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COMBINING GEOGRAPHIC REGION WITH META-ANALYSIS TO MAP THE POTENTIAL ASSOCIATION BETWEEN THREE GENETIC POLYMORPHISMS AND CORONARY ARTERY DISEASE

KOMBINOVANJE GEOGRAFSKOG PODRUČJA S METAANALIZOM RADI MAPIRANJA POTENCIJALNE POVEZANOSTI IZMEĐU TRI GENETSKA POLIMORFIZMA I KORONARNE ARTERIJSKE BOLESTI

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Summary

Background: Coronary artery disease (CAD) is a complex trait influenced by genetic and environmental factors. Geographic isolation and natural selection present fundamental forces to diversify genetic backgrounds during human evolution and migration. In this study, we attempted to assess whether human geographic isolation affects the genetic predisposition of CAD.

Methods: We first included 21 genetic association studies of the methylenetetrahydrofolate reductase (MTHFR) gene polymorphism C677T and CAD from 16 geographic regions consisting of 9,008 participants and performed a meta-analysis based on the distributions of these studies.

Results: It was found that the positive signals for the association of C677T with CAD were mainly enriched in the regions of northern Africa (pooled OR=1.73, 95% CI=1.45–2.06, Z=3.17, P<0.0001) and India (pooled OR=1.61, 95% CI=1.30– 2.00, Z=4.38, P<0.0001). To validate the potential geographic effects on the genetic polymorphism of CAD, we then carried out two additional meta-analyses involving 30 and 13 studies on genetic associations of the angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) and APOA5 gene T1131C polymorphisms for CAD consisting of 22,190 and 12,322 participants, respectively. We found that the associations of T1131C with CAD were concentrated in East Asia (pooled OR=1.35, 95% CI=1.22–1.49, Z=6.00, P<10–5), whereas the associations of I/D polymorphism with CAD were clustering in Europe and America (pooled OR=1.20, 95%

Kratak sadržaj

Uvod: Koronarna arterijska bolest (KAB) kompleksno je oboljenje na koje utiču genetski faktori kao i faktori sredine. Geografska izolacija, zajedno s prirodnom selekcijom, ima najveći uticaj na diverzifikaciju genetskog porekla tokom ljudske evolucije i migracije. Mi smo u ovoj studiji pokušali da utvrdimo da li geografska izolacija ljudi utiče na genetsku predispoziciju za KAB.

Metode: Najpre smo uključili 21 studiju o genetskoj povezanosti genskog polimorfizma C677T metilenetetrahidrofolat-reduktaze (MTHFR) sa KAB, sa 16 geografskih područja, kojima je obuhvaćeno 9.008 učesnika i sproveli metaanalizu na osnovu distribucija u ovim studijama.

Rezultati: Utvrđeno je da su pozitivni signali za povezanost C677T sa KAB bili većinom koncentrisani u područjima severne Afrike (pooled OR=1,73, 95% CI=1,45–2,06, Z=3,17, P<0,0001) i Indije (pooled OR=1,61, 95% CI=1,30–2,00, Z=4,38, P<0,0001). U cilju validacije potencijalnog geografskog uticaja na genetski polimorfizam KAB, mi smo potom sproveli dve dodatne metaanalize koje su obuhvatile 30, odnosno 13 studija o genetskoj povezanosti između genskog prisustva/ odsustva (I/D) angiotenzin-konvertujućeg enzima (ACE) i polimorfizama T1131C gena APOA5 za KAB, koje su obuhvatile 22.190, odnosno 12.322 učesnika. Otkrili smo da je povezanost T1131C sa KAB bila koncentrisana u istočnoj Aziji (pooled OR=1,35, 95% CI=1,22–1,49, Z=6,00, P<10–5), dok je povezanost polimorfizma I/D sa KAB pokazala klastere u Evropi i Americi (pooled OR=1,20, 95% CI=1,04–1,39, Z=2,49, P=0,01)

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CI=1.04–1.39, Z=2.49, P=0.01) and Turkey (pooled OR=1.33, 95% CI=1.05–1.69, Z=2.40, P=0.02).
Conclusions: Our results showed that geographic isolation might have potential effects on the genetic polymorphism of human complex diseases, such as CAD.

Keywords: genetic predisposition, geographic isolation, coronary artery disease, meta-analysis

i Turskoj (pooled OR=1,33, 95% CI=1,05–1,69, Z=2,40, P=0,02).
Zaključak: Naši rezultati ukazuju na to da geografska izolacija potencijalno može uticati na genetski polimorfizam složenih bolesti kod ljudi, kakva je KAB.

Ključne reči: genetska predispozicija, geografska izolacija, koronarna arterijska bolest, metaanaliza

Introduction

Human complex diseases, such as hypertension, coronary artery disease (CAD), diabetes and cancers, are thought to be diseases in which a combination of risk alleles from different susceptibility genes predisposes to the development of the disease, following exposure to as yet unknown environmental factors. However, the direct correlation between some gene polymorphisms and the diseases remains a controversy among various human ancestries reported. Some previous studies approved the importance of human genetic variation in complex disease which can cause alleles to occur at a greater frequency in people from specific geographic regions (1, 2). Geographic isolation and natural selection can be considered as two reasons for the genetic variation existing between populations in different geographic regions. Alleles under natural selection are likely to occur only in those geographic regions where they confer an adaptive advantage.

The recent theory that humans migrated out of Africa supports the idea that the increasing possibility of genetic drift may have had an important influence in the neutrality differences of mutations between populations in different geographic regions. Some genetic analyses results have shown that human genetic variation is geographically structured, in accord with historical patterns of gene flow and genetic drift (3). For example, Rosenberg et al. (4) reported that genetic distance increased in a linear manner as geographic distance increased, consistent with population structure. They approved their earlier results: if enough markers are used with a sufficiently large worldwide sample, individuals can be partitioned into genetic clusters that match major geographic subdivisions of the globe, with some individuals from intermediate geographic locations having mixed membership in the clusters that correspond to neighboring regions. Furthermore, it has also been reported that genetic variation comes from mutations in genetic material, migration between populations, and the reshuffling of genes through sexual reproduction. Long et al. (5) have argued that if variations in many genes between populations are investigated simultaneously, they often correspond to population migrations due to, for example, new sources of food, improved transportation, or shifts in political power. Ongoing genetic research has investigated how ancestral human populations migrated in the ancestral geographic environment into different geographic regions. Also, some published studies found that approximately 25% of genes showed different levels of gene expression between populations of European and Asian descent. This difference in gene expression was considered to be caused by SNPs in gene regulatory regions of DNA (6). Currently, many scientists believe that the genetic diversity variation is mirrored by the variation in phenotype with migratory distance, which can explain the discrepant reports of genetic association between gene polymorphisms and disease risk. The controversies focus on how to interpret the genetic data and whether conclusions based on it are reasonable. Some researchers argue that self-identified race can be used as an indicator of geographic ancestry for certain disease risk. Therefore, it is possible to analyze the potential genetic association differences among populations involved in different geographic regions by genetic analysis.

As an exploration, we used three coronary artery disease (CAD) related gene polymorphisms reported frequently in recent years, methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphism (C677T), the insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (*ACE*) gene and *APOA5* gene polymorphism (T1131C) to detect their association with CAD in different geographic regions using stratified meta-analysis. In the past years, although there were some meta-analyses to explore the association of *MTHFR* C677T, *ACE* I/D and *APOA5* T1131C with the susceptibility of CAD, only a few original investigations were conducted considering the geographic and racial difference which are potentially important factors to affect the association. Therefore, in this paper, we combined human migration routes and geographic regions with a meta-analysis of available case-control studies to assess whether the combined evidence showed an association between these gene polymorphisms and the risk of CAD for populations involved in different geographic regions. The results showed that the association displayed obvious varieties in populations of different geographic regions, suggesting that the geographic isolation and natural selection during human expansion may influence the genetic contribution to CAD. Our analysis provided the assumption that the migration and geographic isolation might affect genetic predisposition of human complex diseases, such as CAD.

