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Association Between Apolipoprotein E Genotype and Carotid Intima-Media Thickness May Suggest a Specific Effect on Large Artery Atherothrombotic Stroke

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Abstract

Background and Purpose—Apolipoprotein E genotype (*APOE*) influences cholesterol levels and ischemic heart disease. Although there is no convincing overall association with ischemic stroke, *APOE* may influence large artery (atherothrombotic) stroke, for which carotid intima-media thickness (CIMT) is an informative intermediate phenotype. We therefore performed a systematic review and meta-analysis of the association between *APOE* and CIMT.

Methods—We sought all published studies assessing the association between *APOE* and CIMT. From each study, we extracted available data on study methods, subjects' characteristics, and mean (and standard deviation) CIMT for each genotype or genotype group. We calculated study-specific and random effects pooled differences in mean CIMT between genotype groups, and assessed heterogeneity between studies and predefined subgroups using I^2 and χ^2 statistics.

Results—Meta-analysis of 22 published studies (30 879 subjects) showed a significant association between *APOE* and CIMT (pooled mean difference ε4-versus ε2-allele containing genotypes 46 μm, 95% CI 29 to 62, P<0.00001). We found evidence of small study (mainly publication) bias, with a diminished (but still highly statistically significant) association in studies of >1000 subjects (pooled mean difference 17 μm, 95% CI 12 to 23, P<0.00001). The association was larger among high vascular risk and eastern Asian populations, but this may simply reflect the smaller size of these studies.

Conclusion—Our results show a clear association of *APOE* with CIMT, even though publication bias means that this is overestimated by the published literature. These findings suggest the possibility of a specific association with large artery ischemic stroke.

Keywords

apolipoprotein E; association; carotid intimal medial thickness; genetics; meta-analysis

Twin and family history studies have shown that the incidence of ischemic stroke is likely to be influenced by genetic factors.1 However, case-control candidate gene association studies have so far had limited success in consistently identifying potentially causative genes.2-4

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Disclosures
None.

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Inadequate study size, control selection bias, and lack of distinction between different ischemic stroke subtypes have all been suggested as reasons.4,5

The apolipoprotein E gene (apoE=protein, *APOE*=gene) is a widely studied gene in vascular and neurodegenerative diseases, including stroke.4,6 Its protein product, the glyco-protein apoE, has 3 common isoforms, E2, E3 and E4, encoded by the alleles ε2, ε3 and ε4, giving rise to 6 genotypes, with ε3/ε 3 occurring in about one half to two thirds of people in most populations. The 3 common protein isoforms interact differently with specific lipoprotein receptors, ultimately altering circulating levels of cholesterol through different effects on lipoprotein metabolism, mediated through the hepatic binding, uptake, and catabolism of chylomicrons, chylomicron remnants, very low density lipoprotein and high density lipoprotein subspecies. The ε4 allele is associated with increased total cholesterol levels and the ε2 allele with decreased levels, and so *APOE* genotype would be expected to influence the development of atherosclerosis and atherosclerotic vascular diseases.6 Possession of an ε4 allele increases risk of ischemic heart disease by about one third.7 Our recent meta-analysis found no convincing overall association between *APOE* and ischemic stroke (OR 1.11, 95% CI 1.01 to 1.22), but results from a few studies with information on ischemic stroke subtypes suggested that ε4 allele-containing genotypes may specifically increase the risk of large artery (atherothrombotic) ischemic stroke (OR 1.33), with no effect on other subtypes (ORs between 0.86 and 1.06).4

Another way of assessing the association between *APOE* (or other genes) and large artery atherothrombosis is to assess its effect on the quantitative intermediate phenotype of carotid intima-media thickness (CIMT).8 CIMT is a good surrogate measure for subclinical atherosclerosis, and increasing CIMT is directly associated with an increased risk both of myocardial infarction and of stroke. Each 1 SD (0.2 mm) increase in common carotid artery (CCA) IMT is associated with about a one third increase in the risk of myocardial infarction and stroke.9 The association with stroke may arise mainly from a specific association with large artery ischemic stroke, as CIMT has been found to be significantly higher in patients with large artery compared with small artery (lacunar) ischemic stroke.10 Lowering LDL cholesterol has also been shown to produce a reduction in CIMT.11 CIMT has reasonably high heritability, although estimates vary between 30% and 86%.12-14

Many studies have now investigated the association between *APOE* genotype and CIMT. We aimed to assess any association reliably, with a systematic review and meta-analysis.

