Research Article   · Open Access ·

Association between T174M polymorphism in the angiotensinogen gene and risk of coronary artery disease: a meta-analysis

Wen-Zhu Wang

Department of Neurology, Saint Petersburg Pavlov State Medical University, St. Petersburg 197022, Russia

Abstract

   Background   Angiotensinogen (AGT) T174M gene polymorphism has been suggested to be linked to risk of coronary artery disease, however, results from studies of this association have been inconsistent. In this study, we assess the relationship between AGT T174M gene polymorphism and coronary artery disease. **Methods**   We conducted a meta-analysis of 18 case-control studies with 8,147 coronary artery disease cases and 5,344 controls in Google scholar, PubMed, Cochrane Library and China National Knowledge Infrastructure (CNKI) databases to identify eligible studies published by July, 2012. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated from these studies. **Results**   Overall, a significant association was found between angiotensinogen T174M polymorphism and coronary artery disease risk when all studies were pooled into the meta-analysis (TT *vs*. MM: OR = 0.53, 95% CI = 0.40–0.71; dominant model: OR = 1.16, 95% CI = 1.01–1.35; recessive model: OR = 0.54, 95% CI = 0.40–0.72). In a stratified analysis, the results indicate a significant association in Caucasians suffering from coronary stenosis (TT *vs*. MM: OR = 0.38, 95% CI = 0.23–0.63; recessive model: OR = 0.39, 95% CI = 0.23–0.64). No significant increased risk for coronary artery disease was found in Asians. **Conclusions**   The meta-analysis indicate a significant association of T174M polymorphism with coronary stenosis risk in Caucasians.

1   Introduction

   Coronary artery disease (CAD) continues to be a major cause of morbidity and mortality among adults in both Europe and North America.[1] Projections show that by 2030 an additional eight million people could have CAD, a 16.6% increase in prevalence from 2010 in the United States.[2] In 2008, the overall CAD death rate was 122.7 per 100,000 population. From 1998 to 2008, the annual death rate due to CAD declined 28.7%, and actual number of deaths declined 11.9%. In the United States, the death rate was 161.7 for white males and 183.7 for black males, while the rate was 91.9 for white females and 115.6 for black females.[3] A study found that previous CAD risk factor exposures were common among those who developed CAD. About 90% of patients with CAD have prior exposure to at least one of the major risk factors, which include high total blood choles-terol level or current medication with cholesterol-lowering drugs, hypertension or current medication with blood pressure-lowering drugs, current cigarette use, or a clinical report of diabetes.[4]

   There is increasing evidence that gene-environment interaction plays a vital role in the genetic studies of complex trait diseases. Human angiotensinogen (AGT) is produced by the liver and changed to angiotensin I via renin. Afterwards, angiotensin I is converted to angiotensin II, which leads to myocardial hypertrophy, fibrosis and vasoconstriction. The AGT gene is located at lq42–43 and consists of five exons.[5] A threonine to methionine substitution at amino acid 174 is a common polymorphism called T174M (rs699), designating the T and M alleles, respectively.[6] Over the past decade, the AGT gene T174M polymorphism has been investigated for its association with the risk of developing CAD. Tiret *et al.*[7] were the first to investigate the association between AGT T174M polymorphism and myocardial infarction (MI), the study population comprised 630 cases and 741 controls. The result showed that the T174M genotype distribution did not differ between the case group and control group, and they did not find a significant association between T174M and CAD risk.[7] Later, several studies, but not all, confirmed the relationship between AGT

T174M polymorphism and susceptibility to CAD. Meta-analysis is a useful tool to evaluate rare allele frequency polymorphisms.[7] The present meta-analysis was conducted to obtain a more precise estimation of the associations between AGT T174M gene polymorphism and CAD.

2 Methods

2.1 Selection of studies

Google scholar, PubMed, Cochrane Library and China National Knowledge Infrastructure (CNKI) databases were searched for all articles on the association between AGT T174M polymorphism and CAD risk available before July, 2012 without language restrictions, using the following key words: "angiotensinogen", "myocardial infarction", "coronary heart disease", "coronary artery disease", "gene" and "T174M polymorphism." Only articles that had complete data on the comparison of T174M polymorphism frequency between CAD and control were selected in our meta-analysis. Reference lists of review articles and included articles were identified through hand searches to find other potentially eligible papers. The criteria includes: CAD, ischemic heart disease and atherosclerotic heart disease.

