Meta Analysis

M235T polymorphism in the angiotensinogen gene and cardiovascular disease: An updated meta-analysis of 39 case–control comparisons

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Abstract

Objective: M235T polymorphism of the angiotensinogen (AGT) gene has been linked with cardiovascular disease (CVD). The aim of this meta-analysis was to investigate whether combined evidence supports this association.

Methods: A systematic search was conducted for studies published up to October 2018 that evaluate the association between AGT M235T polymorphism and risk of CVD. Case–control studies were identified, and the association between AGT M235T polymorphism and CVD risk was assessed using genetic models.

Results: Thirty-nine comparisons from 38 studies were collected, and a meta-analysis and subgroup analysis was performed based on ethnicity. In the overall population (9225 cases and 8406 controls), the occurrence of CVD was found to be associated with AGT M235T polymorphism in both allelic [T vs. M: odds ratio (OR)=1.16] and recessive (TT vs. MT+MM: OR=1.14) models. In subgroup analyses, a significant association was identified between AGT M235T polymorphism and CVD risk in East Asian subgroups in allelic (T vs. M: OR=1.46), homozygous (TT vs. MM: OR=1.78), dominant (MT+TT vs. MM: OR=1.47), and recessive (TT vs. MT+MM: OR=1.68) models, but there was no significant association in Caucasian populations.

Conclusion: Among East Asians, the AGT variant M235T is associated with CVD risk. However, current evidence suggests that there is no such association in the Caucasian population. (Anatol J Cardiol 2019; 21: 222-32)

Keywords: angiotensinogen, genetic polymorphism, cardiovascular disease

Introduction

Cardiovascular disease (CVD) is the main cause of death and leads to over 30% of mortality annually worldwide (1). The general risk factors for CVD include smoking, high body mass index, hypertension, lipid metabolism disorders, and diabetes mellitus, among several other factors (2). Emerging evidence has demonstrated that genetic and environmental factors and polymorphisms also play a crucial role in the occurrence and development of CVD (3, 4). The advancement in single-nucleotide polymorphism (SNP) and genome-wide sequencing technologies has led to an increased number of in-depth studies on the genetics of CVD, and a number of candidate genes have been identified, such as those involved in the regulation of lipid metabolism (5), inflammatory cytokines (6), and the renin–angiotensin–aldosterone system (RAAS) (7).

The RAAS plays a critical role in the pathogenesis of coronary heart disease, and previous studies have determined that it is involved in the progression of hypertension and vascular and left ventricular remodeling (8). Much accumulated evidence has indicated that the RAAS is significantly associated with the initiation and progression of coronary atherosclerosis and thrombogenesis (9). In addition, studies involving angiotensin-converting enzyme (ACE) inhibition and angiotensin II receptor blockade have highlighted the vital role of the RAAS, and gene polymorphism of the RAAS may also affect the efficacy of drug (10). Recently, several genetic variants in the RAAS have been found to be significantly associated with
CVD risk, such as an insertion/deletion polymorphism in the ACE gene, and T175M and M235T polymorphisms in the angiotensinogen (AGT) gene (11-13). AGT is a crucial determinant of angiotensin II levels, which is an important component of the RAAS. Furthermore, polymorphism in the AGT gene may contribute to atherogenesis in the coronary artery and may be related to the development of CVD (14, 15). The M235T polymorphism has been most widely studied; however, several inconsistent results regarding this polymorphism and CVD risk have been reported. Raygan et al. (16), Bonfim-Silva et al. (17), and Isorda-Salas et al. (18) detected positive correlations, whereas Renner et al. (19), Ranjith et al. (20), and Erbas et al. (21) determined that the AGT M235T polymorphism has no significant effect on the development of CVD. Meta-analyses have been performed to resolve these discrepancies; however, these analyses have been compromised by deficiencies in the sample size, and the results have been either inconclusive or only weakly significant (16). Some of the studies have been limited to Asian populations (22, 23), and several of the most recent studies have not been considered. The aim of the present study was to compile case–control research and updated meta-analyses to explore the association between AGT M235T polymorphism and susceptibility for CVD in a range of populations for more accurate assessment.

