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Genetic Effects on Carotid Intima-Media Thickness : Systematic Assessment and Meta-Analyses of Candidate Gene Polymorphisms Studied in More Than 5000 Subjects

Lavinia Paternoster, Nahara A. Martinez-Gonzalez, Rebecca Charleton, Mabel Chung, Steff Lewis and Cathie L.M. Sudlow

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Genetic Effects on Carotid Intima-Media Thickness

Systematic Assessment and Meta-Analyses of Candidate Gene Polymorphisms Studied in More Than 5000 Subjects

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Background—Carotid intima-media thickness (CIMT) is highly heritable and associated with stroke and myocardial infarction, making it a promising quantitative intermediate phenotype for genetic studies of vascular disease. There have been many CIMT candidate gene association studies, but no systematic review to identify consistent, reliable findings.

Methods and Results—We comprehensively sought all published studies of association between CIMT and any genetic polymorphism. We obtained additional unpublished data and performed meta-analyses for the 5 most commonly studied genes (studied in at least 2 studies in a total of >5000 subjects). We used a 3-step meta-analysis method: meta-analysis of variance; genetic model selection; and random effects meta-analysis of the mean CIMT difference between genotypes. We performed subgroup analyses to investigate effects of ethnicity, vascular risk status, and study size. We accounted for potential reporting bias by assessing qualitatively the possible effects of including unavailable data. Polymorphisms in 3 of the 5 genes (apolipoprotein E, angiotensin I converting enzyme, and 5,10-methylenetetrahydrofolate reductase) had an apparent association with CIMT, but for all these, we found evidence of small study bias. Apolipoprotein E $\epsilon 2/\epsilon 3/\epsilon 4$ was the only polymorphism with a persistent, statistically significant but modest association when we restricted analysis to larger studies (>1000 subjects).

Conclusions—Of the most extensively studied polymorphisms, apolipoprotein E $\epsilon 2/\epsilon 3/\epsilon 4$ is the only one so far with a convincing association with CIMT. Larger studies than have generally been performed so far may be needed to confirm the associations identified in future genome-wide association studies, and to investigate modification of effect according to characteristics such as ethnicity and vascular risk status. (*Circ Cardiovasc Genet.* 2010;3:15-21.)

Key Words: carotid arteries ■ genetics ■ meta-analysis

Studying intermediate, quantitative traits is a potentially useful approach to identify genetic risk factors for ischemic stroke and ischemic heart disease.¹ Carotid intima-media thickness (CIMT) is an intermediate phenotype for early atherosclerosis, is a strong predictor of future vascular events, including myocardial infarction and ischemic stroke, and is significantly greater in large artery (atherothrombotic) than small artery ischemic stroke.²⁻⁴ Estimates of its heritability range from 30% to 86%.⁵⁻⁷

Clinical Perspective on p 21

There have been many studies on the effects of a range of candidate genes on CIMT, the results of which could provide useful insights into genetic influences on atherothrombotic disease and on large artery ischemic stroke and ischemic heart disease. We aimed to identify all published studies of the influence of any genetic polymorphism on CIMT and, for the most commonly studied polymorphisms, to carry out

detailed methodological appraisals and meta-analyses of relevant studies. In doing so, we aimed to establish which candidate gene polymorphisms have been most extensively studied, and of these, which have shown reliable associations with CIMT, and how studies in this area might be improved in the future.

Methods

Identification of Studies

We used a comprehensive, 2-stage search strategy. In stage 1, we used an electronic search in Medline (1966 to end of 2007) and Embase (1980 to end of 2007) combining general genetics terms with terms for CIMT and carotid atheroma (see Methods in the online-only Data Supplement). From an initial screening of the titles, abstracts, and occasionally full articles, we identified all potentially relevant studies.