Materials and Methods

Study selection

In this analysis, we obtained literature evidence using Gene Prospector (http://www.hugenavigator.net/HuGENavigator/home.do) which provides an online gateway for searching the evidence about human genes in relation to disease. In addition, we also performed a systematic literature search in PubMed and EMBASE database, using terms as follows: »gene«, »polymorphism«, »coronary artery disease«, »genetic variant«, »heart failure«, »coronary heart disease«, »association«, »MTHFR C677T«, »ACE I/D« and »APOA5 T1131C«. No restrictions were placed on language, race, ethnicity or geographic region. In the present study, we considered the published time of the selected literature when performing the search strategy. On one hand, recent works are usually more reliable than initial studies due to improved techniques and avoidance of earlier errors. To ensure the stability of studies, we selected literature published after December 2000 for *MTHFR* C677T and *APOA5* T1131C. For *ACE* I/D, considering a lot of the relevant literature was published before 2000 and a sufficient number of participants (cases and controls) must be guaranteed to enter the meta-analysis, we therefore extended the selected literature to published after 1995. On the other hand, our analysis also included new studies up to 2011, as this will help to expand the sample size and improve the statistical test power. Eligible studies included in this meta-analysis had to fulfill the following rules: (i) association studies using an unrelated case-control design, (ii) cases were coronary artery disease with the diagnosis based on clinical criteria, (iii) enough data were provided to calculate Odd Ratio (OR) values.

Selection and quality assessment

In this process, we excluded the studies that contained overlapping data, and two authors independently assessed trial eligibility and quality, and they reached a consensus on all items. For each study, that met our criteria, the following information was collected: first author, year of publication, ethnicity, population, number of genotypes in cases and controls respectively.

Statistical analysis

Considering population stratification, genetic heterogeneity and demographic history, we divided the populations to different groups according to their geographic regions and conducted a stratified meta-analysis of genetic association studies to assess whether *MTHFR* C677T, *ACE* I/D and *APOA5* T1131C are associated with CAD in combination with geographic regions and migration routes. In this paper, we applied RevMan (http://ims.cochrane.org/revman) and R software (http: //www.r-object.org) to perform the meta-analysis. Consider a meta-analysis including different case-control studies, not necessarily typed using the same genotyping product or imputed to the same reference panel; we therefore assumed that studies have been filtered for appropriate quality control metrics to exclude poorly genotyped or imputed SNPs. We tested for heterogeneity with the Cochrane Q test and measured inconsistency with I^2 which is the percentage of total variance across studies that is due to heterogeneity rather than chance. The I^2 is ranged from 0 to 100% (I^2=0–25%, no heterogeneity; I^2 = 25–50%, moderate heterogeneity; I^2 = 50–75%, large heterogeneity; I^2 = 75–100%, extreme heterogeneity) (7). According to heterogeneity, we selected a random effect model or a fix effect model to perform the meta-analysis. Consider the minor allele of C677T polymorphism of *MTHFR* gene increased the susceptibility to CAD; we therefore mainly investigated the association between this genetic variant and CAD risk of different regions in an allelic model (T vs C). For the *ACE* I/D polymorphism, because a number of studies have suggested a high prevalence of the DD genotype in patients with CAD (8), we performed a stratified meta-analysis under a genetic model (DD vs DI+II). Taking into account the universality of the dominant model for *APOA5*, a stratified meta-analysis under a genetic model (CC+CT vs TT) was performed. The results for other models will be described simply in the Discussion section. The significance of the pooled OR was determined by the Z-test ($P<0.05$ suggests a significant association).

Results

Stratified meta-analysis for MTHFR C677T

To ensure the stability of studies, we selected literature published after December 2000. As a result, 56 relevant references with *MTHFR* polymorphism and CAD were retained by applying the Gene Prospector tool and PubMed or EMBASE search. All studies were published in English. Full text articles were then retrieved for assessment in detail. Because we only limited our studies to the *MTHFR* polymorphism C677T, 29 studies were excluded for other *MTHFR* polymorphisms. For consistency, we included studies of only case-control design. Finally, 21 studies from 16 geographic regions consisting of 9,008 participants met the inclusion criteria for this meta-analysis (9–29) (see Supplementary *Table I*). We found that C677T is overall significantly associated with CAD in an allelic model (pooled OR=1.33, 95% CI=1.13–1.57, Z=3.42, P=0.0006). A cumulative meta-analysis showed a trend of association as information accumulated. The stability in the relative changes in OR values indicates that there is enough evidence to draw a stable conclusion about the risk effect of the C677T MTHFR polymorphism variant in CAD (see Supplementary *Table II*).

To clarify the discrepancy caused by migration history and geographic regions, we performed a stratified meta-analysis of available case-control studies to assess whether C677T is associated with CAD in combination with geographic regions and migration routes. We divided all the literature into three main groups according to the geographic regions of populations, which are as follows: Europe (Germany, Corsica, Poland and Silesia) and America (Brazil and America) group, Asia group including India, East Asia (China, China Tai Wan and Korea) and Turkey, and North Africa group (Tunisia, Morocco and Egypt). A Kruskal-Wallis test approved that there was no significant difference for minor allele frequency (MAF) in the three main groups (MAF for case: $P=0.420$; MAF for control: $P=0.174$; MAF for total: $P=0.204$). The stratified meta-analysis under the allelic model showed that there was no association between C677T and CAD for Europe and America group (pooled OR=1.05, 95% CI=0.95–1.17, Z=1.00, P=0.32), and this is supported by Lewis et al. study (30), in which they found no significant association between this *MTHFR* polymorphism and CAD in the European, North American and Australian group. Although association existed in the Asia group (Z=2.17, P=0.03), the high genetic heterogeneity could not assure its rationality. We therefore further divided the Asia group into India (heterogeneity test: $p=0.43$), East Asia (China, China Tai Wan and Korea; heterogeneity test: $p=0.50$) and Turkey (heterogeneity test: $p=0.08$). We found that strong association evidence existed in India (pooled OR=1.61, 95% CI=1.30–2.00, Z=4.38, P<0.0001) and a lighter association in Turkey (pooled OR=1.49, 95% CI=1.02–2.17, Z=2.07, P=0.04). Although a study of 362 Japanese male patients found an association between this mutation and coronary artery disease, the combined evidence did not show any association in East Asia (pooled OR=1.01, 95% CI=0.82–1.24, Z=0.07, P=0.95), and this is also confirmed by Lewis et al. study (30). It has been suggested that the observed geographical differences relate to nutritional habits, widespread use of vitamin supplements, or fortification of breakfast cereals with folate in North America and Europe in contrast with more unfavorable intakes of folic acid in other regions. If increased folate intake in some parts of the world does explain the heterogeneity of the associations, folic acid as a preventive intervention would be unlikely to have any major role to play in the regions where there is no *MTHFR*-coronary artery disease association. A similar meta-analysis of *MTHFR* and ischemic stroke also found a greater increase in risk among TT homozygote in Japan compared with other countries and regions (31). The inference that the *MTHFR*-disease association is greatest in Japan because folate intake is low seems to be incongruous with the low incidence of folic acid deficiency and neural tube defects in Japan relative to other countries (32, 33). Moreover, significant association was also seen for North Africa (pooled OR=1.73, 95% CI=1.45–2.06, Z=3.17, P<0.0001). The greater association difference between Europe (America) and North Africa can be explained by the larger genetic distance between Africa and America for the longest geographic distance (see *Table I*).

