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A1166C genetic variation of the angiotensin II type I receptor gene and susceptibility to coronary heart disease: Collaborative of 53 studies with 20,435 cases and 23,674 controls

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ABSTRACT

Objective: Angiotensin II induces vasoconstriction and vascular smooth muscle growth via stimulation of the angiotensin II type I receptor (AGTR1). Some studies have reported an association between a genetic variant (A1166C) in the 3′ un-translated region of AGTR1 and increased risk of coronary heart disease (CHD), but other have yielded apparently conflicting results.
Methods: Literature-based meta-analyses were performed on 48 papers including 53 studies published before June 2008 in relation to the A1166C polymorphism (NCBI, dbSNP: rs5186) of the AGTR1, involving a total of 20,435 CHD cases and 23,674 controls. We also explored potential sources of heterogeneity and conducted appropriate stratified analyses.
Results: In a combined analysis, the per-allele odds ratio (OR) for CHD of the A1166C polymorphism was 1.11 (95% confidence interval: 1.03–1.19), but there is an indication of publication bias and heterogeneity among the 53 studies. Sample size and study quality were significant sources of heterogeneity among studies of the A1166C polymorphism with possibly overestimates in studies of smaller sample-size and poor-quality. When the analyses were restricted to 11 larger studies (≥500 cases), and to 8 high-quality studies (quality score: ≥11 points), the summary per-allele odds ratios were 0.992 (95% confidence interval, 0.944–1.042) and 0.990 (95% confidence interval, 0.915–1.072), respectively.
Conclusions: An overall weak association between the A1166C polymorphism and CHD is observed but this is likely to be due to publication bias and heterogeneity between studies. There were no significant associations among the larger sample-size and high-quality studies which are less prone to selective publication and have greater power to detect a true association.

1. Introduction

Coronary heart disease (CHD) is a leading cause of morbidity and mortality all over the world, affecting millions of people in both developed and developing countries. Despite much investigation, the causes are not yet fully discovered. Lines of evidence suggest that coronary heart disease is determined by a complex interaction of both genetic and environmental factors. There is increasing evidence that predisposition to CHD is associated with vascular tone and blood pressure [1–4]. Therefore, the enzymes involved in the physiological control of blood pressure have received much attention.

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One of the most important physiological pathways affecting the cardiovascular system and fluid and electrolyte balance is the renin angiotensin system (RAS), a two-enzyme cascade, which, in parallel with kinins, has diverse regulatory roles in the regulation of blood pressure, vascular remodeling, and sodium homeostasis [1,2]. Angiotensin II is the most important active component of the RAS, which is not only a very powerful vasoconstrictor but also a potent growth-promoting factor for vascular smooth muscle cells, myointimal proliferation and cardiac hypertrophy. Most of the known actions of angiotensin II are mediated through the angiotensin II type 1 receptor (NCBI, Gene Symbol: AGTR1), which is particularly prominent in vascular smooth muscle cells and in the myocardium [5]. A polymorphism located in the 3′ un-translated region (A1166C) was first reported to be important with the risk of coronary heart disease in 1994 [6]. Since then, considerable effort has been poured on exploring the relationships between the AGTR1 polymorphisms and CHD. However, a number of studies yielded apparently conflicting and inconsistent results [6–60]. These disparate findings may partly due to improper study design and insufficient power in most of such studies (which have not involved more than a few hundred disease cases), and are too few to assess reliably any genetic effects that might be realistically expected (such as per-allele odds ratio (OR) increases of about 10–20%). Furthermore, the interpretation of these studies has been further complicated by the use of different type of study designs (prospective vs retrospective), of different ages of onset, of different coronary disease endpoints (e.g., myocardial infarction (MI) and coronary stenosis), of different populations (Caucasian vs other ethnicities), of different genotyping procedures, and of different control groups (e.g., population vs hospital based).

Several polymorphisms in the exons and promoter region of AGTR1 gene have been studied [9,41], but only few studies explored the associations between coronary heart disease and polymorphisms in the exons and promoter region of AGTR1 gene such as A-810T (NCBI, dbSNP: rs275652), C-521T (NCBI, dbSNP: rs1492078), and A-153G (NCBI, dbSNP: rs275653). Most studies have focused on the A1166C polymorphism (NCBI, dbSNP: rs5186) located in the 3′ un-translated region. In an attempt to understand the cumulative evidence on this topic, we reported a comprehensive meta-analysis of published genetic association studies of the AGTR1 gene and the risk of CHD. Due to the limited number of reported other genetic variants in the AGTR1 gene, in this meta-analysis we only focused on the widely reported A1166C polymorphism involving a total of 20,435 CHD cases (including MI patients) and 23,674 controls in 48 published papers with 53 separate studies (counting each study's cases and controls only once). To reliably assess the hypothesized the AGTR1-CHD relationship on the basis of these included data, we provided combined overall estimates and stratified analyses using both a classic random-effects model and a fixed-effects model. We also quantitatively examined sources of heterogeneity in previous studies regarding the direct relation of A1166C genotypes to CHD risk and conducted stratified analyses according to the potential sources of heterogeneity.