2.2 Selection criteria

To be eligible for inclusion in this meta-analysis, the following criteria were established: (1) case-control study design; (2) studies were conducted to evaluate the association between AGT T174M polymorphism and CAD risk; (3) studies that included sufficient genotype data to calculate the Odds ratios (OR) and 95% confidence intervals (95% CI); and (4) genotype distributions of healthy control population were in Hardy-Weinberg equilibrium (HWE). Major reasons for exclusion were: (1) not case-control studies that evaluated the association between AGT T174M polymorphism and CAD risk; (2) case reports, letters, reviews, and editorial articles; (3) no sufficient data were reported; (4) duplicate data; and 5) healthy controls were not in HWE.

2.3 Data extraction

The following characteristics were collected from each study: first author, year of publication, country, gender, end point of assessment, number of cases and controls, polymorphisms of gene, and evidence of HWE of controls. For conflicting evaluations, investigators came to an agreement after discussion.

2.4 Statistical analysis

Meta-analysis was performed using the STATA software version 12.0 (Stata Corporation, College Station, TX, USA). The associations of T174M polymorphisms with CAD risk for co-dominant model (TT vs. MM, TT vs. MT), dominant model (MM + MT vs. TT) and recessive model (TT + MT vs. MM) were examined and evaluated by OR with corresponding 95% CI. The Q test and I^2 test were performed to probe whether the variation was due to heterogeneity or sampling error (chance). Values for I^2 of 25%, 50% and 75% were defined as low, moderate and high estimates, respectively.[8] We used fixed-effects methods if the result of the Q-test was not significant ($P > 0.10$ or $I^2 < 50\%$). Otherwise, the random-effects model was used for pooling all the results. We tested whether genotype frequencies of controls were in HWE using the Pearson χ^2 test and a $P < 0.05$ was considered as significant disequilibrium. Subgroup analysis was performed according to ethnicity, CAD subtypes, and gender. The different ethnicities were categorized as Asians and Caucasians. Begg's funnel plot was used to assess publication bias ($P < 0.05$ was considered statistically significant). Sensitivity analysis was performed by sequential omission of individual studies to ensure the stability of measuring results. All P values were two-sided.

3 Results

3.1 Characteristics of studies

There were 495 papers relevant to the search words. Based on the inclusion criteria, only eighteen case-control studies were selected for this meta-analysis, [9–26] and 477 studies were excluded. The flow chart of the study selection is summarized in Figure 1. These selected studies included 8,147 CAD cases and 5,344 healthy controls. All studies were case-control studies that evaluated the association between AGT T174M polymorphism and CAD risk. The genotype distributions among the controls of all studies aiming at AGT T174M polymorphism were consistent with HWE (Table 1, $P > 0.05$). The study selection and subject characteristics are summarized in Table 1. Of these case-control studies, nine reported on Caucasians,[9,10,16–19,24-26] while the other reports were on Asians.

3.2 Results of meta-analysis

The combined results of AGT T174M polymorphism and CAD risk are summarized in Table 2. The results of the overall meta-analysis indicated a significant association between AGT T174M polymorphism and CAD risk (TT vs. MM: OR = 0.53, 95% CI = 0.40–0.71; dominant model: OR = 1.16, 95% CI = 1.01–1.35; recessive model: OR = 0.54, 95% CI = 0.40–0.72). Similarly, in the subgroup meta-

analysis, a significant association between AGT T174M polymorphism and CAD risk was observed in Caucasians (TT *vs.* MM: OR = 0.41, 95% CI = 0.27–0.62; recessive model: OR= 0.42, 95% CI = 0.27–0.64) and Caucasians with coronary stenosis (TT *vs.* MM: OR = 0.38, 95% CI = 0.23–0.63; recessive model: OR= 0.39, 95% CI = 0.23–0.64) (Figure 2). In addition, no statistical association was found