In addition, larger geographic distances generally increase genetic variation, and genetic distance significantly correlates to geographic distance between populations. In terms of geographic locations, Turkey is near to the North Africa countries, and they all show significant association between C677T *MTHFR* polymorphism and the risk of CAD. This can be explained by some cultures' cuisine. For example, the eastern nations of North Africa, such as Egypt, are heavily influenced by the Ottoman Empire and its Turkish culture, sharing characteristics and similar dishes with much of Turkish and Peninsular Arab cuisine. Interestingly, India showed association similarity with the Middle East (Turkey and North Africa) rather than East Asia, which is consistent with the findings that allele frequencies in India showed detectably greater similarity to those in the Middle East than to those in East Asia. A noticeable observation is that the association trend of geographical regions from south to north is consistent with their corresponding MAF distribution in case and control. With the decreased ratio of T allele frequency in case and control from Indian (case/control=1.49) and North African (case/control=1.47) to East Asian (case/control=1.12) and European (case/control=1.04), the susceptibility to CAD was decreased. Summarizing these results will allow us to observe the different association of C677T MTHFR polymorphism and CAD risk among populations of different geographical regions (see *Figure 1* and *Figure 4*).

Two additional meta-analyses

To validate the potential geographic effects on genetic predisposition of CAD, we then carried out two additional meta-analyses involving genetic associations of angiotensin-converting enzyme (*ACE*) gene insertion/deletion (I/D) and *APOA5* gene T1131C polymorphisms for CAD.

Stratified meta-analysis for ACE I/D

In this process, we performed stratified meta-analysis for the insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene (28, 34–50) to see its association with different geographical regions. To ensure more evidence are included into analysis, we extended the selected literature published after 1995, and finally 30 studies involving 22,190 participants were selected (see Supplementary *Table III* in detail). A strong association between ACE I/D and CAD was seen in the specified model (DD vs DI+II, pooled OR=1.30, 95% CI=1.11–1.52, P=0.0008). A cumulative meta-analysis also showed a trend of association as infor-

Table I The different association of *MTHFR* C677T (T vs C) polymorphism in different geographic regions.

Regions	Total number of studies	Association coefficient	Heterogeneity test-χ^2	P	Meta association – Zvalues	Meta association – Pvalues
Europe and America	7	0.143	7.81	0.25	1.00	0.32
India	3	0.750	2.75	0.43	4.38	<0.0001
East Asia	3	0.000	1.40	0.50	0.07	0.95
Turkey	2	0.500	3.03	0.08	2.07	0.04
North Africa	3	0.667	3.18	0.20	3.17	

Note: Association coefficient indicates the ratio of the number of MTHFR C677T association studies to the number of total studies.

Table II The different association of *ACE* I/D (DD vs DI +II) polymorphism in different geographic regions.

Regions	Total number of studies	Association coefficient	Heterogeneity test-χ^2	P	Meta association – Zvalues	Meta association – Pvalues
Europe and America	12	0.250	20.01	0.05	2.49	0.01
India	4	0.250	12.89	0.005	0.57	0.57
East Asia	6	0.167	9.44	0.09	0.69	0.49
Turkey	5	0.200	1.23	0.87	2.40	0.02
North Africa	3	1.000	22.61	<0.0001	0.57	0.57

Note: Association coefficient indicates the ratio of the number of ACE I/D association studies to the number of total studies.

mation accumulated. According to the stability in the relative changes in OR values, we found that there was enough evidence to support the risk effect of the ACE I/D polymorphism variant in CAD (see Supplementary *Table II*).

Under a genetic model (DD vs DI+II), Europe and America (pooled OR=1.20, 95% CI=1.04–1.39, Z=2.49, P=0.01) and Turkey (pooled OR=1.33, 95% CI=1.05–1.69, Z=2.40, P=0.02) showed significant association, whereas East Asia (pooled OR=1.06, 95% CI=0.89–1.27, Z=0.69, P=0.49), India (pooled OR=1.10, 95% CI=0.67–2.06, Z=0.57, P=0.57) and North Africa (pooled OR=1.44, 95% CI=0.41–5.02, Z=0.57, P=0.57) showed no significant association (see *Table II*, *Figure 2* and *Figure 4*). Population origin and true race-specific genetic effects might explain these results, since functional analyses of variation in the ACE gene have indicated that different loci control ACE levels in particular 'racial' groups (51). For example, it has been reported the inconsistencies in the risk effects of ACE I/D on restenosis between Caucasian and East Asian populations might be due to race-related anatomical differences in coronary arteries, since a smaller total vessel diameter has been described for Asian populations (52). Besides, a recent report also pointed out the role of ACE I/D gene polymorphism for some diseases in Caucasian children is different from that in Asian children (53). However, a small number of included studies for some populations, and a small number of subjects enrolled in most studies in these populations, imply that some negative conclusions could be due to low statistical power. These results should be interpreted with caution.

Stratified meta-analysis for APOA5 T1131C

According to the inclusion criteria, 13 studies about APOA5 T1131C polymorphism and CAD consisting of 12,322 participants were included into our analysis. To our pleasure, this result is completely consistent with a recent report in which a meta-analysis for APOA5 T1131C association with CAD was conducted (see Supplementary *Table V*) (7). Under a dominant genetic model, a significant association exists between APOA5 T1131C and CAD using all of the samples (pooled OR=1.31, 95% CI =1.21–1.43, P<0.0001). Similarly, a cumulative meta-analysis also showed an association trend as information accumulated. Stability in the relative changes in OR values approved the risk effect of the APOA5 T1131C polymorphism variant in CAD (see Supplementary