Methods

Search strategy
A systematic search of MEDLINE, Embase, China National Knowledge Infrastructure, OVID, ScienceDirect, and WanFang databases was performed to identify epidemiological studies on M235T polymorphisms of the AGT gene and CVD that were published up to October 2018. In the literature searches, various combinations of the keywords “angiotensinogen gene,” “AGT,” “M235T gene,” “genetic polymorphism,” “variants,” or “variations,” “coronary heart disease,” “coronary artery disease,” “cardiovascular disease,” “myocardial infarction,” “ischemic heart disease,” and “coronary stenosis” were used. Only studies published in English or Chinese were included in the study. The references of all full text papers were examined to identify additional relevant studies. Secondary searches of gray literature were not performed. All retrieved articles were organized using reference manager software (Endnote 6).

Inclusion and exclusion criteria
Inclusion criteria were the following: (1) the study evaluated AGT M235T and CVD risk, (2) original research (case–control studies) or AGT M235T genotype frequencies were provided by case–control status, (3) the study had sufficient data to allow the association between AGT M235T and CVD risk, (4) the study included original data, independent of other studies, and (5) the language of the report was in English or Chinese. Exclusion criteria were the following: (1) overlapping data, (2) missing information (particularly genotype distributions and studies without controls), after having not received the requested information from the corresponding author, and (3) genome scans investigating linkages without detailed genotype frequencies between cases and controls. Two reviewers independently screened the titles and abstracts for eligibility criteria. Thereafter, the reviewers read the full text of the studies that potentially met the inclusion criteria, and the literature was reviewed to determine the final inclusion of data. For each study, the following information was recorded: first author, year of publication, geographical area, ethnicity, number of cases and controls, genotypes for cases and controls, and evidence of Hardy–Weinberg equilibrium in the controls. If the two reviewers disagreed regarding the inclusions of a study, a consensus was reached through additional review and discussion.

Data extraction
The two reviewers extracted data from each study independently, and any discrepancies were resolved. The information extracted from each article in Tables 1 and 2, including first author, year of publication, country of origin, ethnicity of patients, numbers of cases and controls, AGT genotypes, allele distribution of cases and controls, and outcome, was summarized.

Statistical analysis
Data analysis was conducted using STATA 12.0 software (StataCorp, College Station, TX, USA). The association between AGT M235T polymorphism and CVD susceptibility was assessed in the following genetic models: T versus M (allelic), TT versus MM (co-dominant), MT versus MM (co-dominant), MT+TT versus MM (dominant), and MT+MM versus TT (recessive). Inter-study heterogeneity was tested using Q-statistics. The Mantel–Haenszel method for fixed effects and the DerSimonian and Laird method for random effects were used to estimate pooled effects (24). The fixed effects method was used if the result of the Q test was not significant. Otherwise, the pooled odds ratio (OR) and 95% confidence interval (CI), assuming a random effects model, were calculated. Fixed effects assume that genetic factors have similar effects on CVD susceptibility across all studies, and that the observed variations among studies are caused by chance alone (25). The random effects model assumes that different studies may have substantial diversity and assesses both intra- and inter-study variations (26). A recently developed measure, I², was used to quantify the inconsistency among the studies’ results for values of ≥50%, with large heterogeneity among values of ≥75% (27). Data are expressed as OR with 95% CI and two-tailed p-values. A p-value <0.05 was considered statistically significant. Assessment of publication bias was conducted both visually by using a funnel plot and statistically via Begg’s funnel plots.
and Egger’s bias test, which measures the degree of funnel plot asymmetry (28, 29). The Begg’s adjusted rank correlation test was used to assess the correlation between test accuracy estimates and their variances. The deviation of Spearman’s rho values from zero provides an estimate of funnel plot asymmetry, where positive values indicate a trend toward higher levels of test accuracy in studies with smaller sample sizes. The Egger’s bias test detects funnel plot asymmetry by determining whether the intercept deviates significantly from zero in a regression of the standardized effect estimates against their precision. Meta-regression analysis was applied to evaluate the heterogeneity of the studies.

