

# Association between angiotensinogen T174M polymorphism and the risk of diabetic nephropathy: A meta-analysis

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## Abstract

**Objective:** Although the angiotensinogen (AGT) gene T174M polymorphism has been implicated in the pathogenesis of diabetic nephropathy (DN), study results have been inconsistent. The present meta-analysis was conducted to determine the correlation of AGT T174M polymorphism with DN.

**Methods:** We retrospectively extracted relevant studies from Embase as well as PubMed databases. Additionally, a fixed- or random-effects model was employed for calculation of pooled odds ratio (OR) along with 95% confidence interval (CI).

**Results:** In total, we identified six studies (1179 cases and 927 controls) regarding the AGT gene T174M polymorphism. The pooled ORs for the association between the AGT T174M polymorphism and DN risk were not statistically significant under all genetic models (M vs T: OR = 1.22, 95% CI = 0.84–1.75; MM vs TT: OR = 1.94, 95% CI = 0.93–4.04; MT vs TT: OR = 1.11, 95% CI = 0.76–1.63; the dominant model: OR = 1.19, 95% CI = 0.80–1.77; the recessive model: OR = 1.94, 95% CI = 0.93–4.03). Subgroup analyses based on the type of race showed the M allele of the AGT T174M polymorphism increased DN risk in Asians, but not in Caucasians.

**Conclusions:** Our study indicated that the T174M polymorphism in the AGT gene was associated with DN in Asians.

## Keywords

AGT, diabetic nephropathy, meta-analysis, polymorphism, T174M

## Introduction

Diabetic nephropathy (DN) is a major microvascular complication during the progression both of type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), accounting for 25%–40% of DM-associated morbidity and mortality.<sup>1</sup> Moreover, DN is among the leading causes of end-stage renal disease in Western countries.<sup>2,3</sup> Therefore, earlier diagnosis and prevention of DN is important. However, the mechanism of DN is complicated. In spite of a certain correlation between DN and plasma glucose levels/diabetes duration, it is not definitely clarified. Certain DM patients with well-controlled plasma glucose levels are burdened with DN in the early course of DM, while some of the remaining do not develop DN throughout their life. Therefore, it is likely that hyperglycemia is a critical, but not the only, factor affecting DN development. Recent studies show that genetic factors are critically involved in DN development as well.<sup>4,5</sup>

The renin-angiotensin-aldosterone system (RAAS), playing a vital part in the modulation of renal homeostasis,

sodium and water balance as well as blood pressure, also participates in the pathophysiology of DN.<sup>6,7</sup> In other words, RAAS dysregulation is critically involved in DN pathogenesis. Excessive RAAS activation constricts renal arterioles leading to increased peripheral and renal resistance, increases glomerular capillary pressure leading to proteinuria, augments oxidative stress leading to endothelial dysfunction and so on.<sup>8</sup> All these aggressively damage renal function, eventually leading to DN. Targeted inhibition of the activation of RAAS with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers is

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the most common clinical strategy for slowing disease progression.<sup>9</sup>

Angiotensinogen (AGT) is a liver protein interacting with the protease renin to generate angiotensin I, a prohormone of angiotensin II. Ang II has been demonstrated to participate in cardiac fibrosis, regulation of collagen synthesis and cardiac fibroblast growth as well as progression of cardiomyocyte hypertrophy both in human and animal models. Genetic polymorphisms of *AGT* are likely to influence RAAS activity, subsequently affecting the pathogenesis and progression of DN. The *AGT* gene is located at 17q42–43 and consists of five exons. A threonine to methionine substitution at amino acid 174 is a common polymorphism called T174M (rs699), designating the T and M alleles, respectively.<sup>10</sup>

Various epidemiological research has investigated the relationship of *AGT* T174M polymorphism with DN risk, without consistent conclusions. Meta-analysis can be a useful tool in detecting an association that could otherwise remain masked in smaller sample size studies, especially in those evaluating the frequency of rare allele polymorphisms.<sup>11</sup> Hence, the present meta-analysis aimed at exploring the correlation of the *AGT* T174M polymorphism with DN risk via collection of all available case-control studies.