In stage 2, we carried out a series of supplementary searches in Medline and Embase for genetic polymorphisms that had been studied in at least 2 studies (because of the recognized importance of

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the need for independent replication of genetic associations) in a total of >5000 subjects, combining gene-specific terms with terms for CIMT and carotid atheroma, to ensure that we had identified all potentially relevant articles for the selected polymorphisms (see Methods in the Data Supplement). We used the subject-number cutoff of 5000 to restrict our detailed analysis to a manageable number of polymorphisms for which results were likely to be the most precise and reliable.

We sought studies in all languages, obtaining translations where necessary. We obtained full articles for all potentially relevant studies of the selected polymorphisms, as well as for relevant reviews, and checked the reference lists of these for any further relevant studies.

We included in our analyses any study that had assessed the association between variation in one of the selected polymorphisms and a measure of the thickness of the intima-media of the carotid artery. We excluded data on associations with IMT of arteries other than the carotid, with frank atheroma or plaque in the carotid or other arteries, or with change or rate of change in IMT. We avoided double counting by using only the largest available published dataset from any study described in >1 published article.

Data Extraction

We extracted information from the articles relevant to each selected study on year of publication; total number of subjects studied; country in which the study was conducted; ethnicity of the subjects; types of subjects studied (eg, healthy volunteers, subjects sampled from the general population, subjects with hypertension, or with diabetes); mean age and gender distribution of the subjects; candidate genes and specific polymorphisms studied; whether genotypes were, after calculation or from published data, in Hardy-Weinberg equilibrium; method of CIMT measurement, where possible selecting measurement of the mean of the far wall of the left and right common carotid artery or as close to that as could be achieved; and, for each genotype, number of subjects, and their mean CIMT (and SD). Wherever possible, we treated studies that had presented data separately for groups of subjects defined by criteria such as ethnicity or presence of specific medical condition as separate substudies.

Two authors (L.P. and one among N.A.M.-G., R.C., or M.C.) independently reviewed study eligibility and extracted the information and data from each study, resolving disagreements and uncertainties by discussion and mutual consensus, involving another author (C.L.M.S. or S.L.) if necessary. If key information or data were not presented in the relevant publications, we sought them directly from the authors of the relevant studies.

Statistical Analysis

We used a 3-step approach to investigate the association with CIMT of each genetic polymorphism studied:

1. We first determined whether there was evidence of an overall association between genotype and CIMT, by carrying out a meta-analysis of variance (meta-ANOVA) of CIMT, with study and genotype as categorical variables, weighting studies by the inverse of the square of the SEM CIMT.⁸
2. When we found a statistically significant ($P < 0.05$) overall association from meta-ANOVA, we went on to determine which genetic model (recessive, codominant, or dominant) should be used to determine the size and nature of the association. We used a regression method to estimate the value λ (and its CI), allowing us to select the most appropriate genetic model for meta-analysis, depending on whether λ was closest to 0 (recessive), 0.5 (codominant), or 1 (dominant; see Methods in the Data Supplement). We did not perform further analyses for any polymorphism not reaching statistical significance at the meta-ANOVA stage.
3. Using the selected genetic model, we calculated the study-specific and random effects pooled mean differences in CIMT between genotype groups (CIMT mean difference per single allele change for the codominant model).

For the apolipoprotein E (APOE) genetic polymorphism, the 3 commonly encountered alleles ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$) make up 6 genotypes. For genetic model selection and meta-analysis, we adopted the common convention of grouping these as E2 ($\epsilon 2\epsilon 2$ or $\epsilon 2\epsilon 3$), E3 ($\epsilon 3\epsilon 3$), and E4 ($\epsilon 4\epsilon 4$ or $\epsilon 3\epsilon 4$), such that a codominant model implied equal differences in CIMT between E2, E3, and E4 genotypes.