2. Methods

2.1. Data source

We attempted to conform to Quality of Reporting of Meta-analyses (QUOROM) guidelines in the report of this meta-analysis [61]. Genetic association studies published before June 2008, on CHD and the A1166C polymorphisms in the AGTR1 gene were sought by computer-based searches. The definition of coronary heart disease is based on the World Health Organization criteria (coronary heart disease (CHD), also called coronary artery disease (CAD), ischemic heart disease (IHD), and atherosclerotic heart disease, is the end result of the accumulation of atheromatous plaques within the walls of the arteries that supply the myocardium). The search was supplemented by reviews of reference lists for all relevant studies and review articles, and correspondence with authors. Computer searches of PubMed, EMBASE, and SCOPUS used keywords relating to the relevant genes (e.g., "angiotensin II type I receptor", "AGTR1", "ATG1R", or "AT1R") in combination with words related to CHD (e.g., "coronary heart disease" or "coronary artery disease" or "myocardial infarction" or "ischemic heart disease" or "atherosclerotic heart disease") and polymorphism. All the relevant studies published as full-length articles and English were included. The literature search procedure was conducted by one experienced genetic epidemiologist, and then confirmed by another experienced reviewer and genetic epidemiologist.

2.2. Data extraction

Data extraction was performed independently by two persons and differences were resolved by further discussion. For each included study, The following information was extracted from each report according to a fixed protocol: the first author, published year and month, study design, geographic area, ethnicity, mean age of CHD cases and controls, gender component of cases and controls, case-control match status, definition and numbers of cases and controls, source of controls, genotyping method, frequency of genotypes, and Hardy–Weinberg equilibrium in controls. Relevant clinical outcome included confirmed MI (generally by WHO criteria) and coronary stenosis (defined variously as at least 50%, or 70% stenosis of one or more major coronary arteries on the basis of computer-assisted assessments). For the A1166C polymorphism, there were sufficient data to analyze these outcomes separately. For studies in which data could not be separated according to type of coronary disease from published data, even after contacting the correspondence authors, cases were classified in the more inclusive category of coronary stenosis for the purpose of subsidiary analyses. If multiple published reports from the same study population were available, we included only the one with largest sample size and the most detailed information. Studies with different ethnic groups were considered as individual studies for our analyses.

2.3. Quality assessment

For genetic association studies with conflicting results on the same genetic variants, the study quality reflecting the study design should be assessed by appropriate criteria to limit the risk of introducing bias into meta-analyses. Because no universal scale is available for measuring quality of genetic association studies, we followed the recommendations of the MOOSE guidelines [62] and the rules used in most of the previously published related meta-analytic papers [93,94] and assessed the quality of key components of design separately rather than generating a single aggregate score. Following these recommendations and rules, we assessed study quality based on the following eight criteria: type of study design; sample size; disease-diagnostic criteria; control of confounding factors between cases and control subjects (such as age, gender, geographic area, and ethnicity); extent of deviation from the Hardy–Weinberg equilibrium in controls; genotyping procedure; bias of genotyping data processing; source of control subjects. As it is known that the results based on large sample size, prospective study design, strict disease-diagnostic criteria, proper matches for known cardiovascular risk factors between cases and controls, undeviating from the Hardy–Weinberg equilibrium in controls, less genotyping errors, blindly double checked raw data, general population-based control source are much more trustable. A procedure known as 'extendable quality score' has been developed to