## Participants and methods

### Identification of studies

The following terms were searched for in the Embase and PubMed databases: “AGT/ angiotensinogen,” “T174M,” “DN/diabetic nephropathy” and “gene polymorphism.” In addition, we also manually screened the references of retrieved researches for a comprehensive collection of eligible studies. We established a flowchart of information concerning identification, screening and eligibility and finally selected datasets in line with Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines.<sup>12</sup>

### Inclusion criteria and data extraction

The inclusion criteria were as follows: 1) case-control research addressing DN cases and controls, 2) research assessing the correlation of *AGT* T174M polymorphism with DN risk, 3) research with adequate genotype information for extraction and 4) the definition of DN was urinary albumin excretion >30 mg/24 hours. Studies were excluded when: 1) articles were not case-control studies assessing the relationship of *AGT* T174M polymorphism with DN risk, 2) reviews, editorial articles, letters, case reports as well as meta-analysis, 3) research was based on insufficient raw data or without useful information, 4) research was duplicated and 5) sample size was less than 10.

### Data extraction

Authors N.L. and Y.W. independently retrieved relevant information from all eligible searches, and consensus was reached on all items. The following data were extracted from every study: first author, year of publication, area, ethnicity, number and genotypes of cases and controls, as well as evidence of Hardy-Weinberg equilibrium (HWE) in controls.

### Statistical analysis

Fisher exact test was applied to evaluate deviation from HWE for each genotype distribution in the control group. The strength of the associations between the T174M polymorphism and susceptibility to DN were estimated by odds ratio (OR) and 95% confidence interval (95% CI) under M vs T, a homozygote comparison (MM vs TT), a heterozygote comparison (MT vs TT), a dominant model (MM+MT vs TT) and a recessive mode (TT+MT vs MM) between groups. The heterogeneity among these articles was checked via the  $I^2$  test. When  $I^2 > 50\%$  indicated heterogeneity across studies, the random-effects model was used, otherwise the fixed-effects model was used. The sensitivity analysis was used by omitting each individual article, and an individual article was suspected of excessive sensitivity if the point estimate of its omitted analysis was outside the 95% CI of the pooled analysis. To assess potential publication bias, visual inspection of a Begg funnel plot was performed. STATA version 12.0 was employed for statistical analysis.