Results

Search results and characteristics of included studies

Initially, 427 potentially relevant articles were obtained; however, after screening the abstracts, most were determined to be irrelevant to our analysis. Of the remaining 51 articles, 13 articles were removed because of an insufficient number of cases or unusable data. Eventually, 23 studies in English (12-21, 30-42) and 15 in Chinese (43-57), including 39 comparisons of the AGT M235T polymorphism that all adopted the observational study design, satisfied the eligibility criteria (Fig. 1). A total of 39 comparisons from the 38 studies of the AGT M235T polymorphism were included in this updated meta-analysis.

The relevant studies included 9225 cases and 8406 controls (Tables 1 and 2). Reference to the “overall population” indicates meta-analysis without ethnic subdivisions. Ethnicity-specific meta-analysis was categorized by Caucasian, East Asian, and other races (miscellaneous subgroup).

Association of the AGT M235T polymorphism with CVD risk in the overall population

As shown in Figure 2, significant heterogeneity among studies was observed for the overall population ($P<0.10$ or $I^2\geq50\%$). Using the random effect models, M235T was found to be associated with an increased risk of CVD in the allelic (T vs. M: OR=1.16, 95% CI=1.05–1.27, $p<0.001$) and recessive (TT vs. MT+MM: OR=1.14, 95% CI=1.06–1.23, $p<0.001$) models.

Association of the M235T polymorphism of the AGT gene with CVD risk in subgroups analysis

When analyses were subdivided according to ethnicity, no associations were noted for Caucasians using any of the five genetic models. However, for the East Asian subgroup, M235T was significantly associated with CVD risk in allelic (T vs. M: OR=1.46, 95% CI=1.13–1.90, $p<0.001$), homozygous (TT vs. MM: OR=1.78, 95% CI=1.18–2.67, $p=0.01$), dominant (MT+TT vs. MM: OR=1.47, 95% CI=1.05–2.04, $p=0.02$), and recessive (TT vs. MT+MM: OR=1.68, 95% CI=1.25–2.27, $p<0.001$) models. In miscellaneous populations, a significant association between M235T and CVD risk was observed in the allelic model (T vs. M: OR=1.21, 95% CI=1.07–1.36, $p<0.001$), but no association was observed in the other four genetic models. In subgroup analysis, neither moderate nor large heterogeneity was observed among Caucasians, but true heterogeneity was noted among East Asians (T vs. M: $P_h<0.10$, $I^2=83\%$ and TT vs. MT+MM: $P_h<0.10$, $I^2=81\%$) and miscellaneous populations (MT vs. MM: $P_h=0.05$, $I^2=51.1\%$) (Table 3).

Publication bias and sensitivity analysis

Publication bias was not detected in the analyses of the homozygote, heterozygote, or dominant models ($p>0.05$, for all). However, publication bias was noted in the analyses of the associations between M235T polymorphisms and CVD risk (allelic model: $P_{Begg}=0.01$, $P_{Egger}=0.02$ and recessive model: $P_{Egger}=0.01$) (Table 4). Sensitivity analyses showed that the present meta-analysis was relatively stable and credible (Fig. 3).