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.⁹ We performed prespecified subgroup analyses to assess the effects of study size (above or below the mean number of subjects per eligible study or substudy and in a post hoc analysis above or below 1000 subjects), ethnicity (Eastern Asian, Southern Asian, white, or black), and high or low vascular risk status (where studies among subjects with a history of vascular disease or included on the basis of one or more vascular risk factors such as hypertension or diabetes were considered high risk, and studies among healthy volunteers or those from the general population were considered low risk). We used χ^2 tests to assess the significance of differences between subgroups in the size of the association between genotype and CIMT. We assessed the potential for publication bias through subgroup analyses based on study size (above) and visual examination of funnel plots.

We carried out all statistical analyses in Stata version 7.0. We were unable to include in formal meta-analyses several otherwise eligible studies for which the necessary data remained unavailable even after attempts to obtain it from the studies' authors. For each polymorphism selected for analysis, we quantified the proportion of all subjects from eligible studies for whom data were unavailable for meta-analysis. Because qualitative statements about the presence or absence of an association between genotype and CIMT were generally available from the relevant articles for the studies with incomplete data, we attempted informally to assess how our results and overall conclusions might have been affected if we had been able to include these studies in our meta-analyses.

Results

Identification and Selection of Genetic Polymorphisms and Studies for Meta-Analyses

Our stage 1 search strategy yielded 2319 articles, 384 of which seemed to be potentially relevant from titles and abstracts. Polymorphisms in 5 genes (*APOE*, apolipoprotein E; *ACE*, angiotensin I converting enzyme; *MTHFR*, 5,10-methylenetetrahydrofolate reductase; *NOS3*, nitric oxide synthase 3 [endothelial cell]; *ADD1*, adducin 1) had been studied in at least 2 studies in a total of >5000 subjects. We selected these genes for stage 2 gene-specific searching and analyses.

For each of the 5 selected genes, Table 1 gives summary information on the polymorphisms studied, the function of their protein products, and the numbers of relevant studies (and subjects), together with information on numbers of studies (and subjects) that could not be included in meta-analyses because the necessary data were unavailable.

We identified 95 independent studies (77 118 subjects) that analyzed the association between CIMT and a polymorphism or polymorphisms in 1 of the 5 genes of interest (Supplemental references W1–W95). Twenty-nine studies (including 12 982 subjects) did not have all the necessary data available for analysis in the relevant published articles (supplemental references W4, W5, W15, W16, W25, W27, W29, W36, W47, W50, W53, W54, W59, W61, W63, W64, W67, W69, W70, W72, W76, W78, W80, W82, W84, W86, W92, and W94), and authors from 9 of these were able to provide us with additional unpublished data (Supplemental references W15, W16, W27,

Table 1. Five Selected Genes With Polymorphisms Studied for Association With CIMT in >5000 Subjects: Function of Gene Protein Products and Number of Studies (and Subjects) Identified

Gene (Polymorphism)*	No. of Studies/ Substudies (Subjects) in Analysis†	No. of Studies Fulfilling Inclusion Criteria (Subjects)	No. of Studies (Subjects) With Data Available for Analysis	No. of Studies (Subjects) With Incomplete Data, Excluded From Analysis
<i>APOE</i> (ε2, ε3, ε4)	Lipid metabolism	30 (32 995)	27 (32 253)	3 (742)
<i>ACE</i> (I/D)	Renin-angiotensin system (BP/fluid balance)	39 (20 105)	30 (17 038)	9 (3067)
<i>MTHFR</i> (677 C/T)	Homocysteine metabolism	20 (10 487)	13 (7945)	7 (2542)
<i>NOS3</i> (Glu298Asp)	Vascular smooth muscle + endothelial function	12 (7475)	6 (4390)	6 (3085)
<i>ADD1</i> (Gly460Trp)	Endoskeletal protein involved in BP regulation	4 (6056)	3 (5636)	1 (420)

APOE indicates apolipoprotein E; ACE, angiotensin I-converting enzyme; MTHFR, 5,10-methylenetetrahydrofolate reductase; NOS3, nitric oxide synthase 3; ADD1, adducin 1 (α); BP, blood pressure.

*Polymorphisms defined using their common name, 677 C/T notation denotes DNA base change, and Glu298Asp denotes amino acid change.