in other stratified analyses (Figure 2). Sensitivity analysis was performed by sequential omission of individual studies. The significance of pooled OR in all individual analyses and subgroup analyses was not excessively influenced by omitting any single study. Publication bias of the literature was assessed by the Begg test. The results are shown in Table 2 and Figure 3.
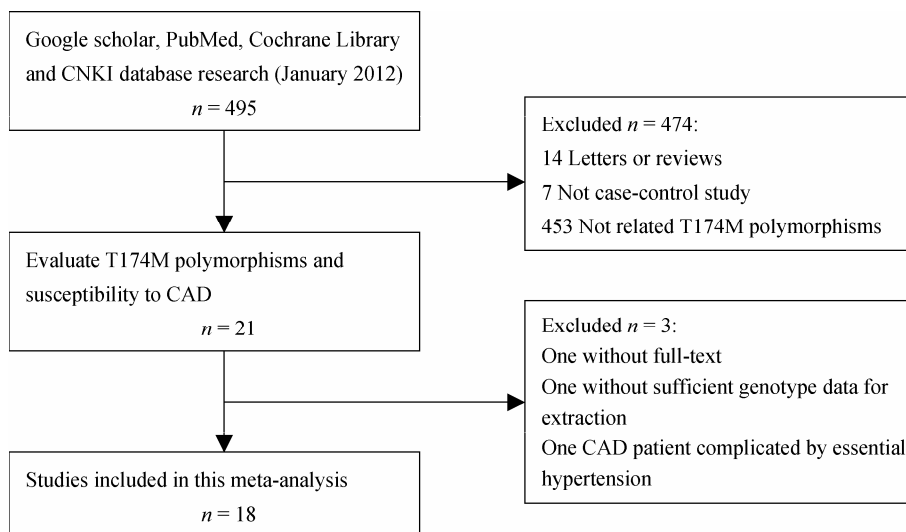

Figure 1. Flow chart showing study selection procedure. CAD: coronary artery disease; CNKI: China National Knowledge Infrastructure.

Table 1. Study selection and subject characteristics of included studies in meta-analysis.

Author	Year	Country	End point	Cases	Controls	Genotypes for cases			Genotypes for controls			P for HWE
						MM	MT	TT	MM	MT	TT	
Tiret, *et al*.[7]	1995	France	MI	630	741	12	125	493	9	154	578	0.73
Wenzel, *et al*.[10]	1997	Germany	CAD	111	102	3	26	82	1	21	80	0.77
Ichihara, *et al*.[11]	1997	Japan	CAD	327	352	6	47	274	4	57	291	0.53
Ko, *et al*.[12]	1997	China	CAD	268	336	1	45	222	2	64	270	0.39
Sheu, *et al*.[13]	1998	China	CAD	102	145	0	18	84	0	18	127	0.43
Frossard, *et al*.[14]	1998	UAE	CAD	40	61	0	8	32	0	13	48	0.35
Cong, *et al*.[15]	1998	Japan	CAD	104	170	2	13	89	2	32	136	0.94
Gardemann, *et al*.[16]	1999	Germany	CAD	1,739	511	24	362	1353	7	115	389	0.65
Fatini, *et al*.[17]	2000	Italy	CAD	205	209	5	61	139	3	46	160	0.88
Spiridonova, *et al*.[18]	2001	Russia	CAD	94	122	3	36	55	0	19	103	0.35
Babunova, *et al*.[19]	2003	Russia	CAD	229	90	7	57	165	1	21	68	0.66
Nair, *et al*.[20]	2003	India	CAD	136	131	0	25	111	2	27	102	0.89
Zhang, *et al*.[21]	2005	China	MI	105	201	8	19	78	3	32	166	0.32
Renner, *et al*.[22]	2005	Austria	CAD	2,583	733	14	174	545	49	610	1924	0.94
Tsai, *et al*.[23]	2007	China	CAD	735	519	15	195	525	5	111	403	0.38
Freitas, *et al*.[24]	2008	Portugal	CAD	298	510	4	59	235	3	107	400	0.14
Abboud, *et al*.[25]	2010	Tunisia	CAD	341	316	28	39	274	4	44	268	0.17
Konopka, *et al*.[26]	2011	Poland	MI	100	95	5	41	54	1	27	67	0.34

CAD: coronary artery disease; HWE: Hardy-Weinberg equilibrium; MI: myocardial infarction.

Table 2.   Summary of Odds ratios (ORs) and 95% confidence intervals (95% CI) of AGT T174M polymorphism and CAD risk.