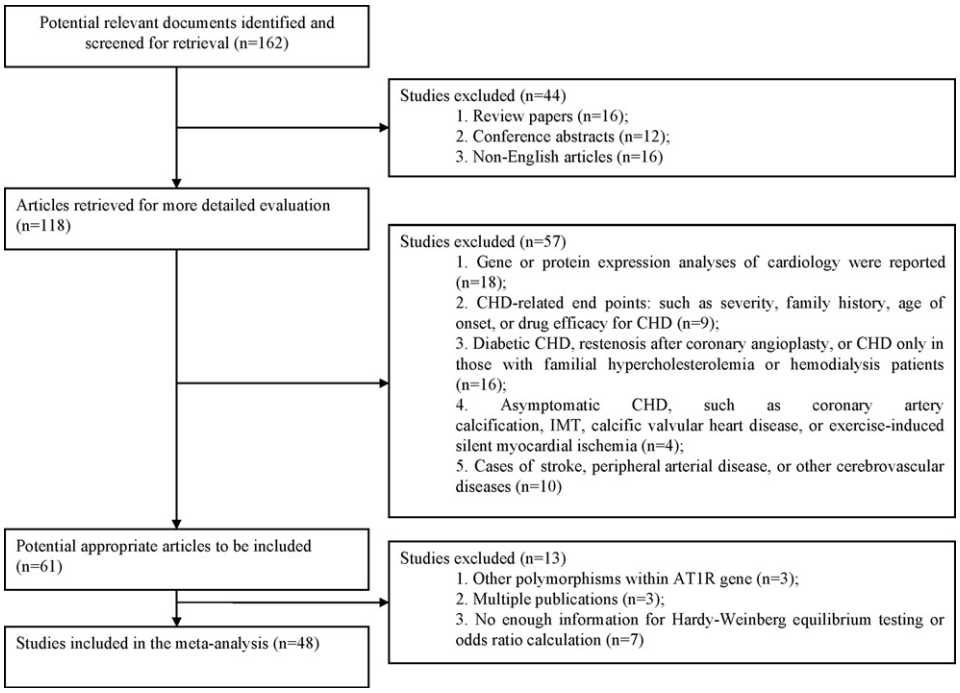

Fig. 1. Study flow diagram.

assess the quality of the included genetic association studies. The procedure with eleven items scores each paper categorizing it as having 'high', 'median' or 'poor' quality. The quality assessment score system is described in Supplement Table 1.

2.4. Statistical analyses

Deviation from Hardy–Weinberg equilibrium was examined by χ^2 tests with 1 degree of freedom. The per-allele odds ratio ("odds ratio (OR)") of the putative risk allele (1166C) was compared between cases and controls by assigning scores of 0, 1, and 2 to common homozygote, heterozygote, and rare homozygote, respectively, and calculating odds ratios per unit score by logistic regression as an additive genetic model. The calculation procedure for the reported per-allele odd and its 95% confidence interval was described in detail previously [4,95,96]. Additional pooled estimates were also given with corresponding results under dominant and recessive genetic models. Random-effects and fixed-effect summary measures were calculated as inverse-variance weighted average of the log odds ratio. The results of random-effects summary were reported in the text because it takes into account the variation between studies. Sensitivity analysis, which determines the influence of individual studies on the pooled estimate, was determined by sequentially removing each study and recalculating the pooled relative risk and 95% confidence interval. Heterogeneity was assessed using the Q statistic test [63], and I^2 test, which describes the proportion of variation in the log odds ratios that is attributable to genuine differences across studies rather than to random error [64]. Subsidiary analyses included subgroup analyses or random-effects meta-regression with restricted maximum likelihood [65]. Publication bias was assessed using the funnel plot [66] and Trim and Fill method [67]. Because funnel plots have several caveats and represent only an informal approach to detect publication bias [68], we further carried out quantitative testing by use of Egger's regression test [69]. Study design (prospective vs retrospective studies), geographic region (Europe, East Asia, North America, and others), ethnicity (Caucasian, East Asian, and others), mean age of cases (>55, ≤55, or unknown), types of CHD endpoints (MI vs coronary stenosis), gender (male, female or mixed), status of Hardy–Weinberg equilibrium (yes or no), genotyping procedures (RFLP vs other), study size (≥500 cases, 200–499 cases, and 200 cases), source of controls (population vs hospital based), and study quality (high, middle, or poor) were pre-specified as characteristics for assessment of heterogeneity; other potentially relevant subgroups (such as age of onset, haplotypes containing A1166C, and more detailed disease outcomes, e.g. cardiovascular mortality, fatal, non-fatal events, or the extent and number of stenosis of major coronary arteries) could not be reliably investigated since individual participant data were not available for this meta-analysis. Ethnic group was defined as Caucasian (i.e., people of white European origin), East Asian, or others (e.g., African Indian, and African American). All p-values are two-sided and $p < 0.05$ is considered as statistically significant. All statistical analyses were carried out by using the Statistical Analysis System software version 8.2 (SAS Institute Inc., Cary, North Carolina, USA). In the figures, areas of squares of individual studies are inversely proportional to the variances of the log odds ratios, and the horizontal lines represent CIs.