†Gene functions obtained from UniProtKB/Swiss-Prot database.

W29, W59, W76, W78, W80, and W84), enabling us to retrieve 24% of the unavailable data (5457/12 982 subjects).

Characteristics of Included Studies

The summary characteristics of all relevant studies for the 5 selected genes are shown in supplemental Table I. Sample sizes ranged from 47 to 9304. White subjects from Europe, Australia, and the United States made up the majority of subjects; Eastern Asian subjects were mostly from China, Japan, and Taiwan; and 1 study included Southern Asian subjects. One study was carried out in black Americans.

Subjects were mostly middle aged to elderly. Most were from population samples or healthy volunteers, but some were selected groups of patients at high vascular risk. Genotypes were mostly in Hardy-Weinberg equilibrium, and where they were not, the subjects were generally selected patient groups for which Hardy-Weinberg proportions would not necessarily be expected. CIMT measurement methods varied between studies, but the majority measured the far wall of the common carotid artery.

Tests for Overall Association and Selection of Genetic Models

Table 2 shows the results of the 3-step meta-analysis for the 5 selected genes. Meta-ANOVA found an overall association

between genotype and CIMT at $P < 0.05$ for only 3 polymorphisms: *APOE* (ε2/ε3/ε4), *ACE* (I/D), and *MTHFR* (677 C/T). Linear regression showed that *APOE* (ε2/ε3/ε4) and *ACE* (I/D) should be analyzed according to a codominant genetic model (λ=0.4 and 0.5, respectively). For *MTHFR* (677 C/T), λ was estimated to be 0.2 (95% CI, 0.1 to 0.4). Although the estimated 95% CI did not include any of the expected values (0, 0.5, or 1), we analyzed the data using a recessive model, because 0.2 is closest to 0.

Meta-Analyses of Polymorphisms in APOE, ACE, and MTHFR

We found 30 relevant studies (36 substudies, 32 995 subjects) for *APOE* (ε2/ε3/ε4). Full data for meta-analysis were unavailable in relevant publications or from the authors for 3 studies (742 subjects, 2% of subjects; supplemental references W4, W5, and W25). Meta-ANOVA yielded a significant overall association between *APOE* and CIMT ($P < 0.001$), with a pooled random effects estimate of the difference in mean CIMT per step from E2 to E3 or from E3 to E4 genotypes of 25 μm (95% CI, 17 to 33; Table 2 and supplemental Figure I).

We found 39 relevant studies (43 substudies, 20 105 subjects) for *ACE* (I/D). Full data for meta-analysis were unavailable for 9 studies (3067 subjects, 15% of subjects; supplemental references W25, W36, W47, W50, W53, W54, W61, W63, and

Table 2. Results of the 3-Step Meta-Analysis of the Association Between CIMT and Polymorphisms in the 5 Selected Genes

Gene	No. of Studies (Subjects) in Analyses	Step 1 Meta-ANOVA P Value	Step 2		Step 3 Random Effects Pooled Mean CIMT Difference Between Genotypes With Selected Model, μm (95% CI)
			λ (95% CI)	Selected Genetic Model	
<i>APOE</i> (ε2, ε3, ε4)	32 (32253)	<0.001	0.4 (0.3 to 0.6)	Codominant	25 (17 to 33)
<i>ACE</i> (I/D)	34 (17038)	0.005	0.5 (0.4 to 0.6)	Codominant	14 (5 to 22)
<i>MTHFR</i> (677 C/T)	15 (7945)	0.02	0.2 (0.1 to 0.4)	Recessive	29 (0 to 58)
<i>NOS3</i> (Glu298Asp)	8 (4390)	0.3
<i>ADD1</i> (Gly460Trp)	3 (5636)	0.7

W64). Meta-ANOVA yielded a significant overall association between ACE and CIMT ($P=0.005$), the pooled estimate of the per allele (D) difference in mean CIMT being $14 \mu\text{m}$ (95% CI, 5 to 22; Table 2 and supplemental Figure II).