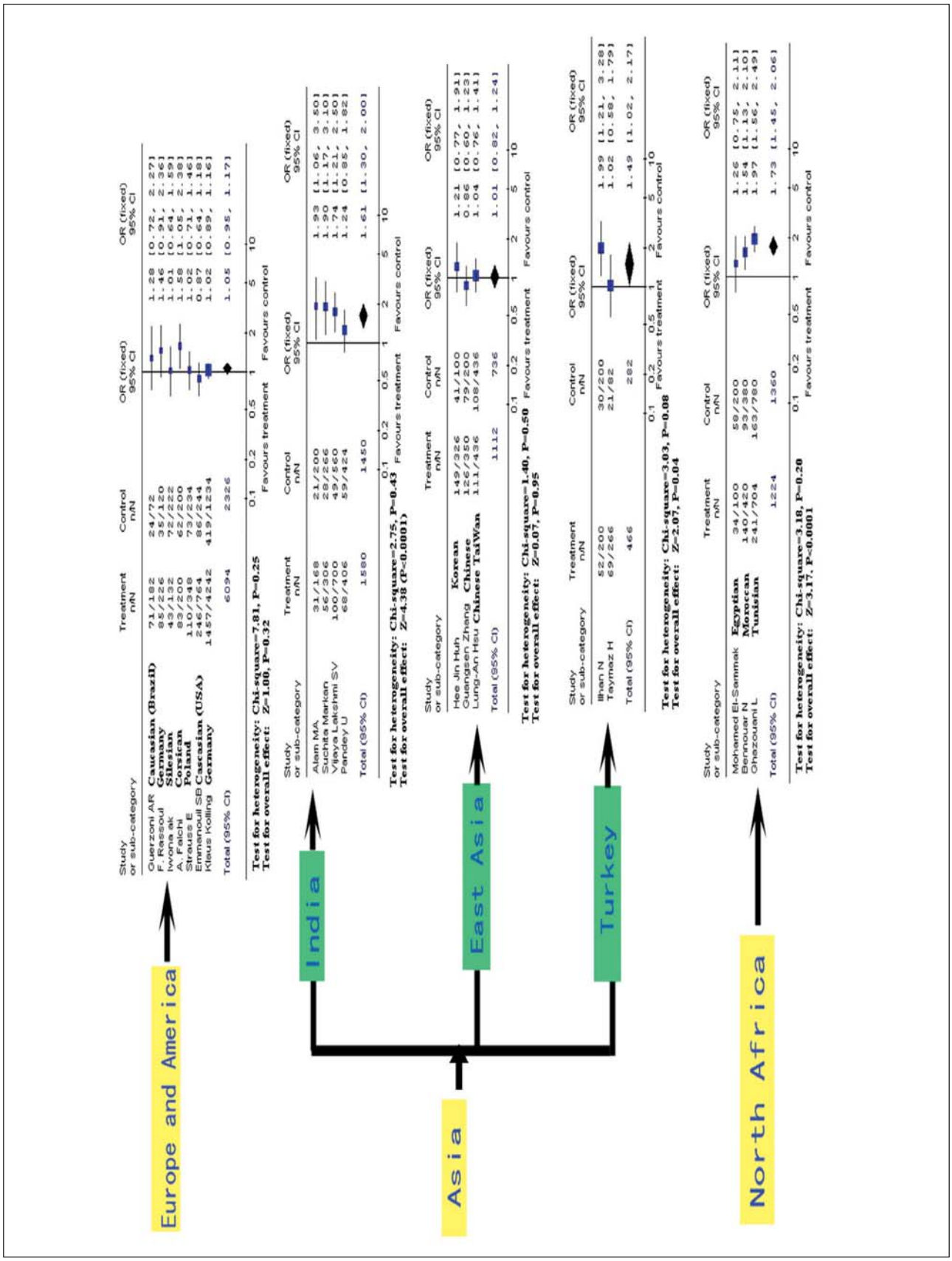

Figure 1 Association between *MTHFR* C677T polymorphism (T vs C) and the risk of CAD in different geographic regions. Pooled estimate is displayed as a diamond.

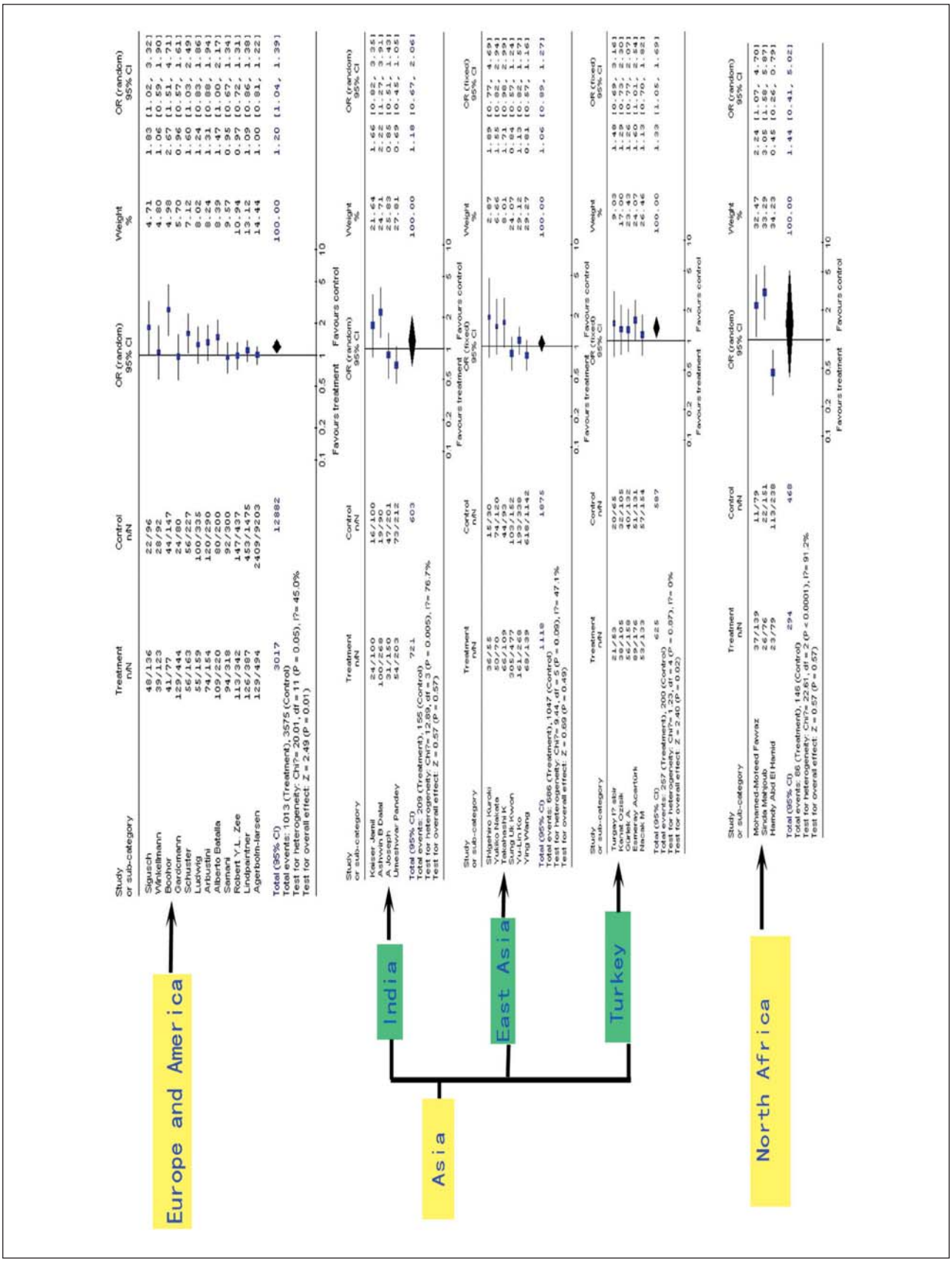

Figure 2 Association between *ACE* I/D polymorphism (DD vs DI+II) and the risk of CAD in different geographic regions. Pooled estimate is displayed as a diamond.