Meta-regression

A meta-regression analysis for several potential sources of heterogeneity, including published year, sample size, age, gender, outcome, and ethnic background, was performed. Single covariates were added in the allelic, homozygote, dominant, and recessive models. The results suggest that the East Asian population (allelic model: $p=0.006$, homozygote model: $p=0.010$, dominant model: $p=0.022$, and recessive model: $p=0.005$) and study size (homozygote model: $p=0.042$ and recessive model: $p=0.010$) contributed to the observed heterogeneity across all studies of the association between AGT M235T polymorphisms and CVD susceptibility.
| Study            | Year | ID | Country | Ethnic  | Sample size | Enthic       | Country         | Sample size | Genotypes and allele distribution |
|------------------|------|----|---------|---------|-------------|--------------|-----------------|-------------|-----------------------------------|
| Tiret et al.     | 1995 | 1  | France  | Caucasian | 630         | 741           | 229             | 301         | 100 759 501 258 372 111 888 594 |
| Kamitani et al.  | 1995 | 2  | Japan   | East Asian | 103         | 103           | 6               | 31          | 66 43 163 10 41 52 61 145       |
| Ko et al.        | 1997 | 3  | China   | East Asian | 150         | 338           | 4               | 22          | 124 30 270 4 54 279 62 612      |
| Chen et al.      | 1998 | 4  | China   | East Asian | 57          | 76            | 4               | 13          | 40 21 93 13 31 32 57 95        |
| Sheu et al.      | 1998 | 5  | China   | East Asian | 102         | 145           | 1               | 26          | 75 28 176 1            | 37 107 39 251 |
| Pastinen et al.  | 1998 | 6  | Finland | Caucasian | 122         | 122           | 48              | 66          | 37 162 140 53 64 34 170 132    |
| Frossard et al.  | 1998 | 7  | UAE     | Caucasian | 40          | 61            | 14              | 18          | 8 46 34 16 26 19 58 64        |
| Gardemann et al. | 1999 | 8  | Germany | Caucasian | 1058        | 511           | 319             | 582         | 157 1220 896 385 585 222 1355 1029 |
| Winkelmann et al.| 1999 | 9  | Germany | Caucasian | 122         | 92            | 38              | 54          | 30 130 114 28 53 11 109 75     |
| Batalla et al.   | 2000 | 10 | Spain   | Caucasian | 220         | 200           | 69              | 99          | 52 237 203 64 96 40 224 176     |
| Fomicheva et al.| 2000 | 11 | Russia  | Caucasian | 198         | 152           | 63              | 85          | 50 211 185 43 75 34 161 143     |
| Olivier et al.   | 2001 | 12 | Italy   | Caucasian | 247         | 245           | 63              | 124         | 60 250 244 54 76 27 184 130     |
| Xie et al.       | 2001 | 13 | China   | East Asian | 106         | 86            | 8               | 29          | 69 45 167 11 30 45 52 120      |
| Fernández-Arcas et al. | 2001 | 14 | Spain   | Caucasian | 212         | 180           | 59              | 121         | 32 239 185 34 97 49 165 195     |
| Ermis et al.     | 2002 | 15 | Turkey  | Miscellaneous | 102        | 114           | 32              | 48          | 22 112 92 39 59 16 137 91      |
| Hooper et al.    | 2002 | 16 | USA     | Miscellaneous | 110        | 185           | 4               | 29          | 67 37 163 2 31 67 35 165       |