Methods

Identification of Studies

We sought all available published studies of the association between *APOE* and CIMT in humans. We used a comprehensive search strategy in Medline (1966 to the end of 2006) and Embase (1980 to the end of 2006), which included MeSH terms and textwords for *APOE* and for CIMT (see Appendix). We also checked the reference lists of all relevant articles for further studies.

We included all studies that had measured the thickness of the intima-media of the carotid artery, but excluded studies which only reported the presence or extent of atheroma or plaque. We included studies among healthy subjects from the general population (low risk) or groups of subjects with existing vascular disease or vascular risk factors, such as hypertension, diabetes, hyperlipidemia, or coronary heart disease (high risk). We sought articles in all languages. We excluded from our analyses otherwise relevant studies from which the data required were not available in the relevant publication(s).

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Data Extraction

For each included study we extracted information on year of publication, country in which the study was conducted, ethnicity of subjects, nature of the study population (eg, patients with diabetes), total number of subjects, mean age and gender distribution of the subjects, genotyping methodology, whether genotyping was done blind to CIMT and vice versa, and the definition of CIMT. In addition, we extracted information on (or where possible tested directly for) Hardy-Weinberg equilibrium. For each genotype or group of genotypes, we extracted the mean CIMT and its standard deviation (SD). Where possible, we treated studies that included both a high and a low risk group or more than one ethnic group as separate substudies.

Two authors (L.P., N.A.M.G.) independently reviewed study eligibility and extracted the information and data from each study, resolving any disagreements by discussion with a third author (C.S.). Some articles reported several different measures of CIMT (eg, from different carotid artery sites). Where a choice of measurement was available we chose the one that was closest to our ideal: the mean CIMT of the left and right far wall of the CCA. We chose this method because the CCA may give more reproducible results than the internal carotid artery (ICA) because of its accessibility, and far wall measurements are more accurate than near wall, which have to be performed at the trailing edge of the ultrasound pulse.15

We defined genotype groups as E2 ($\varepsilon 3/\varepsilon 2$ or $\varepsilon 2/\varepsilon 2$), E3 ($\varepsilon 3/\varepsilon 3$), and E4 ($\varepsilon 3/\varepsilon 4$ or $\varepsilon 4/\varepsilon 4$), and excluded any $\varepsilon 2/\varepsilon 4$ subjects from each study, as they could not be classified as E2 or E4. These were extremely small in number (2% of all subjects), making it unlikely that their inclusion or exclusion would affect the results.

Statistical Analysis

We first calculated the overall mean CIMT for each genotype group across all studies, and then carried out meta-analyses using Cochrane RevMan software (version 4.2) comparing the 2 genotype groups with the highest and lowest overall mean CIMT (E4 versus E2). We calculated the mean CIMT difference between the E4 and E2 genotype groups for each study, and pooled results using a random effects model. We used the I^2 statistic to assess heterogeneity between studies, where I^2 estimates the percentage of variation between studies that cannot be attributed to chance.16

We plotted the study-specific mean CIMT difference between E4 and E2 genotypes against the standard error of this difference (a funnel plot) to check for the possibility of small study bias. To assess the impact of study characteristics on the association we performed prespecified subgroup analyses. We grouped the studies according to risk status of individuals (those of high and low risk of vascular disease), study size (above or below the mean total number of subjects), ethnicity (Eastern Asian, White, or Black African), and method of measuring CIMT (ideal or not, where ideal measurements were those only from the far wall of the CCA). We assessed the significance of differences between subgroups by partitioning heterogeneity and using χ^2 tests.

Results

Study Selection

Figure 1 shows the process of study selection and exclusion. Our search identified 490 articles, of which 32 were potentially relevant.17-48 Four studies were duplicates of other included studies and so were excluded.39-42 Six studies had insufficient data presented to be included in the analysis43-48 (including 2 that combined E2 and E3 genotype

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groups46,48). We therefore included 22 studies (increasing to 25 studies after 3 were split into 2 substudies) in a total of 30 879 subjects in our analyses.

