model to assess the relationship.

Results: There were no statistically significant link between AGT M235T and DN risk in dominant ($P = 0.801$, OR: 0.95; 95% CI: 0.66-1.38), allelic ($P = 0.933$, OR: 1.01; 95% CI: 0.75-1.37) and recessive ($P = 0.374$, OR: 1.21; 95% CI: 0.80-1.83) genetic models. Further, the stratified analysis based on ethnicity did not reveal significant link between AGT M235T and DN risk in Asian (Dom OR: 1.07; 95% CI: 0.63-1.82) and the Caucasian populations (Dom OR: 0.77; 95% CI: 0.49-1.21). In all three models, there was a high degree of heterogeneity between studies. Publication bias was not seen.

Conclusion: Our findings suggest that the AGT gene M235T polymorphism does not contribute to DN risk. However, validation of this association will require multi-center and large population-based studies.

Introduction

Diabetic nephropathy (DN) is one of the common microvascular complications in patients with diabetes mellitus, and it is the leading cause of chronic kidney disease (CKD) and renal failure (1). In the past decade, the incidence of DN is increased and now accounting for approximately 50% of all end-stage renal diseases (ESRDs) (2). According to USA Renal Data System, DN was the most common primary diagnosis in 2018 (3). Both type 1 and type 2 diabetes induce changes in glomerulus, tubulointerstitium and vasculature in different kidney compartments (4). Besides, long-standing DM, poor glycemic control and genetic factors are the major risk factors for DN (5). However, despite the belief that genetic factors may have an impact on the DN risk, none of the genes have yet been clearly defined. The advancement of single-

Key point

Diabetic nephropathy in diabetes patients is positively correlated with end-stage renal disease (ESRD). Previous evidence has shown that RAAS plays an essential role in the development and progression of DN. The present meta-analysis analysed the association between AGT M235T polymorphism nephropathy in T2DM patients. This meta-analysis indicated that the AGT gene M235T polymorphism does not contribute to DN risk.

nucleotide polymorphism (SNP) technology has resulted in an increase in the number of studies being conducted to investigate the relationship between genetic polymorphism and DN risk (6). A number of candidate genes have been identified, including those involved in the regulation of renin–angiotensin–aldosterone (RAAS) and lipid metabolism (7-9).

Previous evidence has shown that RAAS

plays an essential role in the development and progression of DN (10). Recently, some genetic variants such as angiotensin-converting enzyme (ACE) I/D polymorphism, angiotensin II type 1 receptor (AT1R) A1166C (rs5186) gene polymorphism and AGT M235T (rs699) in the RAAS pathway have been significantly associated with the risk of DN (11,12). AGT is a vital component of level II angiotensin, which is a major component of the RAAS. Further, genetic polymorphism in the AGT gene may lead to DN progression in patients with DM.

Several studies have examined the relationship between M235T polymorphism and diabetic nephropathy; however, the results are inconsistent (13-26). Thus, we conducted this meta-analysis to investigate the precise relationship between AGT M235T and diabetic nephropathy.

Materials and Methods
Literature search
A meta-analysis was carried out to investigate the association between DN risk and AGT M235T. In this perspective, MEDLINE, PubMed, EMBASE, Scopus databases, Web of Science, used to conduct analysis using the search keywords of "Angiotensinogen", "AGT M235T", "rs699", "diabetic nephropathy" and "diabetic kidney disease." All Information was collected upto July 2020.

Inclusion and exclusion criteria
The inclusion and exclusion criteria used in our study were as follows. Inclusion criteria; (a) case-control study design; (b) studies that addressed DN cases and diabetic controls; (c) Studies that assessed the link between AGT M235T and DN risk; (d) detailed genotype methods should be provided for the study. Exclusion criteria: (a) not case-control studies; (b) studies with AGT M235T with T1DM and (c) studies overlapped with any other articles. The names of the first author, year of publication, country, ethnicity, genotyping method and genotype data from the T2DM and T2DN groups were collected.