2.5. Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

3.1. Characteristics of the included studies

Fig. 1 describes the literature selection process according to QUORUM criteria. A total of 48 eligible original reports with 53 separate association studies [6–13,15–25,28–40,42–47,50–59], including a total of 20,435 CHD cases and 23,674 controls (weighted mean age of cases 57.61 years), were identified and described in Supplementary Table 2: four [10,11,18] of which conducted separate analyses according to type of coronary disease (coronary

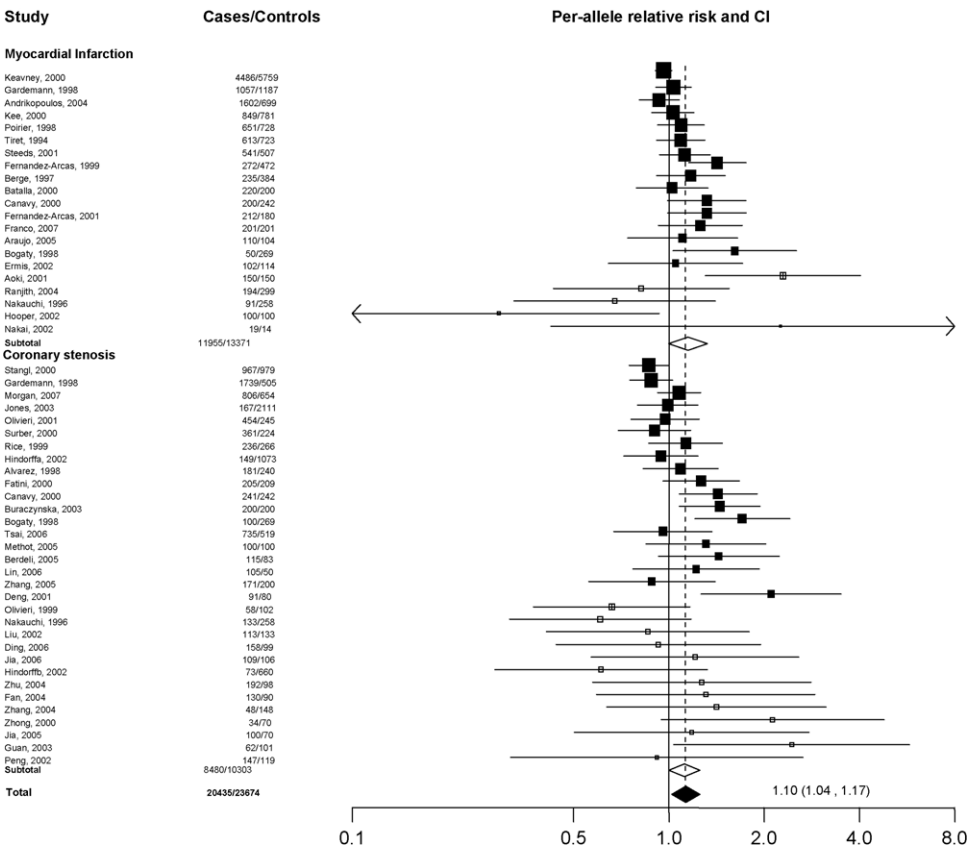

Fig. 2. Meta-analysis of studies of A1166C polymorphism and coronary heart disease.

stenosis and myocardial infarction), which were entered as two separate studies respectively; one [37] of which had two separate case-control studies derived from two different ethnic populations respectively, which were also entered as two separate studies respectively. This polymorphism was found to occur in frequencies consistent with Hardy–Weinberg equilibrium in the control populations of the vast majority of the published studies, except 7 papers with 8 studies [6,9,10,16,29,37,56]. For geographic distribution, 82% of cases were from Europe, 3% North America, 13% East Asia and 3% of other regions (including Africa South American, India, and Turkey). For ethnic distribution, 84% of cases were Caucasian, 13% East Asian and 3% of other ethnic origins (including African American, Indian, and Turk). Of the 53 studies, 49 involved retrospective comparisons [6–10,12,13,15–21,23–25,28–38,40,42–47,50–59] and 4 were prospective in design [11,22,39]. Of the 49 retrospective studies, 22 used population-based controls [6,8–13,16,20,23,30,32,35,36,38,39,42,43,46,50,53] and 31 hospital based controls. All but 15 studies used polymerase chain reaction/restriction fragment length polymorphism (RFLP) with various restriction enzymes for genotyping. Of the remaining studies, 2 used direct sequencing method [30,50], 9 used allele specific amplification (AS-PCR or SB-ASA) [6,17,18,22,23,34,43,53], 2 used matrix assisted laser desorption ionisation-time of flight-mass spectrometry (MALDT-TOF) [35,58], 1 used nested polymerase chain amplification (nested-PCR) [15], and 1 used single strand conformation polymorphism (SSCP) [9]. The extended-quality scores ranged from 6 to 14, and 40 studies were given median quality (≥7 points and <11 points) [6–9,12,13,15–20,22,24,28,29,31–33,35–38,40,42–45,47,50–55,57–59], whereas 8 were given high quality (≥11 points) [11,21,23,25,30,39,46]. Five 'poor quality' studies were found [10,34,37,56].