Study Characteristics

Details of the included studies are shown in the Table. Sample sizes ranged from 52 to 9304 with a mean of 1235. Subjects were mostly middle-aged to elderly. Most studies had approximately equal proportions of males and females, although 5 included only men.21,25,28,35,36 The studies were conducted in several European countries, the USA, Australia, Japan, and China. Most subjects were included in low risk studies (healthy subjects or general populations), whereas a smaller proportion were included in high-risk studies (see Table). Most studies used a polymerase chain reaction/restriction fragment length polymorphism method for genotyping, whereas 3 used isoelectric focusing,17,25,28 2 used polymerase chain reaction/allele specific oligonucleotide hybridization,23,31 and the 2 most recent studies used Taqman.37,38 Genotype group frequencies were fairly consistent across all studies. No studies were found to depart from Hardy-Weinberg equilibrium, although this was not reported and could not be tested directly in 5.20,23,24,33,34

All studies measured CIMT with B-mode ultrasound. The supplemental Table I, available online at http://stroke.ahajournals.org, shows which segment of the carotid artery was measured and how multiple measurements were combined to obtain the final CIMT value. Only 2 studies stated that ultrasonography staff were blind to genotype data,28,34 and only one stated that genotyping was carried out blind to the ultrasonography findings.29

Association Between *APOE* and CIMT

E4 genotypes had the highest mean CIMT across all studies (760 μm), the E3 genotype group had an intermediate mean CIMT (751 μm), and E2 genotypes had the lowest mean CIMT (743 μm; in keeping with their known effects on cholesterol levels). We therefore compared mean CIMT for E4 versus E2 genotypes in our meta-analyses. Figure 2 shows the study-specific and pooled results for the E4 versus E2 comparison in a total of 11989 subjects. Overall there was a highly significant pooled mean CIMT difference of 46 μm (95% CI 29 to 62, $P<0.00001$). There was substantial heterogeneity between the studies ($I^2=80\%$).

The funnel plot for the analysis was markedly asymmetrical, suggestive of small study (presumably mainly publication) bias (supplemental Figure I, available online at http://stroke.ahajournals.org).

Results of our subgroup analyses are shown in Figure 3. We found a substantially larger pooled mean CIMT difference among high-risk compared with low-risk populations and in Eastern Asian compared with White or Black African populations. However, we also found that the pooled mean CIMT difference in smaller studies was more extreme than that in larger studies (smaller studies [mean number of subjects analyzed 58]:93 μm, 95% CI 46 to 140; larger studies [mean number of subjects analyzed 1814]:17 μm, 95% CI 12 to 23, $P<0.00001$; χ^2 test for difference between the 2 subgroups: $P<0.0001$), suggesting the existence of small study bias, consistent with the appearance of the funnel plot (supplemental Figure I). There was little heterogeneity between the results of the larger studies ($I^2=5\%$). Study size could explain the apparent difference in size of association between high and low risk and between ethnic groups, because most studies in high risk populations and among Eastern Asian subjects were small (mean number of subjects analyzed in high-risk group 55, in low-risk group 813, in Eastern Asians 50, in other ethnic groups 682).

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As CIMT was measured at a variety of sites within the carotid artery (supplemental Table I), we split the data into those studies which used our preferred method (measuring only the far wall of the CCA) and those that used any other method. Studies using our ideal CIMT measurement method yielded less heterogeneous results and a smaller pooled mean CIMT difference (16 μm, 95% CI 5 to 27), consistent with the estimated association in the larger studies.

Robustness to Missing Data

We considered the impact of excluding relevant studies without available data for the E4 versus E2 genotype group comparison. Two relevant studies combined E2 and E3 data and so could not be included in our analyses. One found no relationship between *APOE* and CIMT among patients with noninsulin dependent diabetes, but found that ε4 allele-containing genotypes increased CIMT by 0.14 mm compared with other genotypes in a nondiabetic population.48 The other found no significant relationship between *APOE* and CIMT.46 Four studies did not present mean and SD CIMT data by genotype or genotype groups and so could not be included in any of our analyses. Three found no significant association between *APOE* and CIMT,43-45 whereas the fourth found that E4 genotypes were significantly more frequent than E2 genotypes in the subjects with higher CIMT values.47 The total number of subjects in all genotype groups in all of these potentially relevant additional studies was 1612, bringing to 32 491 the total possible number of subjects that could have been included if data had been available. “Missing data” therefore comprised only 5% of the total, making it very unlikely that including these studies would have made a material difference to our results. Furthermore, the largest of these “missing data” studies included 511 subjects.46 Therefore, none would have been categorized as a large and so more reliable study (>1000 subjects).