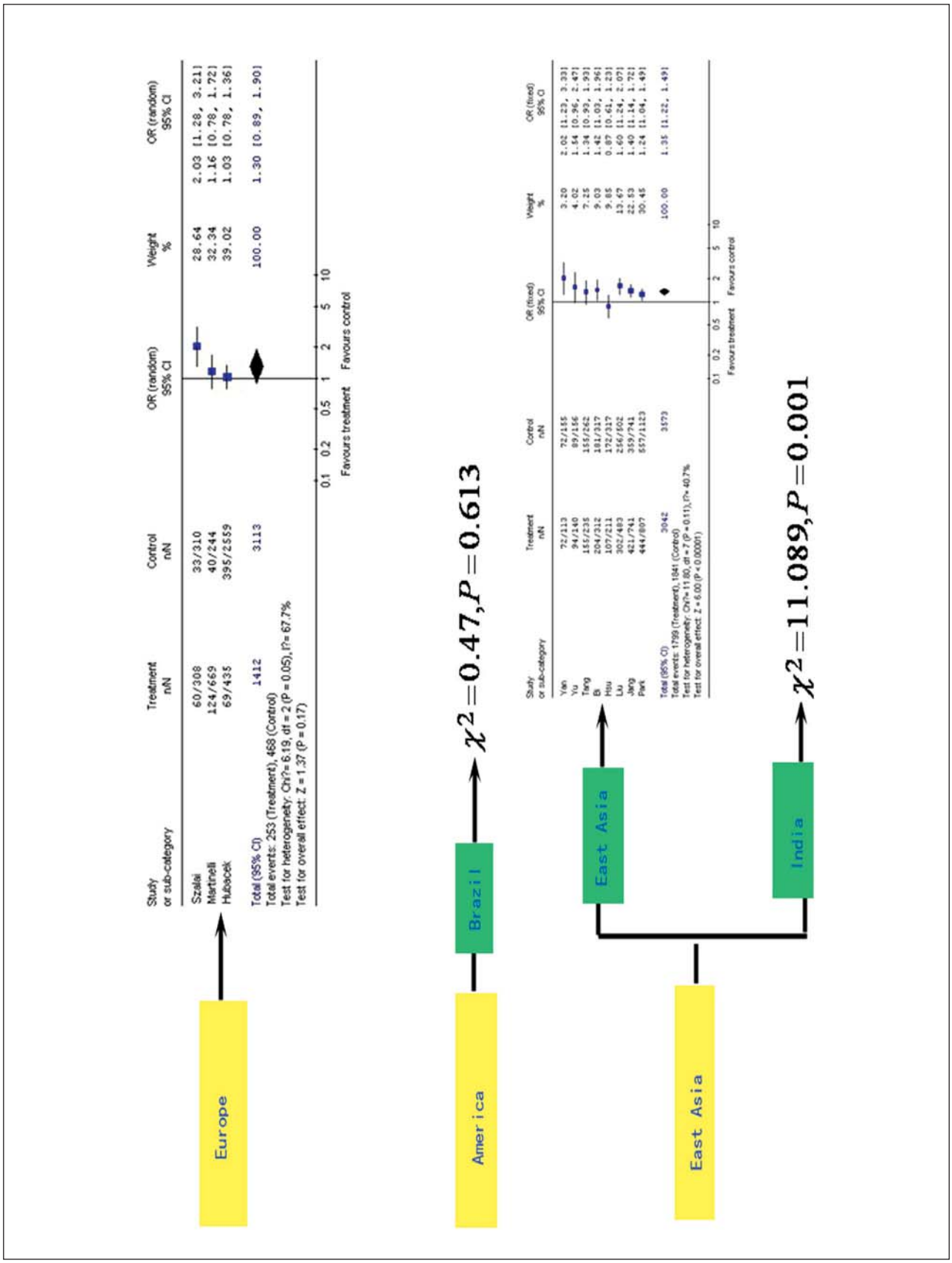

Figure 3 Association between *APOA5* T1131C polymorphism (CC+CT vs TT) and the risk of CAD in different geographic regions. Pooled estimate is displayed as a diamond.

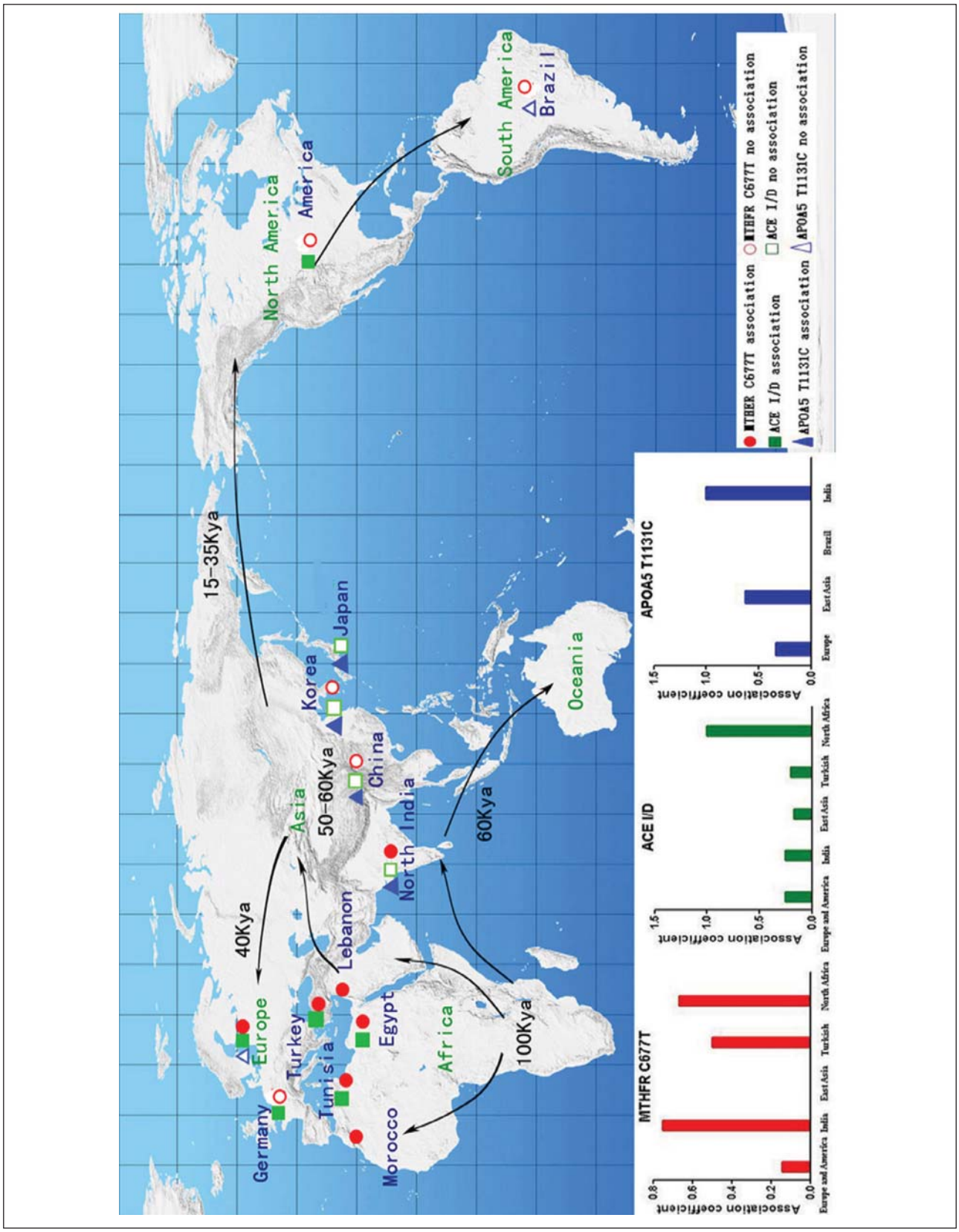

Figure 4 An integrated graph for human migration routes, geographic regions and stratified meta-analyses. In this graph, the red filled circles, green filled squares and blue filled triangles indicate the *MTHFR* C677T, *ACE* I/D and *APOA5* T1131C associated geographic regions respectively. The red hollow circles, green hollow squares and blue hollow triangles indicate an opposite situation. The black curves with arrows show the human migration routes.

Table III The different association of APOA5 T1131C (CC+CT vs TT) polymorphism in different geographic regions.

Regions	Total number of studies	Association coefficient	Heterogeneity test-χ^2	P	Meta-association – Zvalues	Meta-association – Pvalues
Europe	3	0.333	6.19	0.05	1.37	0.17
East Asia	8	0.625	11.80	0.11	6.00	<0.00001
Brazil	1	0	NA	NA	χ^2=0.47	P=0.613
India	1	1	NA	NA	χ^2=11.089	P=0.001

Note: Association coefficient indicates the ratio of the number of APOA5 T1131C association studies to the number of total studies. NA: Not available.

Table VI). Under a dominant genetic model (CC+CT vs TT), East Asia showed an extreme significant association (pooled OR=1.35, 95% CI=1.22–1.49, Z=6.00, P<10^{-5}), whereas Europe showed no association (pooled OR=1.30, 95% CI=0.89–1.90, Z=1.37, P=0.17). Because only one study reported the APOA5 T1131C polymorphism in relation to CAD in Brazil and India respectively, a chi-square test in the dominant model was used, and the results showed a strong association in India (χ^2=11.089, P=0.001) but no association in Brazil (χ^2=0.47, P=0.613) (see *Table III*, *Figure 3* and *Figure 4*).