Statistical analysis
The association between AGT M235T and DN risk was measured in three genetic models (allelic, dominant and recessive) by estimating ORs and corresponding 95% CIs. The Cochran's Q test and I^2 values were applied to assess the degree of heterogeneity between studies (27). The results were pooled using a random-effects model. To test the robustness of this meta-analysis, sensitivity analysis was used, which involved removing one study at a time. Begg's funnel plot and Egger's test were used to investigate publication bias. Ethnicity based subgroup analysis was performed. All analysis were performed by MetaGenyo web tool (28).

Results
Study characteristics
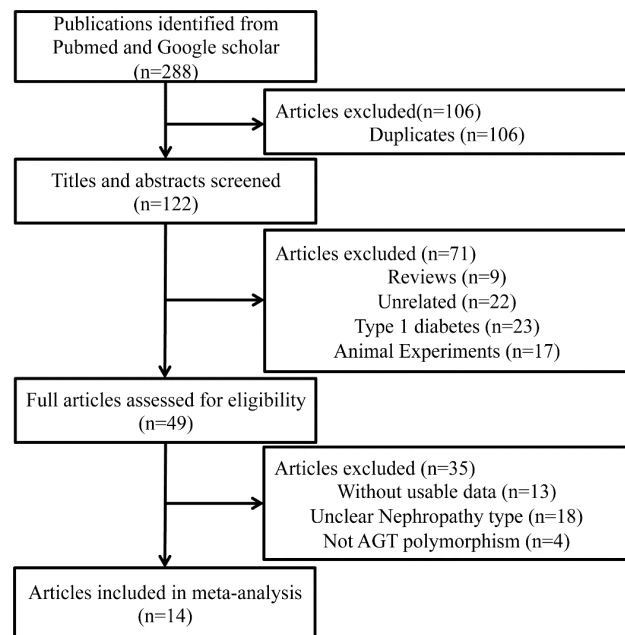The flow of the literature search strategy is shown in

Figure 1. Process of Study selection for meta-analysis.

Figure 1. Based on the systematic search we have identified 14 papers that were analyzing the association between AGT M235T polymorphism nephropathy in T2DM patients (13-26). The essential features of each study and their genotype frequency of AGT M235T polymorphism were depicted in Table 1. The years of publication ranged from 1996 to 2017. Four studies have been deviated from Hardy-Weinberg equilibrium (HWE) in control groups of the AGT M235T polymorphism (14,18,20,23).

Association of AGT M235T polymorphism with susceptibility to diabetic nephropathy
To explore the association between AGT M235T gene polymorphisms with the risk of DN, 14 studies were enrolled. Forest plot and overall pooled effect show the relationship between AGT M235T polymorphism and DN an independent studies in the dominant model (Figure 2). The current meta-analysis shows that there was significant heterogeneity between studies (I^2 = 73% to 94%). The random-effect model was used to estimate the pooled ORs. The measures of the association between DN and AGT M235T polymorphism were documented in Table 2. Meta-analysis results suggest that there were no statistically significant association between AGT M235T and DN risk in dominant (P = 0.801, OR: 0.95; 95% CI: 0.66-1.38), allelic (P = 0.933, OR: 1.01; 95% CI: 0.75-1.37) and recessive (P = 0.374, OR: 1.21; 95% CI: 0.80-1.83) genetic models. Moreover, subgroup analyses of AGT M235T based on ethnicity did not show a statistically significant association between DN risk in Asian (Dom OR: 1.07; 95% CI: 0.63-1.82) and the Caucasian populations (Dom OR: 0.77; 95% CI: 0.49-1.21).