Discussion

The results of our meta-analysis show a clear association between *APOE* and CIMT. In keeping with the known effects of *APOE* on cholesterol levels, E2 genotypes had the lowest CIMT, E3 genotypes an intermediate CIMT, and E4 genotypes the highest CIMT.

The overall pooled mean difference between E4 and E2 genotypes was 46 μm, but we found evidence of small study bias, both from a funnel plot and a subgroup analysis based on study size. Larger studies were far less heterogeneous, and found a substantially smaller—but still highly statistically significant—mean CIMT difference between E4 and E2 genotypes of 17 μm. Thus, although the published literature taken as a whole probably overestimates the size of the association, the consistency of direction of the association observed across the studies, and the highly statistically significant findings for the larger, more reliable studies, strongly suggests the presence of a true association.

Our subgroup analyses also suggested that the association between *APOE* genotype and CIMT might be larger in high-versus low-risk subjects and in Eastern Asian populations. A recent meta-analysis of the association between the angiotensin-converting enzyme insertion/deletion polymorphism and CIMT also found a larger association in high risk populations, and suggested that this may be attributable to an interaction with smoking.49,50 But, although the vascular risk and ethnicity subgroup differences in our meta-analysis could conceivably be real, they may have arisen from confounding by study size, because the studies in high-risk and Eastern Asian subjects were generally smaller than those in low-risk and other ethnic groups, respectively.

The results of studies using ideal CIMT measurement methods were far less heterogeneous than those using a nonideal measurement, suggesting that a consistent approach to CIMT

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measurement (using the mean of the right and left far wall measurements 1 cm below the bifurcation) may lead to more reliable results that are comparable between studies. Although the size of the association between *APOE* and CIMT varied with the location of measurement, the results of individual studies showed that the direction of association was consistent.

Several different genotyping methods were used in the studies, but any variations in the resulting genotyping accuracy should not introduce any systematic error, and indeed we did not find any influence of genotyping method on the size of the association between *APOE* and ischemic stroke in a previous meta-analysis.4

Although our results concur with what we would expect from the known effect of *APOE* on cholesterol levels, there may be other pathways through which *APOE* influences atherosclerosis and so CIMT. Some studies included in our meta-analysis adjusted for covariates, including cholesterol levels, in their analyses of the association between *APOE* and CIMT. Whereas in some this resulted in loss or diminution of the association between *APOE* and CIMT,25,32 in others the association between *APOE* and CIMT was preserved, 17,18,26,28,35,37,38 suggesting that *APOE* genotype may also influence CIMT independently of its effects on cholesterol levels.

Summary

Our results have shown a modest association between *APOE* genotype and CIMT, even though small study bias means that the published literature tends to overestimate the size of this association. This suggests that *APOE* might modestly influence risk of large artery ischemic stroke, and would support large case-control studies specifically to examine this hypothesis. Further work is also required to determine whether or not the association we have observed varies among different types of subjects according to ethnicity and vascular risk status, and to further elucidate the mechanisms by which *APOE* may influence CIMT.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

Search strategy in Medline (similar strategy was designed for Embase)

1. carotid artery diseases/ or carotid artery thrombosis/ or carotid stenosis/ or moyamoya disease/
2. carotid artery disease/ge or carotid artery thrombosis/ge or carotid stenosis/ge or moyamoya disease/ge
3. carotid arteries/ or exp carotid artery, common/

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4. (carotid adj5 [atherosclero$ or arteriosclero$ or steno$ or imt or cimt or intima media$ or ultrasound or plaque or sclero$ or atheroma$ or fatty streak or disease$ or disorder$]).tw.
5. 1 or 3 or 4
6. apolipoproteins/ or apolipoproteins e/
7. ([apolipoprotein$ adj e] or [apoprotein$ adj e] or apo-e or apo e or apoe).tw.
8. 6 or 7
9. 5 and 8
10. 2 or 9
11. limit 10 to humans

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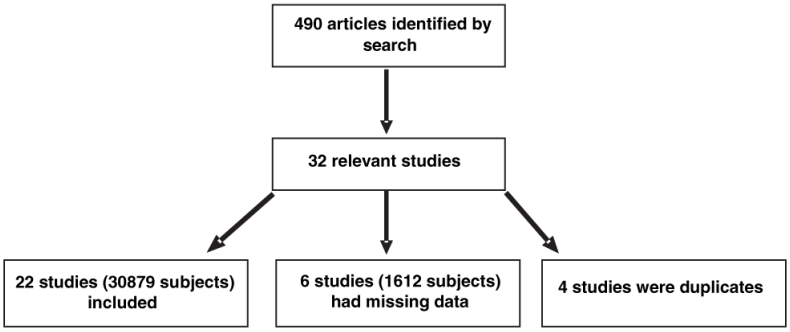

Figure 1.
Flow diagram of study selection and exclusion process.