An obviously different distribution of the APOA5-1131C allele can also be seen from our study. The ratio of C allele frequency in case and control in Indians (case/control=1.33) is higher than in Caucasians (case/control=1.00). Similarly, a higher prevalence of the *APOA5*-1131C allele in cases also presents in other Asian ethnic groups, such as Korean (case/control=1.14) and Chinese (case/control=1.23) in contrast to Caucasian populations (case/control=1.00). These findings were supported by a previous report in which the -1131C allele in *APOA5* was considerably more common in Indians than in UK white subjects (54). We can see this phenomenon is fully consistent with the different association of APOA5 T1131C in different geographic regions in which an association exists in East Asia and India, but not in Europe. Indeed, conditions associated with hypertriglyceridemia, such as pancreatitis, insulin resistance, type 2 diabetes and coronary artery disease are highly prevalent in Asian Indian populations (54). The relatively high *APOA5*-1131C allele frequency within subjects in these regions will mean it is possible to assess whether a life-long increase in triglyceride concentrations as a result of inheriting this variant increases the risk of these disorders (55).

Publication bias

We used funnel plots (56) to show the potential publication bias (see *Figure 5*), and the funnel plot asymmetry tests (57) showed no statistical evidence for publication bias for *MTHFR* C677T (p=0.288) and *APOA5* T1131C (p=0.848). Although a lighter publication bias was detected for the *ACE* I/D polymorphism (p=0.041), this cannot essentially affect the stratified meta-analysis results.

Discussion

In this paper, we provided some of the accumulating evidence of a different association between three genetic polymorphisms (*MTHFR* C677T, *ACE* I/D and *APOA5* T1131C) and CAD across different geographic regions. There are two main reasons why we have decided to use these three genetic polymorphisms to perform the analysis. One reason is that we had to select the same polymorphism for the same gene, which decreased the amount of literature. Also, we had to ensure that the selected studies cover different geographic regions, which greatly reduced the number of studies that met these strict inclusion criteria. Another reason is that we found these selected genetic polymorphisms displayed an obviously different association across different geographic regions. For example, *MTHFR* C677T showed an association with CAD in northern Africa but not in Europe. In contrast, *ACE* I/D showed an association with CAD in Europe but not in Asia. These evidence further support the potential effects of geographic isolation on the genetic polymorphism of CAD. In fact, other genes, such as *APOE*, *APOCIII*, *CETP*, are also important CAD-related genes. We will perform stratified meta-analyses for them in the future as the number of relevant studies is increased.

The present meta-analyses differ from previous ones by the inclusion of recently published articles; new studies published up to 2011; studies on populations in different geographic regions; and most importantly, our subgroup analysis identified ethnicity and geographic region as two potential factors contributing to heterogeneity of association. Specifically, we compared our results for *MTHFR* C677T with Klerk et al. (58) study which included 40 studies published before 2002, most of them published before 2000. In their analysis, individuals with the TT genotype had significantly higher odds of coronary heart disease compared with individuals with the CC genotype in Europe but not in North America. They found

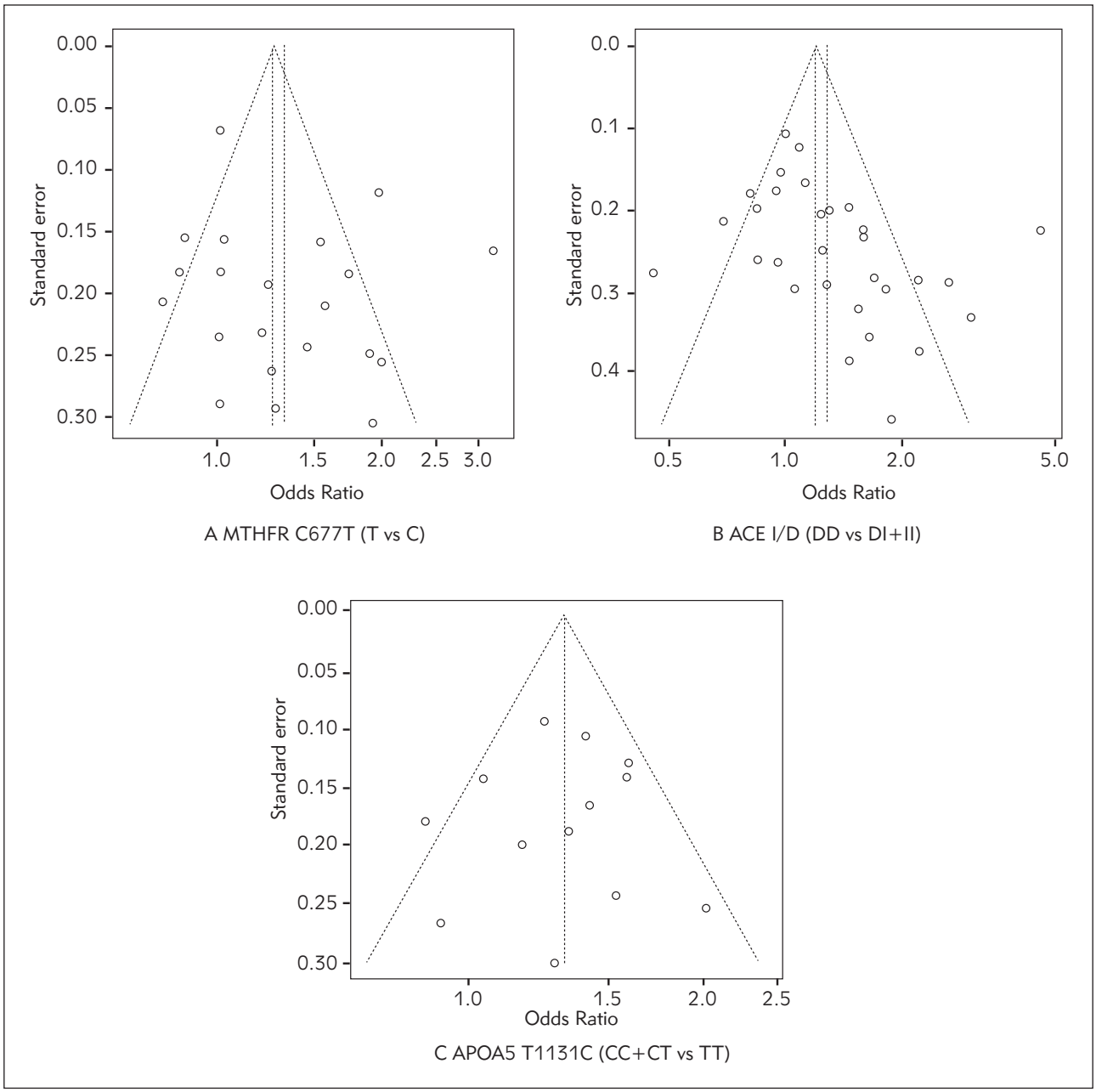

Figure 5 Funnel plots for publication bias tests of three gene polymorphisms:
(A) MTHFR C677T (T vs C), (B) ACE I/D (DD vs DI+II), (C) APOA5 T1131C (CC+CT vs TT).
Each point represents an individual study for the indicated association.

there was no heterogeneity within European studies or North American studies, but there was significant heterogeneity between Europe and North America. In our analysis, we integrated the Caucasian population distributed in Europe and America, and no significant heterogeneity was found (χ^2=7.01, P=0.25). A stratified meta-analysis under an allelic model showed that there was no association between *MTHFR* C677T and CAD for the Europe and America group (pooled OR=1.05, 95% CI=0.95–1.17, Z=1.00, P=0.32), which is supported by Lewis et al. (30), who used 80 studies published up to 2004 to perform a meta-analysis and also found no significant association between this *MTHFR* polymorphism and CAD in Europe and North America. In addition, Klerk et al. (58) mainly focused on individuals in Europe and North America, and Lewis et al. did not distinguish individuals in East Asia from those in India. Different from their studies, we divided Asia into India and East Asia, and found an obviously different association of the *MTHFR* C677T polymorphism in these two different geographic regions.