We found 20 relevant studies (22 substudies, 10 487 subjects) for MTHFR (677 C/T). Full data for meta-analysis were unavailable for 7 studies (2542 subjects, 24% of subjects; supplemental references W50, W63, W67, W69, W70, W72, and W82). Meta-ANOVA yielded a significant overall association between MTHFR and CIMT ($P=0.02$). The pooled estimate of the difference between TT and CT/CC genotypes was $29 \mu\text{m}$ (95% CI, 0 to 58; Table 2 and supplemental Figure III).

For each of these 3 genetic polymorphisms, there was substantial heterogeneity between studies (I^2 values $\approx 80\%$; supplemental Figure I through III). The results of prespecified subgroup analyses to investigate possible causes of this heterogeneity were strikingly similar for all 3 polymorphisms (Figure). We found substantially larger pooled mean CIMT differences among Eastern Asian compared with white population (the subgroup difference was highly significant for APOE), in high-risk populations compared with low-risk populations (subgroup differences clearly significant for all 3 polymorphisms), and in smaller compared with larger studies (subgroup differences clearly significant for all 3 polymorphisms), suggesting the existence of small study bias. For each polymorphism (especially APOE and ACE), there was less heterogeneity between the results of the larger studies. Study size could explain the apparent difference in size of association between different ethnicities and risk groups, because studies in high-risk populations and among Eastern Asian subjects were on average much smaller than among low-risk populations and white subjects (Figure). In keeping with the results of subgroup analyses based on study size, funnel plots of the study-specific mean difference versus its standard error were asymmetrical for all 3 polymorphisms, suggestive of small study bias, most likely publication bias.

Focusing attention on just the larger (and presumably more reliable) studies, an association between CIMT and APOE remained, albeit much smaller than the overall pooled estimate, with an estimated mean CIMT difference of $8 \mu\text{m}$ (95% CI, 6 to 11) per step from E2 to E3 to E4 genotype groups, but associations between CIMT and both ACE (I/D) and MTHFR (677 C/T) were smaller and no longer statistically significant (for ACE, mean CIMT difference per additional D allele: $4 \mu\text{m}$, 95% CI, 0 to 8; for MTHFR, mean CIMT difference between TT and CT/CC: $-9 \mu\text{m}$, 95% CI, -32 to 13). In a further post hoc subgroup analysis, we restricted analyses to studies in >1000 subjects and found a similar, significant result for APOE and similar, nonsignificant results for ACE and MTHFR (data not shown).

Of the 3 studies with data unavailable for the APOE meta-analysis, one found an association between E4 genotypes and higher CIMT (W4), another found a similar association in the nondiabetic subgroup only (W5), and the third found no association (W25). All 3 were small (66, 206, and 470 subjects) and so would not have contributed to the analysis including only larger studies (supplemental Figure I).

Most of the 9 studies with data unavailable for the ACE meta-analysis reported no association. Three would have contributed to our "larger studies" analysis, using the mean study size cutoff criterion (W25, W61, and W63). Of these, 2 found that the D allele was associated with increased CIMT, so their inclusion could potentially have strengthened the association between ACE and CIMT. However, none of the studies with data unavailable for meta-analysis would have been large enough to merit inclusion in our analysis of studies including >1000 subjects (supplemental Figure II).

Of the 7 studies with data unavailable for the MTHFR meta-analysis, 2 were larger than the mean study size cutoff for the study size subgroup analysis (W63 and W69). The smaller of these found an association between MTHFR (677 C/T) and CIMT whereas the other did not, and so their inclusion would seem unlikely to materially alter the results of any of the MTHFR analyses (supplemental Figure III).

Other Selected Genes

There was no association between CIMT and polymorphisms in nitric oxide synthase 3 or adducin 1 (Table 2). Although the proportion of data unavailable for meta-analysis was $>40\%$ for nitric oxide synthase 3 (Table 1), almost all studies with unavailable data reported either no association or association only in a particular subgroup (eg, in males or black diabetics only). Thus, it seems unlikely that the inclusion of these data would have materially affected the results for these polymorphisms.