3.2. Association of A1166C variant with CHD

There was substantial heterogeneity among the 53 studies of the A1166C polymorphism ($\chi^{252} = 105.1$, $I^2 = 50.50\%$, $p < 0.001$). Sample size ($\chi^{22} = 25.7$, $p < 0.001$) and study quality ($\chi^{22} = 9.88$, $p = 0.007$) explained large part of the heterogeneity, whereas study design ($\chi^{21} = 3.43$, $p = 0.064$), geographic region ($\chi^{23} = 6.46$, $p = 0.091$), ethnic group ($\chi^{22} = 3.38$, $p = 0.185$), source of controls ($\chi^{21} = 1.23$, $p = 0.267$), genotyping procedures ($\chi^{21} = 0.13$, $p = 0.721$), status of Hardy–Weinberg equilibrium ($\chi^{21} = 5.18$, $p = 0.023$), mean age of case ($\chi^{22} = 0.41$, $p = 0.814$) and gender ($\chi^{21} = 3.59$, $p = 0.166$) explained little heterogeneity. Overall, the per-allele odds ratio (OR) of the 1166T variant for total CHD was 1.10 (95% CI: 1.04–1.17;

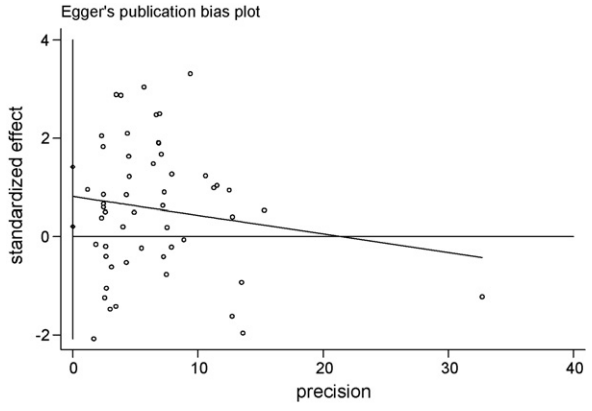

Fig. 3. Test publication bias of studies of A1166C polymorphism and CHD using Egger's test. $p = 0.009$.

Table 1
Stratified meta-analysis of the effect of A1166C polymorphism of the angiotensin II type I receptor gene on risk for CHD.

Subgroup: no. of studies included	No. of subjects		Per allele risk			Test for heterogeneity within group			Test for heterogeneity between subgroup	
	Cases	Controls	OR(95% CI)	*z*	*p*-value	*Q*	*p*-value	I^2	*Q*	*p*-value
Study design										
Prospective: 4 studies	3324	4027	0.965(0.886–1.051)	0.82	0.414	2.85	0.416	0%	3.42	0.064
Retrospective: 49 studies	17,111	19,647	1.127(1.057–1.201)	3.64	<0.001	98.84	<0.001	51.40%		
Geographic region										
Europe: 25	16,754	18,040	1.069(1.009–1.133)	2.27	0.023	52.7	0.001	54.50%	6.46	0.091
North America: 6 studies	572	2471	1.212(0.995–1.477)	0.53	0.593	17.42	0.004	71.30%		
East Asia: 18 studies	2588	2563	1.102(0.772–1.574)	1.91	0.056	26.27	0.07	35.30%		
Others: 4 studies	521	600	1.132(0.895–1.432)	1.03	0.303	2.25	0.523	0%		
Ethnicity										
Caucasian: 30 studies	17,263	19,855	1.088(1.026–1.153)	2.84	0.005	66.51	0	56.40%	3.38	0.185
East Asian: 18 studies	2588	2563	1.212(0.995–1.477)	1.91	0.056	26.27	0.07	35.30%		
Others: 5 studies	584	1256	0.874(0.571–1.337)	0.62	0.535	8.94	0.063	55.25		
Gender										
Male: 10 studies	3659	4684	1.171(1.024–1.339)	2.3	0.021	24.69	0.003	63.50%	3.59	0.166
Female: 2 studies	331	541	1.206(0.751–1.937)	0.78	0.438	4.12	0.042	75.70%		
Mixed: 41 studies	16,445	18,449	1.069(1.000–1.143)	1.96	0.05	68.43	0.003	41.50%		
Mean age of cases										
≥55 years: 33 studies	12,093	11,694	1.061(0.985–1.143)	1.55	0.121	58.26	0.003	45.10%	0.41	0.814
<55 years: 15 studies	6756	8696	1.235(1.091–1.397)	3.34	0.001	38.91	0	64.00%		
Unknown: 5 studies	1586	3284	0.995(0.831–1.192)	0.05	0.959	7.52	0.111	46.80%		
End point										
Coronary stenosis: 32 studies	8480	10,303	1.097(1.002–1.201)	2	0.045	60.26	0.001	48.60%	0.03	0.852
Myocardial infarction: 21 studies	11,955	13,371	1.108(1.026–1.196)	2.6	0.009	44.8	0.001	55.40%		
Experimental method										
RFLP: 38 studies	11,160	12,956	1.109(1.021–1.205)	2.44	0.015	70.28	0.001	48.80%	0.13	0.721
Others: 15 studies	9275	10,718	1.097(1.010–1.192)	2.19	0.028	34.7	0.003	56.80%		
Hardy–Weinberg equilibrium										
Yes: 45 studies	18,635	20,723	1.079(1.019–1.144)	2.58	0.01	80.09	0.001	45.10%	5.18	0.023
No: 8 studies	1800	2951	1.219(0.987–1.504)	1.84	0.065	19.84	0.006	64.70%		
Source of control subjects										
Population-based: 22 studies	7845	8674	1.098(1.015–1.189)	2.32	0.02	35.42	0.025	40.70%	1.23	0.267
Hospital-based: 31 studies	12,590	15,000	1.102(1.013–1.198)	2.27	0.023	68.45	0	56.20%		
Sample size for patients										
≥500 cases: 11 studies	14,046	13,041	0.992(0.944–1.042)	0.34	0.735	13.16	0.215	24.00%	25.7	<0.001
200–499 cases: 12 studies	3037	3065	1.206(1.100–1.321)	4.01	<0.001	15.57	0.158	29.30%		
<200 cases: 30 studies	3352	7568	1.147(1.007–1.307)	2.06	0.04	50.64	0.008	42.70%		
Quality score										
High-quality: 8 studies	7134	6949	0.990(0.915–1.072)	0.24	0.807	12.57	0.083	44.30%	9.88	0.007
Medium-quality: 40 studies	12,873	15,377	1.133(1.056–1.215)	3.48	0.001	69.38	0.002	43.80%		
Poor-quality: 5 studies	428	1348	1.104(0.707–1.726)	0.44	0.663	13.28	0.01	69.90%		
Overall	20,435	23,674	1.100(1.038–1.165)	3.23	0.001	105.1	<0.001	50.50%		