Subgroup	Genetic model	Sample size		Effects model	Test of heterogeneity		Test of association		Test of publication bias	
		Case	Control		I^2	P	OR	95% CI	z	P
Overall	TT *vs.* MM	8,147	5,344	Fixed	29.4%	0.13	0.53	0.40–0.71	0.77	0.44
	TT *vs.* MT			Fixed	47.7%	0.01	0.95	0.86–1.03	0.98	0.33
	Dominant model			Random	56.0%	0.00	1.16	1.01–1.35	1.14	0.26
	Recessive model			Fixed	24.7%	0.18	0.54	0.40–0.72	0.86	0.39
Caucasians	TT *vs.* MM	3,747	2,696	Fixed	28.0%	0.20	0.41	0.27–0.62	1.98	0.05
	TT *vs.* MT			Random	65.5%	0.00	0.84	0.66–1.06	2.40	0.02
	Dominant model			Random	69.4%	0.00	1.32	1.03–1.68	2.61	0.01
	Recessive model			Fixed	22.3%	0.24	0.42	0.27–0.64	1.98	0.05
Caucasians-	TT *vs.* MM	3,017	1,860	Fixed	37.5%	0.14	0.38	0.23–0.63	1.20	0.23
coronary stenosis	TT *vs.* MT			Random	68.6%	0.00	0.83	0.62–1.12	1.50	0.13
	Dominant model			Random	71.6%	0.00	1.33	0.99–1.80	1.80	0.07
	Recessive model			Fixed	34.7%	0.16	0.39	0.23–0.64	1.20	0.23
Caucasians-MI	TT *vs.* MM	730	836	Fixed	25.7%	0.25	0.50	0.23–1.10	0.00	1.00
	TT *vs.* MT			Random	75.8%	0.04	0.790	0.41–1.53	0.00	1.00
	Dominant model			Random	79.5%	0.03	1.35	0.67–2.73	0.00	1.00
	Recessive model			Fixed	0.0%	0.33	0.52	0.23–1.14	0.00	1.00
Asians	TT *vs.* MM	4,400	2,648	Fixed	28.3%	0.21	0.70	0.47–1.05	0.60	0.55
	TT *vs.* MT			Fixed	13.4%	0.32	0.96	0.85–1.09	0.10	1.00
	Dominant model			Fixed	33.1%	0.15	1.07	0.94–1.21	0.31	0.75
	Recessive model			Fixed	23.9%	0.25	0.71	0.47–1.06	0.60	0.55