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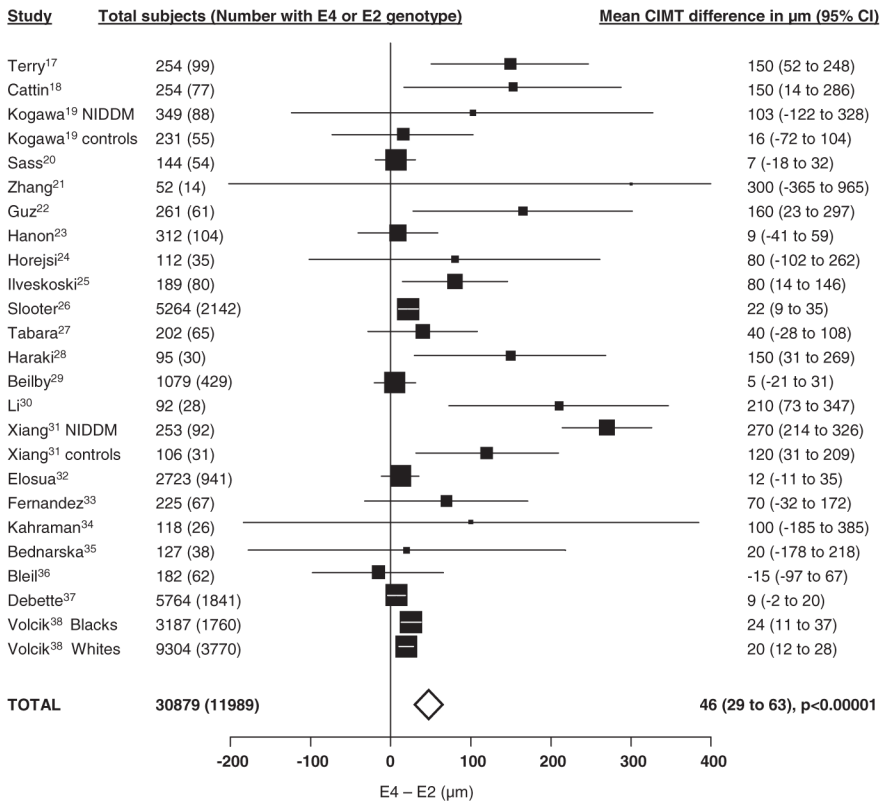

Figure 2.

Study-specific and pooled mean differences of the CIMT between E4 and E2 genotypes (ordered by publication date). The sizes of the squares are proportional to the statistical weight given to each study. The horizontal lines represent 95% CI. The width of the diamond represents the 95% CI of the pooled estimate. Heterogeneity between studies: I^2=80%.

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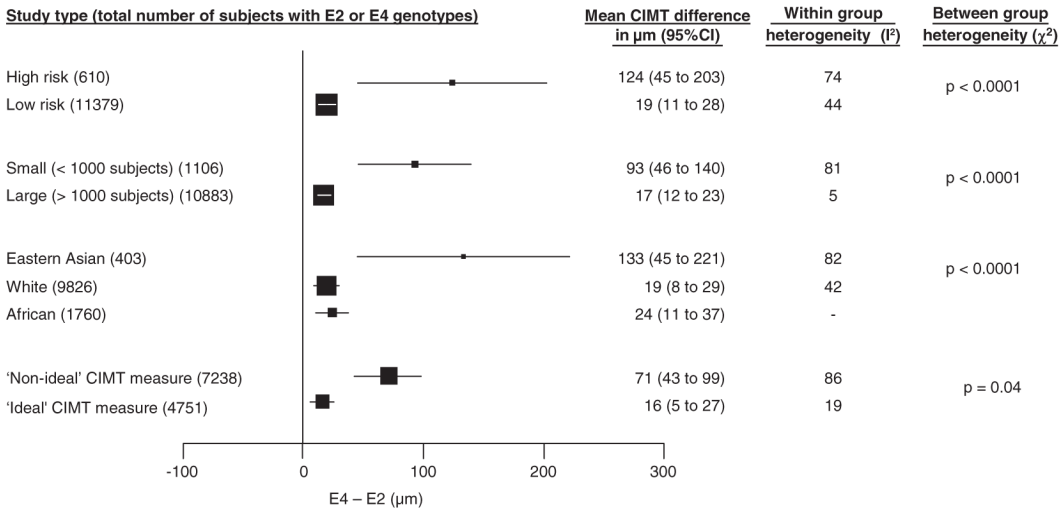

Figure 3.