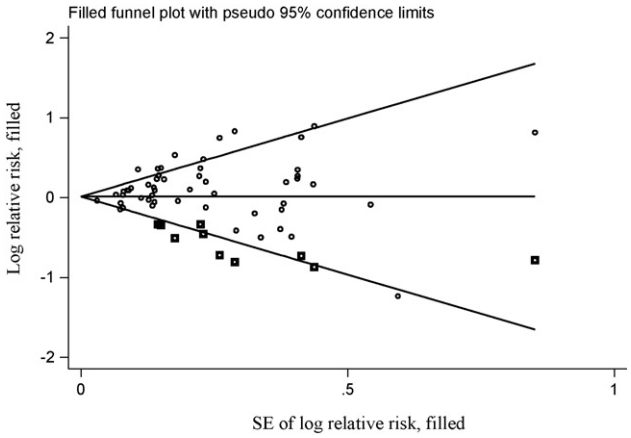

Fig. 4. Trimmed and Filled funnel plot of A1166C polymorphism and CHD. The hollow ovals are the actual studies included in the meta-analysis, the sold square are the Trimmed and Filled studies required to achieve symmetry.

Fig. 2) under additive model, with corresponding results under dominant and recessive genetic models of 1.28 (95% CI: 1.12–1.47) and 1.09 (95% CI: 1.01–1.17), respectively. Subsidiary analyses of specific CHD endpoints yielded a per-allele odds ratio (OR) for MI of 1.11 (95% CI: 1.03–1.26; Table 2) and for coronary stenosis of 1.097 (95% CI: 1.002–1.201; Table 2), with no clear difference between these subendpoints ($\chi^{21} = 0.03$, $p = 0.852$). Via sensitivity analyses, we did not find any large influence of any individual study on the pooled estimates in general or in subgroups. Egger test for asymmetry of the funnel plot of these 53 studies was significant (Egger's test, $p = 0.009$, Fig. 3), which suggested a possibility of the preferential publication of positive findings in smaller studies. Further evidence of selective publication was suggested by the results of the Trim and Fill approach, which indicated that 10 missing studies are required to make the funnel plot symmetrical (Fig. 4). When the 10 missing studies included, the per-allele odds ratio (OR) of the 1166T variant for total CHD was 1.03 (95% CI: 0.970–1.100; $p = 0.310$), indicating the re-calculated combined result was not significant.