4   Discussion

The renin-angiotensin system is one of the major regulators of blood pressure and cardiovascular homeostasis. AGT is an important component of renin-angiotensin system. AGT is a precursor of angiotensin II, which causes drastic vasoconstriction and stimulates proliferation of vascular muscle cells and cardiomyocytes. Many studies have evaluated the relationship of AGT T174M polymorphism with CAD risk, but the above observed associations consisted of a small number of samples, and the results may reflect chance observations rather than true associations. A previous meta-analysis in 2007 conducted by Xu, *et al*.[27] investigated the relationship between T174M and CAD risk in sixteen studies, which did not support an association between the T174M polymorphism and susceptibility to CAD. However, there was no stratified analysis to estimate the relationship between T174M/M235T polymorphism and risk of CAD.[27] Hence, the results might have been affected by ethnicity or end point of assessment differences. Our meta-analysis assessed the association between T174M polymorphism and the susceptibility to CAD by subgroup analysis. The results of the meta-analysis indicated T174M polymorphism may be a potential risk factor for CAD in Caucasians. However, Table 2 and Figure 3 shows evidence for publication bias ($P < 0.05$), when we perform a subgroup analysis with CAD aiming at coronary stenosis and MI. However, the results of the Begg's funnel plot test showed that there was no publication bias, and the data led us to the conclusion that the heterogeneity was closely related to end point of CAD. Furthermore, we need to point out that T174M polymorphism may be related to Caucasian patients with coronary stenosis, not MI. The results agree with the latest studies that the occurrence of MI and coronary atherosclerosis is due to different genetic variants.[28,29] In the subgroup analysis by ethnicity, no significant association was found between T174M polymorphism and susceptibility to CAD in Asians, suggesting a possible role of ethnic differences in genetic backgrounds and the environment in which they live. As the eligible study number was small in this meta-analysis of T174M polymorphism, the results still need further investigation.
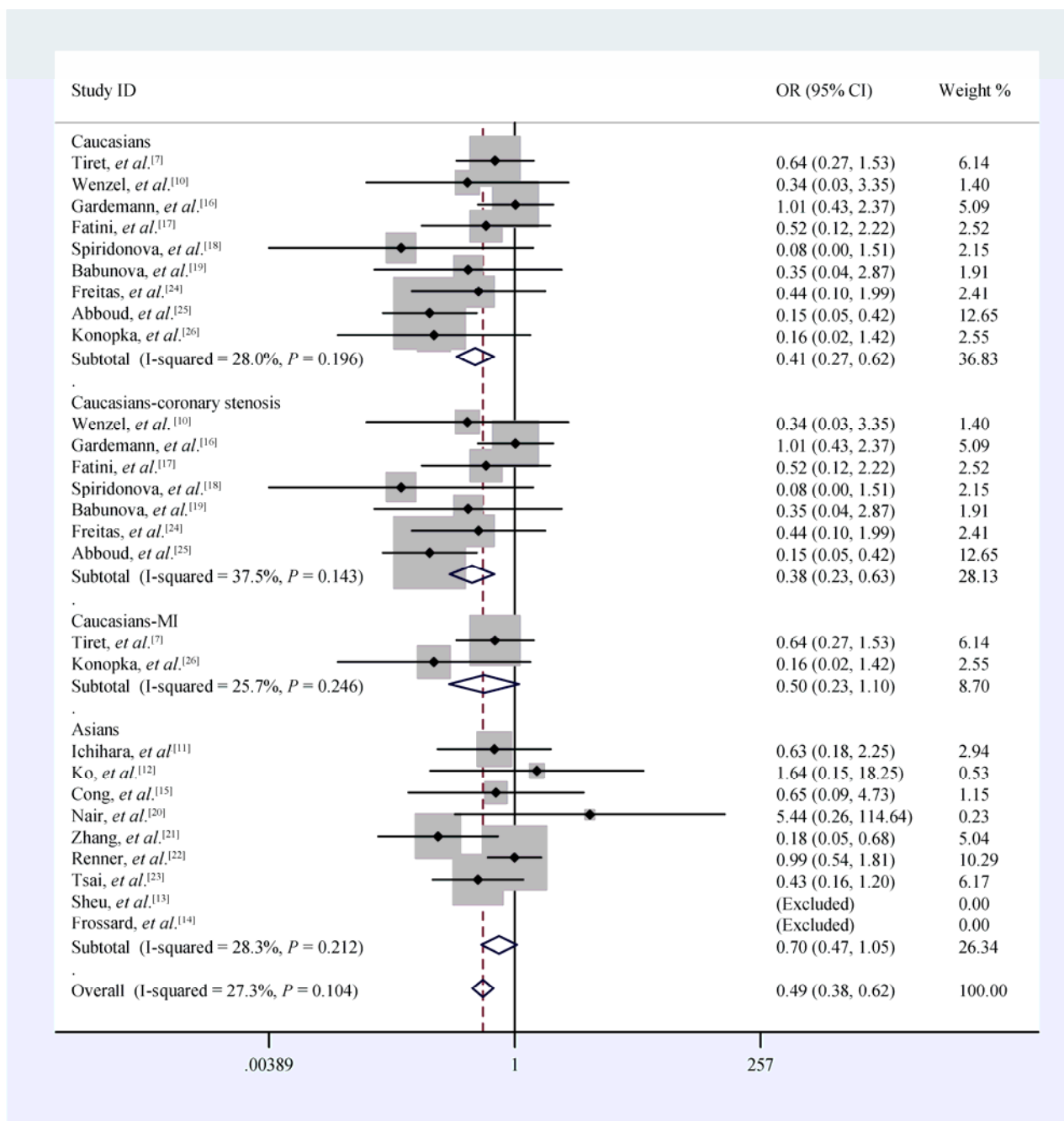
Study ID	OR (95% CI)	Weight %
Caucasians		
Tiret, et al.[7]	0.64 (0.27, 1.53)	6.14
Wenzel, et al.[10]	0.34 (0.03, 3.35)	1.40
Gardemann, et al.[16]	1.01 (0.43, 2.37)	5.09
Fatini, et al.[17]	0.52 (0.12, 2.22)	2.52
Spiridonova, et al.[18]	0.08 (0.00, 1.51)	2.15
Babunova, et al.[19]	0.35 (0.04, 2.87)	1.91
Freitas, et al.[24]	0.44 (0.10, 1.99)	2.41
Abboud, et al.[25]	0.15 (0.05, 0.42)	12.65
Konopka, et al.[26]	0.16 (0.02, 1.42)	2.55
Subtotal (I-squared = 28.0%, P = 0.196)	0.41 (0.27, 0.62)	36.83
Caucasians-coronary stenosis		
Wenzel, et al.[10]	0.34 (0.03, 3.35)	1.40
Gardemann, et al.[16]	1.01 (0.43, 2.37)	5.09
Fatini, et al.[17]	0.52 (0.12, 2.22)	2.52
Spiridonova, et al.[18]	0.08 (0.00, 1.51)	2.15
Babunova, et al.[19]	0.35 (0.04, 2.87)	1.91
Freitas, et al.[24]	0.44 (0.10, 1.99)	2.41
Abboud, et al.[25]	0.15 (0.05, 0.42)	12.65
Subtotal (I-squared = 37.5%, P = 0.143)	0.38 (0.23, 0.63)	28.13
Caucasians-MI		
Tiret, et al.[7]	0.64 (0.27, 1.53)	6.14
Konopka, et al.[26]	0.16 (0.02, 1.42)	2.55
Subtotal (I-squared = 25.7%, P = 0.246)	0.50 (0.23, 1.10)	8.70
Asians		
Ichihara, et al[11]	0.63 (0.18, 2.25)	2.94
Ko, et al.[12]	1.64 (0.15, 18.25)	0.53
Cong, et al.[15]	0.65 (0.09, 4.73)	1.15
Nair, et al.[20]	5.44 (0.26, 114.64)	0.23
Zhang, et al.[21]	0.18 (0.05, 0.68)	5.04
Renner, et al.[22]	0.99 (0.54, 1.81)	10.29
Tsai, et al.[23]	0.43 (0.16, 1.20)	6.17
Sheu, et al.[13]	(Excluded)	0.00
Frossard, et al.[14]	(Excluded)	0.00
Subtotal (I-squared = 28.3%, P = 0.212)	0.70 (0.47, 1.05)	26.34
Overall (I-squared = 27.3%, P = 0.104)	0.49 (0.38, 0.62)	100.00