Our data indicate that the *MTHFR* C677T association with CAD is mainly present in Northern African and Indian populations, which is also validated by the dominative model (North Africa: $Z=5.37$, $P<10^{-5}$; India: $Z=4.38$, $P<0.0001$) and recessive model (North Africa: $Z=4.13$, $P<0.0001$; India: $Z=3.62$, $P=0.0003$). The *ACE* I/D association is mainly present in European, American and Turkish populations. The *APOA5* T1131C association is mainly present in East Asia, which is also validated by the recessive model ($Z=4.65$, $P<10^{-5}$) and the allelic model ($Z=6.64$, $P<10^{-5}$). These results are in agreement with the current theory that the expansion of humans from Africa affected the distribution of genetic variation which can be explained by the decreased gene flow and the increased genetic distance between geographical groups. On one hand, founder populations experience greater genetic drift due to the increased fluctuations in neutral polymorphisms; and on the other hand, new polymorphisms that arose in one group were less likely to be transmitted to other groups as gene flow was restricted.

Certainly, we cannot omit those genes which keep consistent association with CAD in different geographic regions, such as the 9p21 identified by the recent Genome-Wide Association Study (GWAS). The first four GWA studies were carried out with Caucasian populations to confirm the findings that the 9p21 region is significantly associated with CAD; however, several recent replication studies conducted in Korean, Japanese and Chinese populations also demonstrated that the same CAD-associated genetic variants on chromosome 9p21 are consistently observed in East Asians (59). A meta-analysis about GWAS of 9p21 variant (60) carried out not long ago provided enough evidence to show that alleles on chromosome 9p21 are associated with CAD for different populations. We should, however, also note the variation risk which could be due to environmental exposure across different populations. It has been reported the strongest interaction of 9p21 SNPs with diet was found in Latin Americans and South Asians, the same ethnic groups that had stronger protective effects of fruit and vegetable consumption (61, 62) which was inversely associated with fasting glucose and the metabolic syndrome (61). Therefore, the difference in geographical factors and diet environments will put forward a challenge for investigations of the important interplay of genes and environment that rely on replication studies of different populations in the etiology of CAD, and shed light on the underlying pathophysiology of 9p21.

Taken together, although an obvious association difference can be seen among different populations, and ethnicity or race may in some cases provide useful information in biomedical contexts, one should be cautious when using geographic or genetic ancestry to make inferences about individual phenotypes. Moreover, other factors such as periods of geographic isolation, socially reinforced endogamy, cultural reasons and eating habits may affect allele frequencies and give rise to genetically differentiated populations. Therefore, controlling for demographic influences on diversity will present future challenges for the mapping of functionally important variations associated with complex diseases in natural populations.

Study limitations

The potential bias of the inclusion/exclusion criteria used to select studies for our meta-analysis should be pointed out. A small proportion of studies can increase the instability of meta-analysis. When we divide all the populations into different subgroups according to different geographic regions, the number of studies included in each subgroup will inevitably be decreased as well as the statistical test power of the meta-analysis. Therefore, a small number of included studies for some geographic regions might influence the results, such as Africa in our analysis. In practice, this is also the case in other subgroup meta-analyses (63). Therefore, a larger sample size will be needed to validate this assumption in future studies. Moreover, if a trial is poorly done and is part of a meta-analysis, the results of the meta-analysis can certainly be impacted by that trial. Another limitation of the present meta-analysis is that data were taken directly from published articles and not from the original data sets provided by the various authors. In addition, the discrepancy of the observed results could be due to a series of factors, including heterogeneity of enrolled cases, outcome definition variability, genotyping errors, limited statistical power, and different study methods and so on. For each gene polymorphism, therefore, despite the undertaking of more than 10 genetic association studies published in the past 10 years designed to test this hypothesis, the exact role of the association of genetic variation with CAD remains an unresolved issue.

Noteworthy are the population classification limitations of our analysis. The genetic distances that on average increase in a continuous manner with geographic distance might cause an artificial discontinuity in racial classification. That is why some studies on population genetic structure have yielded varying results depending on the methodology used. Also, we have to acknowledge that different genetic effect sizes per population ancestry are partly attributed to other factors, such as gender differences, age, and environmental factors.

In addition, for the limitations of meta-analysis, our estimates of effect are still likely to represent overestimation through publication bias. Mechanisms are clearly needed for sharing data, archiving all relevant findings whether positive or negative, and avoiding duplication of effort. Because of limitations and potential bias, more well-designed studies with a larg-

er sample size, especially focused on Asian and African populations, should be performed in the future. Furthermore, we will consider adding genome-wide association analysis to our study in the future, and further evaluate whether gene-gene interactions contributing to complex diseases are affected by migration routes and geographic isolation.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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Supplementary Table I Characteristics of the individual study of *MTHFR* C677T polymorphism included in this meta-analysis.

First Author	Year	Population	Genotype for case/control (n)		
			CC	CT	TT
Guangsen Zhang	2001	Chinese	72/37	80/47	23/16
Lung-An Hsu	2001	Taiwan	120/125	85/78	13/15
Emmanouil SB	2003	Caucasian (USA)	173/53	172/52	37/17
Iwona ak	2003	Silesian	29/47	31/56	6/8
Klaus Kölling	2004	German	915/266	955/283	251/68
Wassim Y. Imawi	2004	Lebanese	27/220	39/166	30/18
Mohamed El-Sammak	2004	Egyptian	22/50	22/42	6/8
Strauss E	2005	Polish	82/58	74/45	18/14
A. Falchi	2005	Corsican	37/42	43/54	20/4
Hee Jin Huh	2006	Korean	57/18	63/23	43/9
Kerkeni M	2006	Tunisian Arab	58/49	55/35	7/16
Taymaz H	2007	Turkish	78/22	41/17	14/2
Bennouar N	2007	Moroccan	101/113	78/61	31/16
Guerzoni AR	2007	Caucasian (Brazil)	26/14	59/20	6/2
Suchita Markan	2007	North Indian	105/105	40/28	8/0
Ilhan N	2008	Turkish	52/72	44/26	4/2
Alam MA	2008	North Indian	57/80	23/19	4/1
F. Rassoul	2008	German	47/32	47/21	19/7
Ghazouani L	2009	Tunisian	157/247	149/123	46/20
Pandey U	2011	Indian	144/156	50/53	9/3
Vijaya Lakshmi SV	2011	Indian	256/231	88/49	6/0

Supplementary Table II Cumulative analyses for the influence of the individual study of MTHFR C677T polymorphism.

First Author	OR	95% OR CI		P	I^2 (%)
		lower	upper		
Adding Guangsen Zhang (k=1)	0.86	0.60	1.23	0.41	–
Adding Lung-An Hsu (k=2)	0.96	0.76	1.21	0.72	0.0
Adding Mohamed El-Sammak (k=7)	1.18	0.86	1.63	0.31	87.3
Adding Emmanouil SB (k=3)	0.93	0.77	1.11	0.41	0.0
Adding Iwona ak (k=4)	0.94	0.79	1.11	0.46	0.0
Adding Klaus Kölling (k=5)	0.99	0.89	1.09	0.80	0.0
Adding Wassim Y. Imawi (k=6)	1.17	0.82	1.67	0.39	89.4
Adding Strauss E (k=8)	1.16	0.87	1.53	0.31	85.2
Adding A. Falchi (k=9)	1.20	0.92	1.55	0.18	84.1
Adding Hee Jin Huh (k=10)	1.20	0.94	1.52	0.14	82.1
Adding Kerkeni M (k=11)	1.16	0.92	1.45	0.21	81.1
Adding Taymaz H (k=12)	1.15	0.92	1.42	0.21	79.2
Adding Bennouar N (k=13)	1.18	0.96	1.44	0.12	79.0
Adding Guerzoni AR (k=14)	1.18	0.97	1.43	0.09	77.3
Adding Suchita Markan (k=15)	1.21	1.00	1.47	0.04	77.2
Adding Ilhan N (k=16)	1.25	1.03	1.50	0.02	77.3
Adding Alam MA (k=17)	1.27	1.06	1.53	0.01	76.7
Adding F. Rassoul (k=18)	1.28	1.07	1.53	0.006	75.5
Adding Ghazouani L (k=19)	1.32	1.10	1.57	0.002	79.0
Adding Pandey U (k=20)	1.31	1.11	1.56	0.002	77.8
Adding Vijaya Lakshmi SV (k=21)	1.33	1.13	1.57	0.006	77.5

Supplementary Table III Characteristics of individual study of the *ACE* I/D polymorphism included in this meta-analysis.