Other Potential Genes of Interest

Our search strategy identified polymorphisms in >140 genes that had been studied for an association with CIMT, but less than half of these had been assessed in >1 study. Of those studied in <5000 subjects, 19 genetic polymorphisms had been studied in a total of ≈ 3000 to 5000 subjects, a further 53 in a total of ≈ 1000 to 3000 subjects, and a further 68 in an estimated total of <1000 subjects. Excluding those included in our meta-analyses, for 46 genetic polymorphisms, the largest study done had >1000 subjects (and so could, according to the results of our analyses based on study size, be considered reliable), but in no more than a handful of cases was the genetic polymorphism assessed in at least 2 studies including >1000 subjects each. Many of the genetic polymorphisms studied but not included in our meta-analyses showed preliminary evidence for an association with CIMT, but most if not all of these would need replication in much larger samples before the results could be considered reliable. For many others, the study or studies performed were too small reliably to detect effects of moderate size.

Discussion

Although there have been narrative reviews of the genetics of CIMT,¹⁰ to our knowledge, there has not previously been an attempt to review the evidence for genetic associations with this phenotype systematically. Narrative reviews draw attention to exciting new findings and may stimulate new research but may selectively emphasize results of particular studies, and so can sometimes be misleading.¹¹

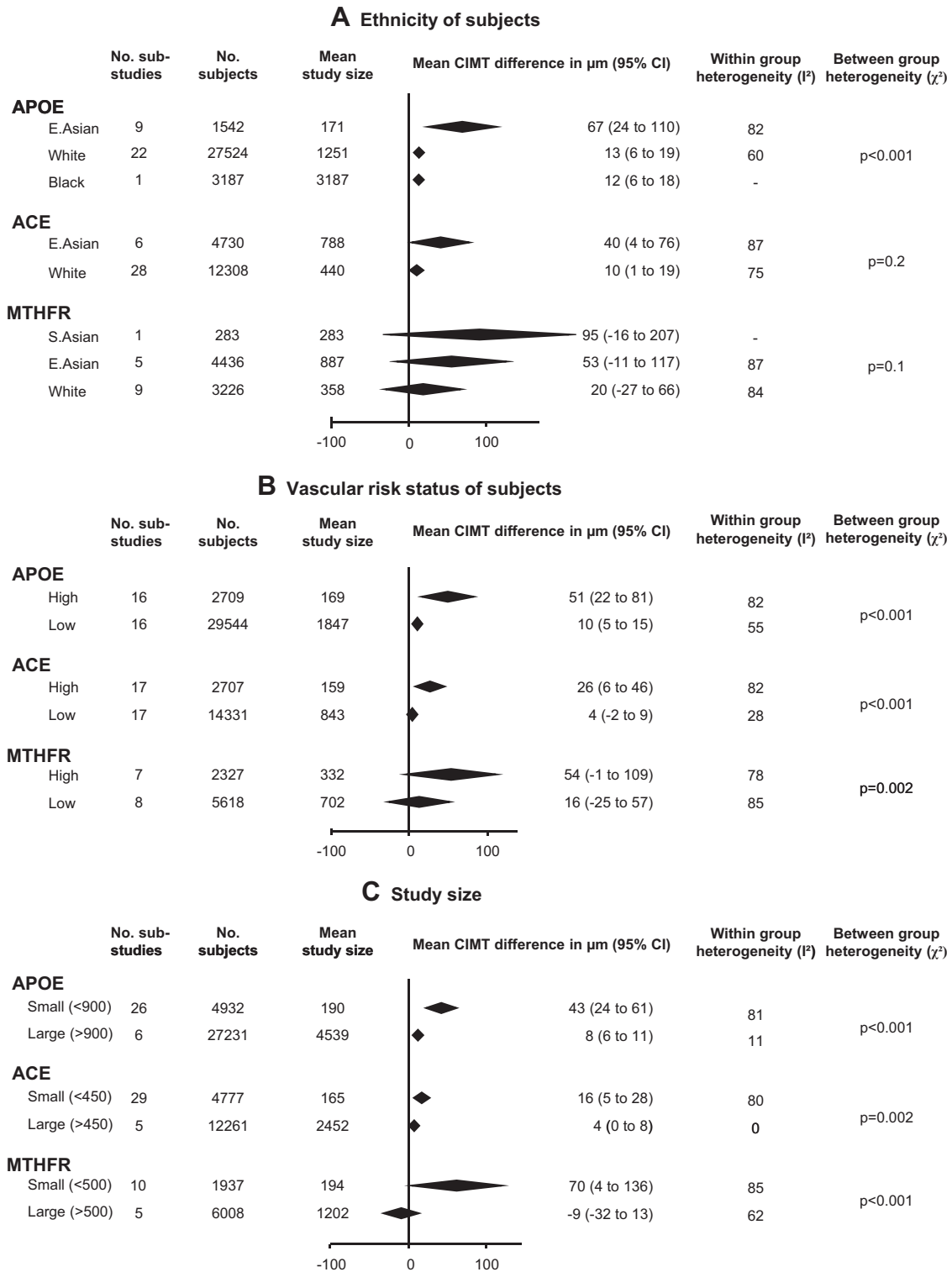


Figure. Meta-analyses of associations with CIMT of APOE ($\epsilon 2/\epsilon 3/\epsilon 4$), ACE (I/D), and MTHFR (677 C/T) by subgroup, according to ethnicity (A), according to the vascular risk status (B), and according to study size (C) (above and below the mean study size for each gene). The data are analyzed according to a codominant genetic model for APOE and ACE and a recessive model for MTHFR. Diamonds represent random effects pooled mean differences and the width of each diamond represents the 95% CI. The horizontal axis refers to the mean difference between genotype groups (E4–E3 and E3–E2) for APOE, the per D allele increase for ACE, and the difference between TT and CT/CC for MTHFR.