.00389   1   257

Figure 2. Meta-analysis with a fixed-effects model for the association between the T174M polymorphism and CAD risk. TT *vs.* MM is illustrated in subgroup analysis. CAD: coronary artery disease.

The mechanism of how AGT T174M polymorphism relates to coronary stenosis risk is still unclear. The serum AGT level was shown to be higher in subjects carrying the T allele.[30] AGT interacts with renin to produce angiotensin II. Angiotensin II activates vascular cell apoptosis, contributing to vascular remodelling and cardiomyocyte loss in ischemia-reperfusion.[31] Furthermore, Angiotensin II has been shown in both human and animal models to be involved in the development of cardiomyocyte hypertrophy, and in cardiac fibrosis, and modulation of cardiac fibroblast growth and collagen synthesis.[32] In addition, the linkage disequilibrium of M235T and T174M in exon 2 of the AGT gene may synergistically increase the risk of CAD.[22] Those evidences suggested that T174M polymorphism might play an important role in the development of coronary stenosis.
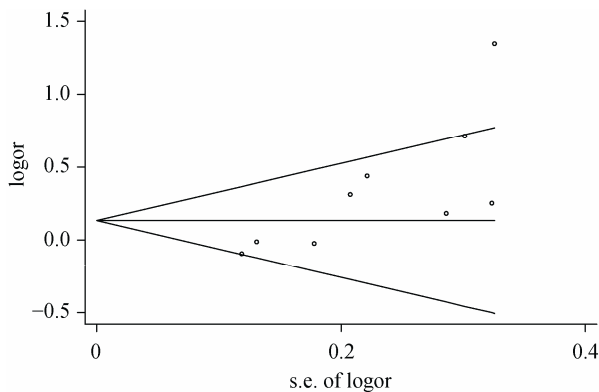
Figure 3. Begg's funnel plot test of publication bias for the association the T174M polymorphism and CAD risk in Caucasians for Dominant model. CAD: coronary artery disease.