Table 1. Baseline characteristics of the AGT M235T studies included in the meta-analysis

| Reference	Country	Ethnicity	Genotyping method	HWE P value <0.001	Diabetic nephropathy			T2DM		
					TT	MT	MM	TT	MT	MM
Tomino et al (14)	Japan	Asian	PCR-RFLP	0.778	507	100	138	277	75	55
Doria et al (26)	USA	Caucasian	PCR-DGGE	0.612	42	86	72	12	48	40
Zychma et al (25)	Poland	Caucasian	PCR-DGGE	0.778	106	228	116	64	116	63
Fradin et al (19)	France	Caucasian	PCR-RFLP	0.485	25	44	49	19	59	40
Eroglu et al (18)	Turkey	Asian	Melt curve analysis	0.010	10	24	12	9	32	15
Prasad et al (22)	India	Asian	PCR-RFLP	0.086	86	86	24	45	85	95
Osawa et al (21)	Japan	Asian	PCR	0.612	22	209	504	15	195	341
Ahluwalia et al (15)	India	Asian	PCR-RFLP	0.528	82	104	54	27	120	108
Tien et al (16)	Taiwan	Asian	PCR-RFLP	<0.001	17	45	31	97	204	131
Manea et al (20)	Romania	Caucasian	PCR-RFLP	0.071	17	60	30	41	92	10
Mtiraoui et al (17)	Tunisia	Asian	PCR-RFLP	0.913	73	138	118	20	176	209
Reis et al (23)	Turkey	Asian	PCR-RFLP	0.049	17	68	23	41	57	19
Shaikh et al (24)	Pakistan	Asian	ARMS-PCR	0.612	40	28	42	62	47	1
Makuc et al (13)	Solvinia	Caucasian	KASpar	0.000	57	144	75	88	180	107

Sensitivity analysis and publication bias

In sensitivity analysis, there was no significant difference in the OR when we excluded one study at a time. Sensitivity analysis pooled ORs were depicted in Figure 3. The results show that there was no difference in pooled ORs when we omit one study each time, indicating that our findings were statistically robust. There is no obvious asymmetry in the funnel plot's shape (Figure 4). Furthermore, Egger's test also showed that there is no evidence for publication bias (T vs. M: Egger's test P=0.445; TM+TT versus MM:

Egger's test P=0.440; TT versus TM+MM: Egger's test P=0.889).

Discussion

Diabetic nephropathy in diabetes patients is positively correlated with ESRD. DN has a multi-factorial etiology that includes both environmental and genetic factors. RAAS can be activated early in the course of diabetes, causing structural and intraglomerular hemodynamic changes in the diabetic kidney at both the glomerular
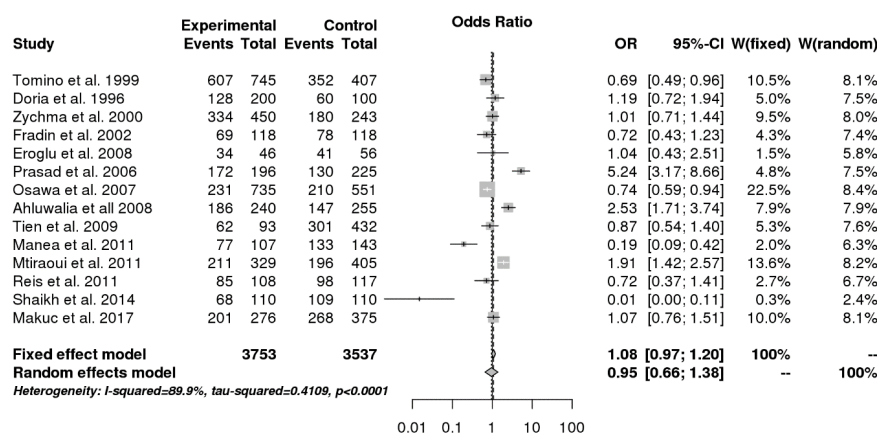
Figure 2. Forrest plot of dominant model for overall comparison of AGT M235T polymorphisms and DN.