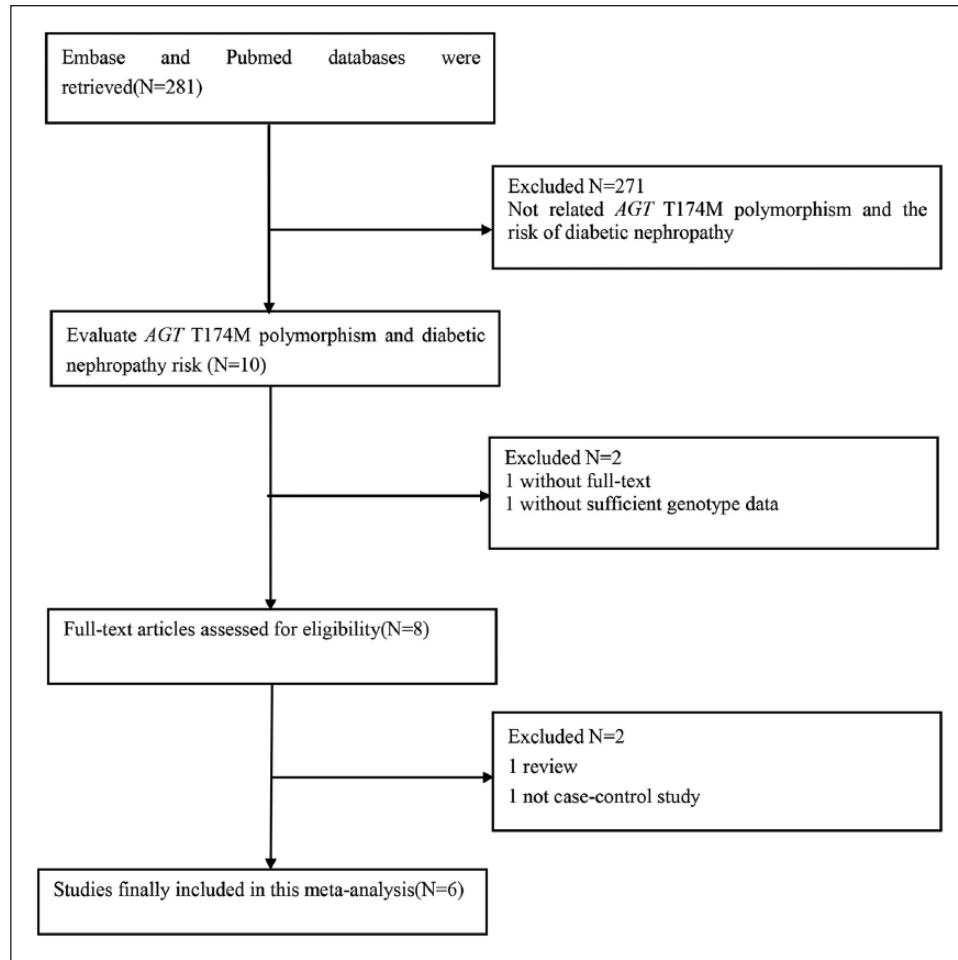
## Results

### Study characteristics

Through data retrieval, 281 possible relevant researches were identified that met the search criteria. After carefully screening according to the criteria, duplicated studies and review articles were excluded. There were only six studies included in the meta-analysis<sup>13–18</sup> comprising 1179 DN patients and 927 controls (Figure 1). Study regions were composed of Denmark, Spain, Italy and China. Participants were grouped into three ethnicities, namely African, Asian and Caucasian (Table 1). Data from the included studies for meta-analysis is included in Table 2.

### Pooled analyses

The evaluation of the relationship of the *AGT* gene T174M polymorphism with DN risk is presented in Figure 2 and Table 3. Meta-analysis results revealed no significant relationship between the *AGT* T174M polymorphism and DN risk (M vs T: OR = 1.22, 95% CI = 0.84–1.75; MM vs TT: OR = 1.94, 95% CI = 0.93–4.04; MT vs TT: OR = 1.11, 95% CI = 0.76–1.63; the dominant model:



**Figure 1.** Flowchart of selection of studies and specific reasons for exclusion from the meta-analysis. AGT: angiotensinogen gene; M: methionine; T: threonine.

**Table 1.** Characteristics of studies included in meta-analysis.

Study first author <sup>ref</sup>	Year	Area	Race	DM type	Matching criteria	Cases/ Controls
Tarnow <sup>13</sup>	1996	Denmark	Caucasian	Type 1	Age, sex, duration, BMI	195/185
Gutiérrez <sup>14</sup>	1997	Spain	Caucasian	–	Sex	193/90
Solini <sup>15</sup>	1999	Italy	Caucasian	Type 2	Age, BMI, duration, HbA <sub>1c</sub> (%)	79/90
Wu <sup>16</sup>	2000	China	Asian	–	–	71/41
Chang <sup>17</sup>	2003	China	Asian	Type 2	Age, sex	129/116
Mtiraoui <sup>18</sup>	2011	Tunisia	African	Type 2	Age, sex, BMI	512/405

BMI: body mass index; DM: diabetes mellitus; HbA<sub>1c</sub>: hemoglobin A<sub>1c</sub>.

OR = 1.19, 95% CI = 0.80–1.77; the recessive model: OR = 1.94, 95% CI = 0.93–4.03). In subgroup analysis by ethnicity, a significant association was found between the *AGT* T174M polymorphism and DN risk in Asians (M vs T: OR = 2.80, 95% CI = 1.59–4.93; the dominant model: OR = 2.73, 95% CI = 1.25–5.96), but not in Caucasians. In assessment of the impact of single literature on final result, sensitivity analysis was performed by omitting

studies each time in turn. As a result, elimination of a single study rarely affected the pooled results, indicating that our findings were robust (Figure 3).