Analysis restricted to the 11 studies with at least 500 cases (total of 14,046 cases and 13,041 controls), which should be less prone to selective publication than smaller studies, yielded a combined per-allele odds ratio (OR) of 0.992 (95% CI: 0.944–1.042; Table 1) under additive model, with corresponding results under dominant and recessive genetic models of 0.981(95% CI: 0.972–1.084) and 0.991 (95% CI: 0.968–1.017). There was no heterogeneity among the 11 larger studies of the A1166C polymorphism ($\chi^{210} = 13.16$, $I^2 = 24.00\%$, $p = 0.215$; Table 1). Further stratified analysis according to study design and quality score found that the combined per-allele odds ratio (OR) for the 4 prospective studies with 3324 cases and 4027 controls was 0.965(95% CI: 0.866–1.051; Table 1) under additive model, with corresponding results under dominant and recessive genetic models of 0.958(95% CI: 0.898–1.117) and 0.978 (95% CI: 0.901–1.081), and the combined per-allele odds ratio (OR) for the 8 high-quality studies with 7134 cases and 6949 controls was 0.990 (95% CI: 0.915–1.072; Table 1) under additive model, with corresponding results under dominant and recessive genetic models of 0.989 (95% CI: 0.910–1.101) and 0.992 (95% CI: 0.921–1.102). There was no heterogeneity among the 4 prospective studies ($\chi^{23} = 2.85$, $I^2 = 0\%$, $p = 0.416$; Table 1), nor among the 8 high-quality studies ($\chi^{27} = 12.57$, $I^2 = 44.30\%$, $p = 0.083$; Table 1).

4. Discussion

The RAS related genes, such as AEC, AGT, and AGTR1, are very important in cardiovascular diseases. Updated meta-analyses of coronary heart disease with AEC and AGT have been well explored. Therefore, in this study, we only re-analyzed the role of AGTR1 genetic variants on the risk of CHD, and mainly focused on the A1166C polymorphism because only this polymorphism was widely investigated worldwide. The present meta-analysis provides the most comprehensive assessment of AGTR1 variants and CHD risk. Overall, there was a nominal weak association between the A1166C variant and CHD risk. However, this association became non-significant when the meta-analysis was restricted to larger studies, well-designed studies, or high-quality studies. Further, we explored potential sources of heterogeneity across studies and the possibility of publication bias. After allowing for publication bias, the overall significant association also disappeared. Our results suggest a publication bias favoring positive results, consistent with the phenomenon known as the "File-Drawer Problem" [70], leading to an overestimation of the true genetic association. The largest, well-designed study with high-quality score in the field conducted by Keavney et al. (Lancet 2000; 355:434–442), which included over 10,000 carefully genotyped subjects, had entirely null findings for the association of the A1166C variant with myocardial infarction [17]. So far, several well-designed genome-wide association studies of coronary heart disease have been performed [71–76], even though the A1166C variant was not tested by these genome-wide association studies because of the limited SNP coverage rate of the existing SNP microarray platforms on the AGTR1 gene, none of them indicated a strong association signal for this gene with coronary heart disease.

The AGTR1 A1166C polymorphism has potential molecular function and during the past 30 years, more than 50 research groups in the world have solely focused on this polymorphism to explore its relationship with CHD, which is the main reason why we conducted the meta-analysis on this single SNP in the ear of GWAS. If a SNP has convinced function and has a big effect size in affecting the susceptibility to CHD, or a SNP with high Linkage Disequilibrium with the functional SNP, the SNP may really play an important role in CHD even though the risk of coronary atherosclerosis is influenced by hundreds of SNPs on the whole genome. Our meta-analysis is based on a debatable SNP with potential function in vascular regulation, through a comprehensive analytic strategy, we ruled out its role in CHD based on a pooled big sample size. Based on the robust results in our meta-analysis, novel studies focusing only on the A1166C polymorphism in the AGTR1 gene cannot be accepted. While the purpose of GWAS is to capture all of the potential susceptibility genes to complex diseases on the human genome through screening out the polymorphic markers significantly associated with the disease phenotypes. After the GWAS-based filtering, replications using enlarged sample size to confirm the individual significant polymorphic markers are warranted. Due to the above reasons, to conduct a meta-analysis on a single SNP in the era of GWAS is still necessary.

Compared with a previous meta-analysis [97], the present study conducted a very comprehensive literature search, strict inclusion criteria and quality assessment of the included studies, and finally pooled 53 independent studies consisting of more than twice as many cases as the older meta-analysis, which extremely improved statistic power. In addition, we tested multiple genetic models regarding the A1166C variant, conducted sensitivity analyses, and assessed potential sources of between-study heterogeneity across studies and the possibility of publication bias. Furthermore, we explored stratified analyses according to the potential sources of between-study heterogeneity, such as study design, geographic region, ethnicity, mean age of cases, types of CHD endpoints, gender, status of Hardy–Weinberg equilibrium, genotyping procedures, sample size, source of controls, and study quality. For the above merits, our study provided more comprehensive and reli-

able assessment of the association between the A1166C genetic variation of AGTR1 gene and CHD risk.