First Author	Year	Population	Genotype for case/control (n)	
			DD	DI+II
Beohar	1995	USA	41/44	36/103
Gardcmann	1995	German	129/24	315/56
Schuster	1995	German	56/56	107/171
Ludwig	1995	USA	55/100	104/235
Arbustini	1995	Italian	74/120	80/170
Lindpaintner	1995	USA	126/453	261/1022
Takahashi K	1995	Japanese	66/44	43/49
Winkellmann	1996	German	39/28	84/64
Samani	1996	UK	94/92	224/208
Yukiko Nakata	1996	Japanese	50/74	20/46
Sigusch	1997	German	48/22	88/74
Agerbolm-larsen	1997	Danish	129/2409	365/6794
Shlgehiro Kuroki	1997	Japanese	36/15	19/15
Yu-Lin Ko	1997	Chinese	161/193	107/145
A Joseph	1998	Indian	31/47	119/154
Turgay Isbir	1999	Turkish	21/20	32/45
Alberto Batalla	2000	Spanish	109/80	111/120
Sung UK Kwon	2000	Korean	305/103	172/49
Gürlek A	2000	Turkish	56/40	102/92
Robert Y.L. Zee	2001	European (Multiple Center)	113/147	229/290
Nacak M	2004	Turkish	53/57	80/97
Ying Wang	2005	Chinese	68/618	71/524
Kanat Ozisik	2005	Turkish	38/32	67/73
Esmeray Acartürk	2005	Turkish	89/51	87/80
Ashwin B Dalal	2006	Indian	100/19	168/71
Kaiser Jamil	2009	Indian	24/16	76/84
Hamdy Abd EI Hamid	2009	Egyptian	23/113	56/125
Sinda Mahjoub	2010	Tunisian	26/22	50/129
Umeshwar Pandey	2011	Indian	54/73	149/139
Mohamed-Mofeed Fawaz	2011	Egyptian	37/11	102/68

Supplementary Table IV Cumulative analyses for the influence of the individual study of *ACE* I/D polymorphism.

First Author	OR	95% OR CI		P	I^2 (%)
		lower	upper		
Adding Beohar (k=1)	2.67	1.51	4.71	0.0007	–
Adding Gardcmann (k=2)	1.52	1.04	2.24	0.030	85.3
Adding Schuster (k=3)	1.55	1.16	2.08	0.003	70.7
Adding Ludwig (k=4)	1.44	1.14	1.82	0.002	60.5
Adding Arbustini (k=5)	1.40	1.15	1.72	0.001	48.5
Adding Lindpaintner (k=6)	1.26	1.08	1.47	0.003	51.4
Adding Takahashi K (k=7)	1.29	1.11	1.50	0.0008	47.1
Adding Winkellmann (k=8)	1.28	1.10	1.47	0.0009	40.4
Adding Samani (k=9)	1.22	1.07	1.39	0.003	43.5
Adding Yukiko Nakata (k=10)	1.23	1.08	1.40	0.002	38.8
Adding Sigusch (k=11)	1.25	1.10	1.43	0.0004	38.8
Adding Agerbolm-larsen (k=12)	1.18	1.06	1.31	0.003	44.6
Adding Shlgehiro Kuroki (k=13)	1.19	1.06	1.32	0.002	42.6
Adding Yu-Lin Ko (k=14)	1.18	1.07	1.31	0.001	38.0
Adding A Joseph (k=15)	1.17	1.06	1.29	0.003	37.6
Adding Turgay I sbir (k=16)	1.17	1.06	1.29	0.002	34.2
Adding Alberto Batalla (k=17)	1.19	1.08	1.31	0.0005	33.5
Adding Sung UK Kwon (k=18)	1.16	1.06	1.28	0.001	36.8
Adding Gürlek A (k=19)	1.17	1.06	1.28	0.0009	33.3
Adding Robert Y.L. Zee (k=20)	1.15	1.05	1.25	0.002	32.8
Adding Nacak M (k=21)	1.21	1.11	1.32	<0.0001	69.0
Adding Ying Wang (k=22)	1.18	1.09	1.29	<0.0001	69.6
Adding Kanat Ozisik (k=23)	1.18	1.09	1.29	<0.0001	68.2
Adding Esmeray Acartürk (k=24)	1.20	1.10	1.30	<0.0001	67.5
Adding Ashwin B Dalal (k=25)	1.21	1.12	1.31	<0.0001	68.1
Adding Kaiser Jamil (k=26)	1.22	1.12	1.32	<0.0001	67.1
Adding Hamdy Abd El Hamid (k=27)	1.19	1.10	1.29	<0.0001	70.5
Adding Sinda Mahjoub (k=28)	1.21	1.12	1.31	<0.0001	71.9
Adding Umeshwar Pandey (k=29)	1.19	1.10	1.28	<0.0001	72.7
Adding Mohamed-Mofeed Fawaz (k=30)	1.19	1.11	1.29	<0.0001	72.5

Supplementary Table V Characteristics of the individual study of *APOA5* T1131C polymorphism included in this meta-analysis.

First Author	Year	Population	Genotype for case/control (n)	
			CC+CT	TT
Bi	2004	Chinese	204/181	108/136
Hubacek	2004	Czech	69/395	366/2164
Szalai	2004	Hungarian	60/33	248/277
Liu	2005	Chinese	302/256	181/246
Tang	2005	Chinese	155/155	80/107
Yan	2005	Chinese	72/72	41/83
Hsu	2006	Chinese	107/172	104/145
Yu	2007	Chinese	94/89	46/67
Martinelli	2007	Italian	124/40	545/204
Jang	2009	Korean	421/359	320/382
Ashokkumar	2009	Indian	225/177	191/239
Prochaska	2010	Brazilian	30/23	150/147
Park	2010	Korean	444/557	363/566

Supplementary Table VI Cumulative analyses for the influence of the individual study of *APOA5* T1131C polymorphism.

First Author	OR	95% OR CI		P	I^2 (%)
		lower	upper		
Adding Bi (k=1)	1.42	1.03	1.96	0.0332	–
Adding Hubacer (k=2)	1.18	0.96	1.46	0.1177	53.2
Adding Szalai (k=3)	1.14	0.94	1.39	0.1897	33.4
Adding Liu (k=4)	1.29	1.11	1.51	0.0011	59.2
Adding Tang (k=5)	1.30	1.13	1.50	0.0003	45.8
Adding Yan (k=6)	1.35	1.17	1.54	<0.0001	51
Adding Hsu (k=7)	1.27	1.12	1.44	0.0003	61.2
Adding Yu (k=8)	1.29	1.14	1.45	<0.0001	56.4
Adding Martinelli (k=9)	1.27	1.13	1.43	<0.0001	50.9
Adding Jang (k=10)	1.30	1.18	1.44	<0.0001	46.8
Adding Ashokkumar (k=11)	1.34	1.21	1.47	<0.0001	46.5
Adding Prochaska (k=12)	1.33	1.21	1.47	<0.0001	41.3
Adding Park (k=13)	1.31	1.21	1.43	<0.0001	37.4