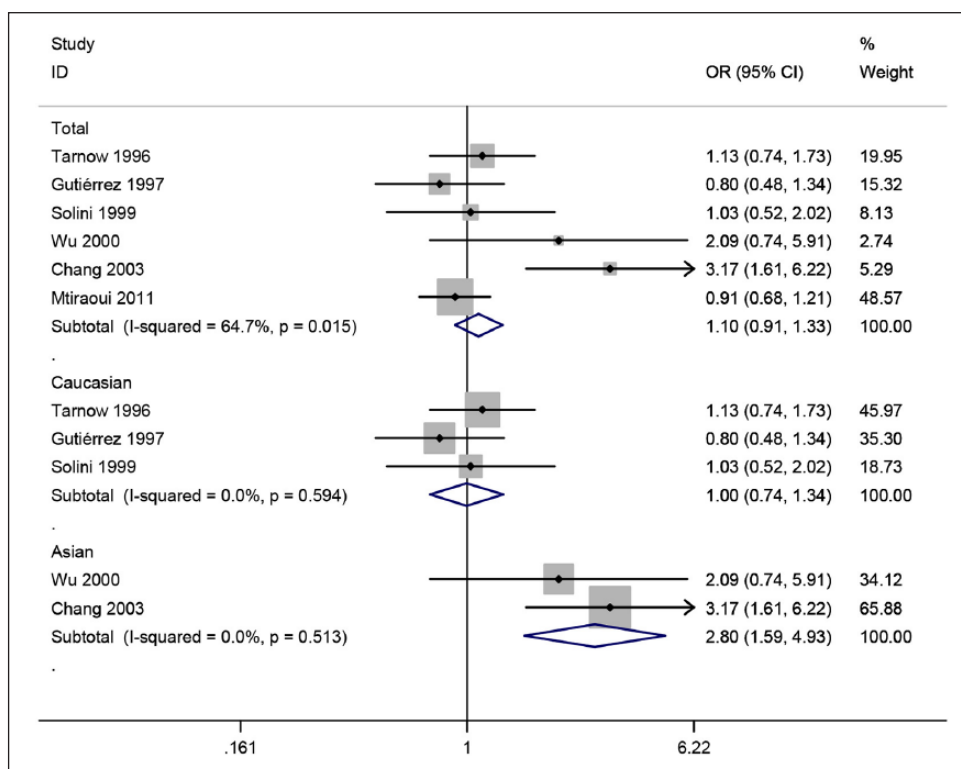
### Publication bias

A Begg funnel plot was used to evaluate whether there was publication bias for the *AGT* T174M polymorphism.

**Table 2.** Data of studies included in meta-analysis.

Study first author <sup>ref</sup>	Allele		Allele		Genotypes for cases			Genotypes for controls			HWE test
	T	M	T	M	TT	TM	MM	TT	TM	MM	
Tarnow <sup>13</sup>	336	54	324	46	148	44	1	141	42	2	0.56
Gutiérrez <sup>14</sup>	340	46	154	26	149	40	4	67	20	3	0.33
Solini <sup>15</sup>	140	18	160	20	62	14	3	71	18	1	0.91
Wu <sup>16</sup>	125	17	77	5	58	9	4	36	5	0	0.67
Chang <sup>17</sup>	220	38	220	12	93	33	3	105	11	0	0.59
Mtiraoui <sup>18</sup>	908	116	710	100	403	99	10	311	91	3	0.19

HWE: Hardy-Weinberg equilibrium; M: methionine; T: threonine.



**Figure 2.** Meta-analysis for the association between DN risk and *AGT* T174M polymorphism (M vs T) as illustrated in subgroup analysis.

*AGT*: angiotensinogen gene; CI: confidence interval; DN: diabetic nephropathy; M: methionine; OR: odds ratio; I-squared: to quantify degree of heterogeneity; T: threonine.

Consequently, the shape of the funnel plot did not reveal any evidence of obvious asymmetry. The results still did not suggest any evidence of publication bias for the *AGT* T174M polymorphism (Figure 4).

## Discussion

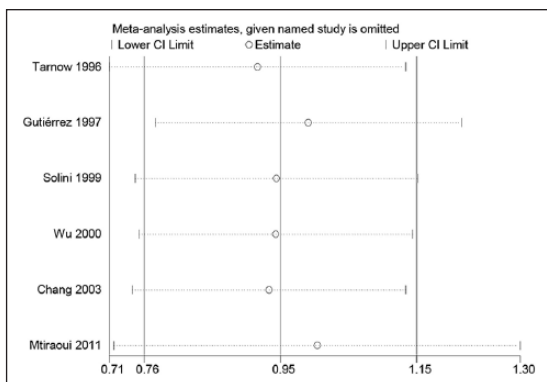
Recent epidemiological studies indicate that the global prevalence of DM has increased to 9.1%. According to a recent report,<sup>19</sup> the total number of adults with diabetes has increased to 415 million by 2015. DN is among the most

common microvascular complications of DM. The pathogenesis of DN is complicated, and poor glycemic control and genetic susceptibility both play an important role. In a population-based case-control study, Tarnow et al.<sup>13</sup> were the first to report the correlation of the *AGT* T174M polymorphism with DN.<sup>13</sup> Their study comprised 195 individuals who survived DN and 185 DM patients with persisting normoalbuminuria, while *AGT* locus did not significantly affect DN risk. In contrast to the above study, several but not all later studies confirmed the association of the T174M polymorphism with increased risk for DN. Nevertheless, it