Several potential limitations of the present study should be noted. First, because of incomplete genotype data[33] or CAD patients with other diseases combined,[34] some relevant studies could not be included in our analysis. Second, the effect of genetic and environmental interactions was not addressed in our meta-analysis. Third, the numerical results may be affected by age, and potentially age-related subgroups could not be reliably investigated because data on individual participants were not available for the meta-analysis.

In conclusion, the results indicate a significant association of AGT T174M polymorphism with coronary stenosis risk in Caucasians. However, further studies of gene–gene and gene–environment interactions should be taken into consideration.

References

1   McGovern PG, Pankow JS, Shahar E, *et al*. Recent trends in acute coronary heart disease: mortality, morbidity, medical care, and risk factors. *N Engl J Med* 1996; 334: 884–890.

2   Heidenreich PA, Trogdon JG, Khavjou OA, *et al*. Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation* 2011; 123: 933–944.

3   Roger VL, Go AS, Lloyd-Jones DM, *et al*. Heart Disease and Stroke Statistics-2012 Update: A Report From the American Heart Association. *Circulation* 2012; 125: e2–e220.

4   Greenland P, Knoll MD, Stamler J, *et al*. Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. *JAMA* 2003; 290: 891– 897.

5   Benigni A, Cassis P, Remuzzi G. Angiotensin II revisited: new roles in inflammation, immunology and aging. *EMBO Mol Med* 2010; 2: 247–257.

6   Sivitskaia LN, Kushnerevich EI, Danilenko NG, *et al*. Gene polymorphism of the renin-angiotensin system in six eth-

nic/geographic regions of Belarus. *Genetika* 2008; 44: 702–709.

7   Tiret L, Ricard S, Poirier O, *et al*. Genetic variation at the angiotensinogen locus in relation to high blood pressure and myocardial infarction: the ECTIM Study. *J Hypertens* 1995; 13: 311–317.

8   Chen D, Liu JL, Liu Y, *et al*. Lack of an association between -308G>A polymorphism of the TNF-α gene and liver cirrhosis risk based on a meta-analysis. *Genet Mol Res* 2011; 10: 2765–2774.

9   Attia J, Thakkinstian A, D'Este C. Meta-analyses of molecular association studies: methodologic lessons for genetic epidemiology. *J Clin Epidemiol* 2003; 56: 297–303.

10 Wenzel K, Blackburn A, Ernst M, *et al*. Relationship of polymorphisms in the renin-angiotensin system and in E-selectin of patients with early severe coronary heart disease. *J Mol Med* 1997; 75: 57–61.

11 Ichihara S, Yokota M, Fujimura T, *et al*. Lack of association between variants of the angiotensinogen gene and the risk of coronary artery disease in middle-aged Japanese men. *Am Heart J* 1997; 134: 260–265.

12 Ko YL, Ko YS, Wang SM, *et al*. Angiotensinogen and angiotensin-I converting enzyme gene polymorphisms and the risk of coronary artery disease in Chinese. *Hum Genet* 1997; 100: 210 –214.

13 Sheu WH, Lee WJ, Jeng CY, *et al*. Angiotensinogen gene polymorphism is associated with insulin resistance in nondiabetic men with or without coronary heart disease. *Am Heart J* 1998; 136: 125–131.

14 Frossard PM, Hill SH, Elshahat YI, *et al*. Associations of angiotensinogen gene mutations with hypertension and myocardial infarction in a gulf population. *Clin Genet* 1998; 54: 285–293.

15 Cong ND, Hamaguchi K, Saikawa T, *et al*. A polymorphism of angiotensinogen gene codon 174 and coronary artery disease in Japanese subjects. *Am J Med Sci* 1998; 316: 339–344.

16 Gardemann A, Stricker J, Humme J, *et al*. Angiotensinogen T174M and M235T gene polymorphisms are associated with the extent of coronary atherosclerosis. *Atherosclerosis* 1999; 145: 309 –314.