| Zhu et al.       | 2002 | 17 | China   | East Asian | 41          | 116           | 2               | 7           | 32 11 71 18 47 51 83 149       |
| Zhu et al.       | 2002 | 18 | China   | East Asian | 118         | 106           | 14              | 48          | 56 76 160 10 42 54 62 150       |
| Bis et al.       | 2003 | 19 | USA     | Caucasian | 208         | 717           | 71              | 98          | 39 240 176 215 348 153 779 655  |
| Gu et al.        | 2003 | 20 | China   | East Asian | 129         | 90            | 12              | 31          | 86 55 203 7 30 53 44 136       |
| Ranjith et al.   | 2004 | 21 | India   | Miscellaneous | 195        | 300           | 24              | 80          | 91 128 262 29 127 144 185 415  |
| Zhu et al.       | 2004 | 22 | China   | East Asian | 192         | 98            | 12              | 75          | 105 99 285 8 36 54 52 144      |
| Li et al.        | 2004 | 23 | China   | East Asian | 120         | 80            | 11              | 60          | 49 82 158 14 41 25 69 91       |
| Tobin et al.     | 2004 | 24 | England | Caucasian | 547         | 505           | 212             | 252         | 83 676 418 197 226 82 620 390   |
| Ren et al.       | 2005 | 25 | China   | East Asian | 100         | 70            | 2               | 10          | 35 14 80 13 26 31 52 88        |
| Araujo et al.    | 2005 | 26 | Brazil  | Caucasian | 110         | 104           | 46              | 52          | 12 144 76 43 51 10 137 71       |
| Renner et al.    | 2005 | 27 | Austria | Caucasian | 1370        | 733           | NA              | NA          | 1537 1203 NA NA NA 832 634      |
| Liang et al.     | 2006 | 28 | China   | East Asian | 133         | 154           | 2               | 30          | 101 34 232 10 60 84 80 228     |
| Tsai et al.      | 2007 | 29 | China   | East Asian | 735         | 519           | 15              | 195         | 525 225 1245 5 111 403 121 917  |
| Niu et al.       | 2008 | 30 | China   | East Asian | 105         | 110           | 8               | 32          | 65 48 162 9 47 54 65 155       |
| Zhu et al.       | 2010 | 31 | China   | East Asian | 151         | 127           | 9               | 27          | 115 45 257 20 51 56 91 163     |
| Peng et al.      | 2011 | 32 | China   | East Asian | 196         | 200           | 14              | 54          | 128 82 155 18 86 96 122 278    |
| Konopka et al.   | 2011 | 33 | Poland  | Caucasian | 100         | 95            | 30              | 46          | 24 106 94 22 44 29 88 102      |
| Mehri et al.     | 2011 | 34 | Tunisia | Miscellaneous | 123        | 144           | 29              | 53          | 41 111 135 53 61 30 167 121     |
| Raygan et al.    | 2016 | 35 | Iran    | Miscellaneous | 155        | 185           | 42              | 79          | 34 163 147 71 85 29 227 143     |
| Bonfim-Silva et al.| 2016 | 36 | Brazil  | Miscellaneous | 153        | 113           | 23              | 69          | 61 115 191 13 63 37 89 137      |
| Erbas et al.     | 2017 | 37 | Turkey  | Miscellaneous | 117        | 106           | 11              | 104         | 2 126 108 16 85 5 117 95       |
| Isordia-Salas et al. | 2018 | 38 | Mexico  | Miscellaneous | 242        | 242           | 138             | 98          | 6 374 110 170 62 10 402 82      |