The findings of this genetic association meta-analysis are rather sobering, suggesting important limitations in the designs of several dozen investigations of the A1166C polymorphism and CHD during the past decade. In particular, the individual studies of the A1166C polymorphism have generally been too small to confirm or to refute reliably genetic effect on CHD of realistic sizes. Genetic association studies should be appropriately powered, acknowledging that large samples are probably prudent when effect sizes cannot be specified with certainty in advance. Problems associated with proliferation of underpowered studies may be reduced by regular review of the available data. The example in this study illustrates general problems that have been noted in the reporting of genetic association studies with complex outcomes [77–81]. As well as a need for much larger and more rigorous studies than is now customary, there is generally a greater need in genetic and genomic epidemiology for quantitative systematic reviews to help minimize random error, identify publication bias, take proper account of differences in study design, and deal with more comprehensively the observed statistical between-study heterogeneity derived from real differences in patient and control populations, study designs, clinical outcomes, etc. The development of high throughput techniques has resulted in an explosion of available genetic and genomic information, which creates challenges in analyzing, synthesizing and finally translating this rapidly accumulating evidence in useful clinical and public health applications. Meta-analysis is a useful quantitative modeling tool in dissecting the genetics of complex diseases and traits by synthesizing and analyzing multi-source diverse data sets. Under the HuGENet framework, prospective collaborative meta-analysis networks in human genome epidemiology have been undertaken by consortia of investigators [82–84] and great efforts have made in Alzheimer's disease, schizophrenia and Parkinson's disease [85–87]. The advent of genome-wide association studies (GWAS) has also given new challenges and opportunities to meta-analysis. Meta-analyses of GWAS have successfully identified additional susceptibility genes to Parkinson's disease, colorectal cancer, bipolar disorder, type I and type II diabetes [88–92].

Recent function study has identified that the A1166C polymorphism located in the 3′UTR of the AGTR1 gene perturbed a microRNA target site, which may potentially affect the interaction between AGTR1 gene and its regulating microRNA gene and then cause allele-specific expression imbalance, which should be the potential biological mechanism regarding how AGTR1 gene plays a role in some cardiovascular diseases, such as hypertension. Hypertension is a main risk factor for CHD, but it might not be able to independently affect CHD.

Several potential limitations of the data sources included in this study should be considered: (1) The quality of the individual studies may largely influence the results of the meta-analysis. Because no universally validated scale of study quality exists, we used a scale that was based on items that were selected based on common sense only. It is possible that a different scale would yield conflicting results and further bring misleading [84]. (2) As with any meta-analysis of published results, the quality of our meta-analysis depends on that of individual studies. Ideally we would like to pool individual-level data. However this is not possible for the present study. (3) Due to limited studies explored for the association of other polymorphisms in this gene with coronary heart disease, meta-analysis could not be performed for other polymorphisms. (4) Due to the limitation of unavailable individual participant data, other potentially relevant subgroups (such as age of onset, haplotypes containing A1166C, and more detailed disease outcomes, e.g. cardiovascular mortality, fatal, non-fatal events, or the extent and number of vessel stenosis of major coronary arteries) as well as the mediating effects of hypertension or high cholesterol could not be reliably investigated.

Limited statistical power is a common problem in complex genetic studies. In this meta-analysis, to obtain as much literature as possible, we put equal emphasis on the positive and negative literature, which reduced potential publication bias and helped maximize statistical power and robustness. To detect a true association gene for CHD might require more accurate phenotype definition and strict selection of patients and healthy controls. Standardization of CHD subtype diagnosis, sample collection methods, DNA marker sets, assessment protocol, and application of demographical statistics methodology would significantly simplify collaboration and comparisons among investigators, and would facilitate future multi-site projects and joint data analyses.

In conclusion, to our knowledge, this may be the first exhaustive and comprehensive meta-analysis of the AGTR1 variants and CHD risk. Strong and consistently negative associations were found in the A1166C variant, which indicates that the AGTR1 genetic locus might not play a role in the etiology of CHD.

Contributors

Ming-Qing Xu had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Ming-Qing Xu initiated the study, conducted the literature search and data extraction and analyses, and wrote the first draft of the manuscript. All investigators were contributed to the analysis, interpretation, and redrafting.

Conflict of interest statement

We declare that we have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2010.07.046.

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