Our systematic review identified >140 genes studied as candidates for association with CIMT. We reviewed in detail 95 independent studies of the association between CIMT and polymorphisms in the 5 most commonly studied genes and

found clear evidence of an association for only one of these. Polymorphisms in the APOE, ACE, and MTHFR genes all showed a significant association with CIMT in meta-ANOVA analysis. But, of these, APOE ($\epsilon 2/\epsilon 3/\epsilon 4$) was the

only polymorphism whose association withstood restricting analysis to larger studies only, suggesting that although there almost certainly is an association, its size is overestimated in the literature because of small study bias. Our subgroup analyses suggest that the apparent associations with CIMT of ACE and MTHFR, and apparent modification of genetic effects according to ethnicity and vascular risk status, may well be due to small study bias. The 95% CI from analyses restricted to the larger and more reliable studies of the relevant polymorphisms in these genes would suggest that, for both ACE I/D and MTHFR 677 C/T, an association with CIMT remains possible, but is unlikely to be larger in magnitude than 8 μm per additional D allele for ACE I/D and could range between approximately -35 and 13 μm for MTHFR 677 TT versus CT/CC.

Our study has some particular strengths. We used a series of explicit, predefined methods to select genes for further investigation, to assess whether there was an overall association between genotype and CIMT, and to choose the most appropriate genetic model for meta-analysis. We also sought additional unpublished data where it was unavailable in publications and considered the implications of any data that remained unavailable for analysis. Publications identified by our search in which the association between a genetic polymorphism and CIMT was not the main feature of the article tended not to identify an association and not to report results in full, although sometimes reported positive results for particular subgroups. Thus, we identified and accounted for reporting bias, where positive results tend to be highlighted and reported in more detail than negative ones. Previous meta-analyses in stroke genetics have used adequate reporting of data within publications as a criterion for inclusion,¹² which might make them prone to reporting bias.