NA - not available
Figure 2. Forest plots of the association between AGT M235T polymorphism and CVD risk. (a) Allelic model, (b) homozygote model, (c) heterozygote model, (d) dominant model, and (e) recessive model.
Discussion

The AGT gene (on chromosome 1q42–43) comprises five exons and four introns spanning 12 kb, with the M235T polymorphism in exon 2. The M235T variant has been demonstrated to alter plasma AGT levels (58, 59), with elevated levels of serum AGT for patients carrying the T allele (60). Furthermore, a positive correlation exists between AGT M235T genotype and cardiovascular disease.

Table 2. Baseline characteristics of the included studies

| Study                 | Ethnic | Outcome | Genotyping-methods | Age                  | Gender (M/F) | HWE |
|-----------------------|--------|---------|---------------------|----------------------|--------------|-----|
| Tiret et al.          | C      | MI      | PCR                 | 53.0±0.3             | 630/0        | 741/0 | Y  |
| Kamitani et al.       | EA     | MI      | PCR                 | 52±1                 | 103/0        | 103/0 | Y  |
| Ko et al.             | EA     | MI      | PCR                 | 61.5±0.6             | NR           | 181/157 | Y |
| Chen et al.           | EA     | MI      | PCR                 | 67.7±8.5             | 50/7         | 69/7  | Y  |
| Sheu et al.           | EA     | CAD    | PCR                 | NR                   | 102/0        | 145/0 | Y  |
| Pastinen et al.       | C      | MI      | PCR                 | 57.7±4.9             | 122/0        | 122/0 | Y  |
| Frossard et al.       | C      | MI      | PCR                 | 55.0±11.3            | 25/15        | 31/30 | Y  |
| Gardemann et al.      | C      | MI      | PCR                 | 62.2±9.5             | 1058/0       | 511/0 | Y  |
| Winkelmann et al.     | C      | MI      | PCR                 | 55.7±9.6             | 122/0        | 92/0  | Y  |
| Batalla et al.        | C      | MI      | PCR                 | 43±5                 | 220/0        | 200/0 | Y  |
| Fomicheva et al.      | C      | MI      | PCR                 | 67 (55-85)           | 11 (6-17)    | 198/0 | 152/0 | Y |
| Olivieri et al.       | C      | MI      | PCR                 | 57.7±12.8            | 160/85       | 221/26 | Y|
| Xie et al.            | EA     | CAD    | PCR                 | 61.4±9.5             | 82/24        | 54/32 | Y  |
| Fernández-Arcás et al.| C      | MI      | PCR                 | 54±13                | 212/0        | 180/0 | Y  |
| Ermis et al.          | M      | MI      | PCR                 | 42.1±11.8            | NR           | NR    | Y  |
| Hooper et al.         | M      | MI      | PCR                 | NR                   | NR           | NR    | Y  |
| Zhu et al.            | EA     | MI      | PCR                 | 59.6±10.4            | 27/14        | 67/49 | Y  |
| Zhu et al.            | EA     | CAD    | PCR                 | NR                   | NR           | NR    | Y  |
| Bis et al.            | C      | MI      | PCR                 | 63.6                 | 128/80       | 371/346 | Y |
| Gu et al.             | EA     | CAD    | PCR                 | 65.8±9.2             | 81/48        | 54/36 | Y  |
| Ranjith et al.        | M      | MI      | PCR                 | 18-45                | NR           | NR    | Y  |
| Zhu et al.            | EA     | CAD    | PCR                 | NR                   | NR           | NR    | Y  |
| Li et al.             | EA     | CAD    | PCR                 | 61.5±11.8            | 80/40        | 47/33 | Y  |
| Tobin et al.          | C      | MI      | PCR                 | 61.9±9.2             | 372/175      | 313/192 | Y |
| Ren et al.            | EA     | CAD    | PCR                 | 60.0±9.8             | 71/29        | 38/32 | Y  |
| Arauji et al.         | C      | MI      | PCR                 | >18                  | 73/37        | 44/60 | Y  |
| Renner et al.         | C      | MI      | PCR                 | 63.1±10.4            | 1081/289     | 378/355 | Y |
| Liang et al.          | EA     | CAD    | PCR                 | 64±8                 | 100/33       | 116/38 | Y |
| Tsai et al.           | EA     | CAD    | PCR                 | 63.8±11.4            | 531/204      | 269/250 | Y |
| Niu et al.            | EA     | CAD    | PCR                 | 59±7                 | 69/36        | 71/39 | Y  |
| Zhu et al.            | EA     | CAD    | PCR                 | 59.7±11.3            | 96/55        | 71/56 | Y  |
| Peng et al.           | EA     | CAD    | PCR                 | 70.0±8.3             | 128/68       | 132/68 | Y  |
| Konopka et al.        | C      | MI      | PCR                 | 57±10                | 79/21        | 76/19 | Y  |
| Mehr et al.           | M      | MI      | PCR                 | 62.3±11.8            | 71/52        | 83/61 | Y  |
| Raygan et al.         | M      | MI      | PCR                 | 62.4±3.2             | 102/53       | 127/58 | Y |
| Bonfim-Silva et al.   | M      | CAD    | PCR                 | 55.7±7.9             | 99/54        | 49/64 | Y  |
| Erbas et al.          | M      | CAD    | PCR                 | 50.2±12.3            | 55/62        | 14/92 | Y  |
| Isordia-Salas et al.  | M      | MI      | PCR                 | 41.0±5.3             | 191/51       | 192/50 | Y |

C - Caucasian; EA - East Asian; M - Miscellaneous; MI - myocardial infarction; CAD - coronary artery disease; PCR - polymerase chain reaction; NR - no reported; HWE - Hardy-Weinberg equilibrium; Y - yes
plasma AGT levels in survivors of myocardial infarction (MI) (14). Elevation circulating AGT levels are associated with an increase in the concentration of angiotensin II, which activates cardio-myocyte hypertrophy and fibroblast proliferation by stimulation of the AT1 receptor (61, 62). In addition, angiotensin II stimulates vascular apoptosis and may promote the retention of low-density lipoprotein in the coronary arteries, oxidize, and be assimilated by phagocytes, ultimately contributing to the dysfunction of the vascular endothelium, myocardial ischemia and rupture of atherosclerotic plaque (63-65). These processes all play critical roles in promoting the pathological development of CVD.