**Table 3.** Summary of different comparative results.

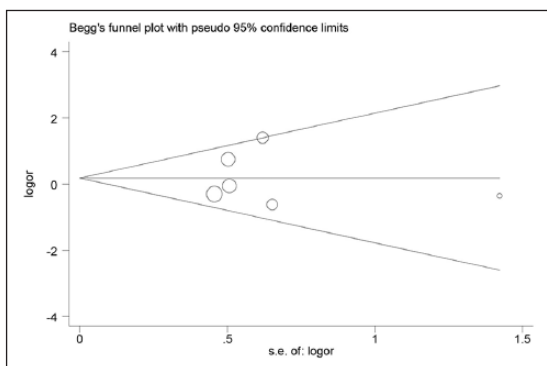
Subgroup	N	OR (95% CI)				
		M vs T	MM vs TT	MT vs TT	Dominant model	Recessive model
<b>Total</b>	6	1.22 (0.84–1.75)	1.94 (0.93–4.04)	1.11 (0.76–1.63)	1.19 (0.80–1.77)	1.94 (0.93–4.03)
<b>Race</b>						
Caucasian	3	1.00 (0.74–1.34)	0.93 (0.32–2.72)	0.95 (0.67–1.33)	0.95 (0.68–1.32)	0.95 (0.33–2.75)
Asian	2	2.80 (1.59–4.93)	6.62 (0.81–54.04)	2.14 (0.73–6.25)	2.73 (1.25–5.96)	5.97 (0.74–48.42)

CI: confidence interval; M: methionine; N: number; OR: odds ratio; T: threonine.



**Figure 3.** One-way sensitivity analysis of the pooled odds ratios and 95% confidence interval (CI) for angiotensinogen gene T174M polymorphism, omitting each dataset in the meta-analysis (M vs T).

M: methionine; T: threonine.



**Figure 4.** Begg funnel plot test of publication bias.

is likely that the reported associations of T174M polymorphism with elevated DN risk might not actually reflect the reality based on the small sample size. To help clarify the inconsistent findings, the present meta-analysis was performed to identify the potential correlation of T174M polymorphism with DN risk by retrieving relevant research.

To our knowledge, this is the first meta-analysis to quantitatively evaluate the correlation of the *AGT* T174M polymorphism with DN risk. In this study, we included and assessed six case-control studies comprising 1179 DN

cases as well as 927 controls. As a result, this polymorphism was insignificantly related with DN risk. In a subgroup analysis stratified by ethnicity, the M allele of the *AGT* T174M polymorphism was significantly correlated with DN risk in Asians, but not in Caucasians, implicating a possible role of ethnic differences in genetic backgrounds and the environment in which they live. A study by Mtiraoui and colleagues<sup>18</sup> aimed at Africans. We cannot perform further subgroup analysis in Africans for only one study. The expression of traits is influenced not only by genotypes, but also by lifestyle, nutritional status, geographical environment, economic level, and the diabetic control group with potential complications, small sample size or lower-power value in some comparisons, all of which potentially affect the results. There have been only two studies on this locus in Asians, so further research is needed to verify our results.<sup>20</sup> Sensitivity analysis further validated that the *AGT* T174M polymorphism is significantly correlated with DN risk. There is no evidence of possible publication bias in the present meta-analysis.

The serum *AGT* level was not changed in individuals with the T174M variant.<sup>21</sup> The mechanism of the relationship of *AGT* T174M polymorphism with DN risk remains undefined. The potential effect of the T174M polymorphism might be influenced by gene-gene interaction. The M235T polymorphism has been associated with elevated levels of *AGT*. *AGT* interacts with renin to produce angiotensin II. Ang II activates vascular cell apoptosis, contributing to vascular remodeling and hypertension,<sup>22</sup> while hypertension is considered as the vital risk factor for DN progression.<sup>23</sup> A recent meta-analysis has demonstrated that the *AGT* M235T polymorphism could significantly enhance DN risk.<sup>24</sup> *AGT* M235T and T174M variants are in linkage disequilibrium, and gene-gene interaction of M235T and T174M might synergistically increase the risk of DN.

There are certain limitations in this meta-analysis. First, our systematic review was based on unadjusted data, as the genotype information stratified for the main confounding variables was not available in the original papers and the confounding factors addressed across the different studies were variable. Second, publication bias is possible because all articles included in the current meta-analysis were



published in English. Finally, the effect of gene-gene and gene-environment interactions was not addressed in this meta-analysis.

In conclusion, our findings implicate a significant association of the *AGT* T174M polymorphism with DN risk in Asians. Large-scale case-control and population-based association studies are warranted to validate the risk identified in the current meta-analysis and investigate potential gene-gene and gene-environment interactions on DN risk.

### Declaration of conflicting interests

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