Table 2. The association between AGT M235T and DN risk in different genetic models

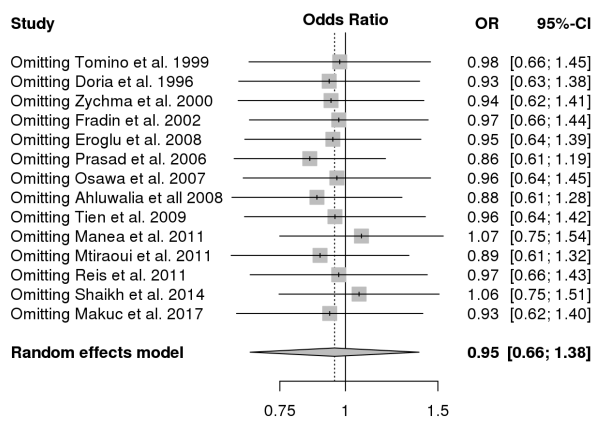| Study group	Allele contrast (T vs. M)		Recessive model (TT vs. TM+MM)		Dominant model (TM+TT vs. MM)	
	OR [95% CI]	P value	OR [95% CI]	P value	OR [95% CI]	P value
Overall (14)	1.01 [0.75-1.37]	0.933	1.21 [0.80-1.83]	0.374	0.95 [0.66-1.38]	0.801
Ethnicity						
Asian (n=9)	1.08 [0.69-1.68]	0.738	1.36 [0.73-2.51]	0.332	1.07 [0.63-1.82]	0.806
Caucasian (5)	0.90 [0.70-1.16]	0.434	0.95 [0.65-1.39]	0.799	0.77 [0.49-1.21]	0.262

Figure 3. Sensitivity analysis results for the AGT M235T polymorphism and DN (dominant model).
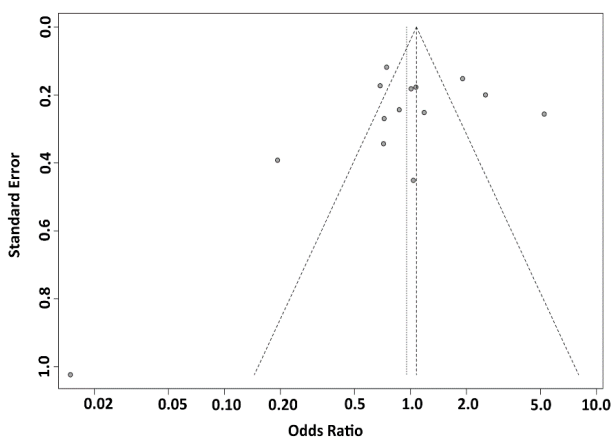

Figure 4. Funnel plot for publication bias test of the AGT M235T polymorphism and DN.

and tubulointerstitial levels (29,30). Microalbuminuria is considered a risk factor for early-stage DN progression and may occur due to endothelial dysfunction and hyperhomocysteinemia. Approximately one-third of diabetic patients are susceptible to nephrotic disease (31). Poor glycemic control has been identified as one of the major causes of developing nephropathy.

Despite the fact that a family history of DN is one of the most likely indicators of a hereditary genetic predisposition to DN, due to the involvement of multiple genetic and environmental risk factors in the pathogenesis of DN, determining an individual's risk of inheriting nephropathy is difficult. Despite extensive and thorough research efforts, the underlying causative genetic components remain elusive (32-34). The M235T variant has been shown to alter the plasma levels of AGT (35, 36) with increased serum AGT levels for patients with T-allele (37). In addition, there is a positive correlation between AGT M235T genotype and AGT plasma levels in myocardial infarction (MI) (38,39). The present meta-analysis revealed that there was no significant association between AGT M235T and DN

risk. We also found that there is a potential heterogeneity between studies. The results of our meta-analysis are in consensus with a previous meta-analysis that provided no evidence of a significant association between AGT M235T polymorphism and DN risk (40). In contrast to this, two independent meta-analyses demonstrated the association between AGT M235T and DN risk in Caucasian (41) and Asian populations (42).

Conclusion
In conclusion, this meta-analysis found no evidence of a link between AGT M235T and DN risk. Since, the majority of the studies in this meta-analysis were Asian and Caucasian, the distribution of AGT M23T varies across ethnic groups and the results cannot be generalised. However, validation of this association will require multi-center and large population-based studies.

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