Pooled mean differences of the CIMT between E4 and E2 genotypes: results for various subgroups. The size of the squares is proportional to the number of subjects. Horizontal lines represent 95% CI.

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Table

Characteristics of Included Studies

Primary Author	Year	Country	Subjects	Sample Size	Genotyping Method	Genotype Frequency			HWE
						E2	E3	E4	
Terry17	1996	US	Referrals for coronary angiography (HR)	254	IEF	0.13	0.61	0.26	√
Cattin18	1997	Italy	Population sample (LR)	254	PCR/RFLP	0.12	0.70	0.18	√
Kogawa19 NIDDM	1997	Japan	NIDDM patients (HR)	349	PCR/RFLP	0.07	0.73	0.18	√
Kogawa19 controls	1997	Japan	Non-diabetic subjects at local check-up (LR)	231	PCR/RFLP	0.09	0.75	0.14	√
Sass20	1998	France	Population sample (LR)	144	PCR/RFLP	0.17	0.62	0.21	?
Zhang21	1998	China	CHD patients (HR)	52	PCR/RFLP	0.08	0.73	0.19	√
Guz22	2000	Turkey	Hemodialysis patients (HR)	261	PCR/RFLP	0.13	0.77	0.11	√
Hanon23	2000	France	Referrals to clinic - normal carotid walls (LR)	312	PCR/ASO	0.12	0.67	0.21	?
Horejsi24	2000	Czech Republic	Lipoprotein disorder patients (HR)	112	PCR/RFLP	0.09	0.69	0.22	?
Ilveskoski25	2000	Finland	Population sample (LR)	189	IEF	0.11	0.58	0.32	√
Slooter26	2001	Netherlands	Population sample (LR)	5264	PCR/RFLP	0.14	0.58	0.26	√
Tabara27	2001	Japan	Population sample (LR)	202	PCR/RFLP	0.13	0.68	0.19	√
Haraki28	2002	Japan	Healthy subjects at annual check-up (LR)	95	IEF	0.11	0.68	0.21	√
Beilby29	2003	Australia	Population sample (LR)	1079	PCR/RFLP	0.14	0.59	0.26	√
Li30	2003	China	Hypertensive patients (HR)	92	PCR/RFLP	0.11	0.70	0.19	√
Xiang31 NIDDM	2003	China	NIDDM patients (HR)	253	PCR/ASO	0.13	0.63	0.23	√
Xiang31 controls	2003	China	Healthy controls (LR)	106	PCR/ASO	0.10	0.70	0.19	√
Elosua32	2004	US	Population sample (LR)	2723	PCR/RFLP	0.13	0.66	0.21	√
Fernandez33	2004	Spain	CHD patients (HR)	225	PCR/RFLP	0.08	0.70	0.22	?
Kahraman34	2004	Turkey	Renal transplant recipients (HR)	118	PCR/RFLP	0.10	0.78	0.12	?
Bednarska35	2005	Poland	Heavy drinkers (HR)	127	PCR/RFLP	0.13	0.70	0.17	√
Bleil36	2006	US	Untreated hypertensives (HR)	182	PCR/RFLP	0.13	0.66	0.21	√
Debette37	2006	France	Elderly population sample (LR)	5764	Taqman	0.12	0.67	0.19	√
Volcik38 blacks	2006	US	Black population sample (LR)	3187	Taqman	0.16	0.59	0.25	√
Volcik38 whites	2006	US	White population sample (LR)	9304	Taqman	0.20	0.45	0.35	√

NIDDM indicates noninsulin dependent diabetes mellitus; CHD, coronary heart disease; LR, low risk; HR, high risk; PCR/RFLP, polymerase chain reaction/restriction fragment length polymorphism; IEF, isoelectric focusing; PCR/ASO, polymerase chain reaction/allele specific oligonucleotide hybridization; HWE, Hardy-Weinberg equilibrium.