In previous studies, the distribution of the AGT M235T variant has been shown to differ significantly among various populations. Katsuya et al. (66) demonstrated that homozygous AGT T235 is an independent risk factor, which carries a two-fold increased risk of CVD. In contrast, Tiret et al. (31) suggested that the AGT genetic polymorphism can lead to predisposition to hypertension, with no relationship to CVD. Renjith et al. (20) and Renner et al. (19) reported similar results showing that AGT genotypes are neither related to CVD nor high blood pressure. Batalla et al. (12) and Bonfim-Silva et al. (17) provided evidence of the synergistic effect between AGT polymorphism and CVD, suggesting that M235T polymorphism is significantly correlated with MI and hypertension. Recently, Raygan et al. (16) reported similar results showing that AGT genotypes are neither related to CVD nor high blood pressure.

Given the disparity of the results, we sought to provide an updated meta-analysis to resolve the discrepancies among studies. Our results clearly demonstrate a difference in the association of M235T polymorphism among Asians and Caucasians, suggesting that there is heterogeneity based on ethnicity. Our results also suggest that the T allele is a genetic risk factor for CVD in Asians, as well as in the miscellaneous population. Previous meta-analyses based on Chinese populations have shown similar positive results (22, 23). Although two recent meta-analyses (16, 67) reported an association between AGT M235T polymorphism and risk of cardiovascular disease, they did not include recently published studies, and the literature sample size was smaller than the current analysis. Thus, an update of previous meta-analyses was warranted. Our results include the largest sample size to date, and we sought to provide an updated meta-analysis.
Figure 3. Sensitivity analyses of the association between AGT M235T polymorphism and CVD risk. (a) Allelic model, (b) homozygote model, (c) heterozygote model, (d) dominant model, and (e) recessive model
ple size to date and provide an ethnicity-based explanation for different results among studies. The association of AGT M235T with stroke was not addressed in the present study but would be an important topic for consideration in future studies to maintain a relatively narrow focus.

The following potential factors may account for differences observed among the various ethnic groups: (1) population diversity (68), (2) different habits among populations (69), and (3) environmental factors leading to differences in susceptibility to CVD (70). Furthermore, we speculate that the non-significant association among Caucasians may be due to the relatively low frequency of the TT genotype in this population. Moreover, owing to the limited number of these studies, the miscellaneous ethnic subgroups were not analyzed further. Therefore, more studies with a larger sample size may reveal factors that influence differences in the association of AGT M235T and CVD, especially among Caucasians and other ethnic populations. The association between AGT M235T and CVD among Asians is evident.

The heterogeneity of associations across all included studies should be noted as it may potentially affect the strength of the present study. Thus, the random effects model was used, and our analysis was based on different ethnic subgroups. Ethnic background and sample size were found to be factors of heterogeneity. However, heterogeneity was still high within the East Asian subgroup. The heterogeneity of results among those included in these studies may be explained by the quality of the included studies, classification of CVD, and sampling criteria. The heterogeneity of genetic effects among individual studies may also be caused by the existence of genetic and environmental or genetic interactions.

**Study limitations**

The primary limitations of our meta-analysis include: (1) significant publication bias in the allelic and recessive models, (2) insufficient genotyping data of AGT M235T in miscellaneous races, which limited the ability to draw conclusions regarding this population, and (3) potential heterogeneity of clinical variables, such as the general condition of subjects, their medical history, medication compliance, complications of CVD, and other factors.

**Conclusion**

The genetic polymorphism of AGT M235T is associated with a critical risk of CVD in East Asian populations, with no detectable association in Caucasian populations. However, further studies with multiple ethnicities and rigorous designs should be performed to confirm these conclusions.

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**Ethical approval:** Ethical approval was not required for this study design.

**Conflict of interest:** None declared.

**Peer-review:** Externally peer-reviewed.

**Authorship contributions:** Concept – H.C.; Design – C.Z., H.C.; Supervision – C.Z., H.C.; Fundings – H.C.; Materials – C.Z., H.Z., K.H.; Data collection &/or processing – C.Z., H.Z., Y.Z., Y.Y.Z.; Analysis &/or interpretation – C.Z., H.Z., K.H.; Literature search – K.H., Y.Z.; Writing – C.Z., Y.Y.Z.; Critical review – Y.Z., Y.Y.Z.

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