17 Fatini C, Abbate R, Pepe G, *et al*. Searching for a better assessment of the individual coronary risk profile: the role of angiotensin-converting enzyme, angiotensin II type 1 receptor and angiotensinogen gene polymorphisms. *Eur Heart J* 2000; 21: 633– 638.

18 Spiridonova MG, Stepanov VA, Puzyrev VP, *et al*. Association between polymorphism T174M of the angiotensinogen gene and coronary atherosclerosis in the Tomsk population. *Mol Biol* 2001; 35: 11–14.

19 Babunova NB, Minushkina LO, Zateishchikov DA, *et al*. Association of the T174M and M235T polymorphisms of the angiotensinogen gene with coronary heart disease in ethnic Russians from Moscow. *Mol Biol* 2003; 37: 52–55.

20 Nair KG, Shalia KK, Ashavaid TF, *et al*. Coronary heart dis-

ease, hypertension, and angiotensinogen gene variants in Indian population. *J Clin Lab Anal* 2003; 17: 141–146.

21 Zhang AP, Ning SC, Li ZQ, *et al*. Polymorphism of angiotensinogen gene is associated with myocardial infarction in Chinese Han population. *J Fourth Mil Med Univ* 2005; 26: 729–731.

22 Renner W, Nauck M, Winkelmann BR, *et al*. Association of angiotensinogen haplotypes with angiotensinogen levels but not with blood pressure or coronary artery disease: the Ludwigshafen Risk and Cardiovascular Health Study. *J Mol Med* (*Berl*) 2005; 83: 235–239.

23 Tsai CT, Hwang JJ, Ritchie MD, *et al*. Renin-angiotensin system gene polymorphisms and coronary artery disease in a large angiographic cohort: detection of high order gene-gene interaction. *Atherosclerosis* 2007; 195: 172–180.

24 Freitas AI, Mendonça I, Brión M, *et al*. RAS gene polymorphisms, classical risk factors and the advent of coronary artery disease in the Portuguese population. *BMC Cardiovasc Disord* 2008; 8: 15.

25 Abboud N, Ghazouani L, Kaabi B, *et al*. Evaluation of the contribution of renin angiotensin system polymorphisms to the risk of coronary artery disease among Tunisians. *Genet Test Mol Biomarkers* 2010; 14: 661–666.

26 Konopka A, Szperl M, Piotrowski W, *et al*. Influence of renin-angiotensin system gene polymorphisms on the risk of ST-segment-elevation myocardial infarction and association with coronary artery disease risk factors. Mol *Diagn Ther* 2011; 15: 167–176.

27 Xu MQ, Ye Z, Hu FB, *et al*. Quantitative Assessment of the Effect of Angiotensinogen Gene Polymorphisms on the Risk of Coronary Heart Disease. *Circulation* 2007; 116: 1356–1366.

28 Lotta LA and Peyvandi F. Addressing the complexity of cardiovascular disease by design. *Lancet* 2011; 377: 356–358.

29 Reilly MP, Li M, He J, *et al*. Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies. *Lancet* 2011; 377: 383–392.

30 Jeunemaitre X, Soubrier F, Kotelevtsev YV, *et al*. Molecular basis of human hypertension: role of angiotensinogen. *Cell* 1992; 71: 169–180.

31 Horiuchi M, Akishita M, Dzau VJ. Recent progress in angiotensin II type 2 receptor research in the cardiovascular system. *Hypertension* 1999; 33: 613–621.

32 Henrion D, Kubis N, Levy BI. Physiological and pathophysiological functions of the AT(2) subtype receptor of angiotensin II: from large arteries to the microcirculation. *Hypertension* 2001; 38: 1150–1157.

33 Sethi AA, Tybjaerg-Hansen A, Grønholdt ML, *et al*. Angiotensinogen mutations and risk for ischemic heart disease, myocardial infarction, and ischemic cerebrovascular disease. Six case-control studies from the Copenhagen City Heart Study. *Ann Intern Med* 2001; 134: 941–954.

34 Liu Y, Jin W, Jiang ZW, *et al*. Correlation between TI74M and M235T Polymorphism of Angiotensinogen Gene and Coronary Heart Disease. *J Diagn Concepts Pract* 2002; 1: 231–236.