Two linkage studies have identified quantitative trait loci for CIMT. One reported a maximum LOD score of 4.1 at 161 cM on chromosome 12 and subsequently found evidence of association with an atherosclerosis candidate gene (SCARB1, a high density lipoprotein receptor, cell-surface glycoprotein) from the region of linkage.¹³ The other identified 2q33-35 as a region with significant linkage (LOD=3.08), including the NOSTRIN, IGFBP2, and IGFBP5 genes, none of which have yet been independently tested for an association with CIMT.¹⁴

In the current era of genome-wide association studies, further promising candidates for CIMT are likely to emerge in the near future. The findings of our systematic review suggest that, if CIMT is to fulfill its promise as an intermediate phenotype that will improve our understanding of the genetics of vascular diseases, studies to confirm associations with promising candidates identified in genome-wide studies may have to be very large. For example, if other polymorphisms influencing CIMT have effect sizes similar to that of APOE, then studies would need to be powered to detect between-genotype differences in CIMT of as small as ≈ 10 μm (the approximate effect size found by pooling data from the larger, more reliable studies). Taking estimates of the population mean and standard deviation of CIMT from a recent relevant review (mean, 700 μm ; SD, 160 μm),² a sample size of >6000 subjects would be needed for 80% power at $P<0.05$ (and of almost 12 000 subjects for 90%

power at $P<0.01$) to detect a per-genotype mean CIMT difference of 10 μm in a codominant model, assuming a minor allele frequency of 0.2. Estimated sample sizes are larger with more stringent power and significance criteria or for polymorphisms with a lower minor allele frequency but are smaller with a higher minor allele frequency, or if we only want reliably to detect larger CIMT differences of up to, say, 100 μm . For example, reliable detection (90% power at $P<0.01$) of a per-genotype mean CIMT difference of 100 μm assuming a minor allele frequency of 0.05 would need ≈ 400 subjects (supplemental Table II). To set this in context, an increase in CIMT of 100 μm is associated with an increased future risk of myocardial infarction and stroke of $\approx 15\%$ and 18% , respectively.² At any minor allele frequency, more than ≈ 2000 subjects would be required reliably to detect a CIMT difference of 25 μm (supplemental Table II), and because it is recommended that findings are replicated in studies that are larger than the discovery dataset, a total of >5000 subjects would be required to detect and confirm such effects confidently. However, our study's stringent cutoff of 5000 subjects for inclusion means that we may have missed genetic polymorphisms with effect sizes much larger than this.

So far, among the most extensively studied genetic polymorphisms, APOE $\epsilon 2/\epsilon 3/\epsilon 4$ is the only one to have shown a convincing association with CIMT, with E4 genotypes associated with an increase and E2 genotypes with a decrease in CIMT. This is consistent with meta-analyses showing an association between the APOE polymorphism and ischemic heart disease,¹⁵ our previous meta-analysis of the association between APOE and stroke subtypes, which suggested that APOE E4 genotypes may be specifically associated with the large artery subtype of stroke but not with other ischemic subtypes,¹⁶ with our previous meta-analysis of APOE and CIMT that used less sophisticated statistical methods,¹⁷ and with our meta-analysis of APOE and white matter hyperintensities on brain imaging (a quantitative phenotype linked to small vessel disease lacunar stroke), which did not identify an association.¹⁸

The genes we reviewed here were those most commonly studied, mainly because they are key genes in known candidate pathways for vascular disease. This method of selecting genes is limited by current knowledge. Recent successes with the genome-wide association approach show the potential for hypothesis-free methods to identify candidate genes in novel pathways,¹⁹ which, if confirmed in studies of adequate size, may lead to new insights into the causes of and treatments for complex disease, including CIMT and so atherothrombotic vascular diseases.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Carotid intima-media thickness (CIMT) is a measure of subclinical atherosclerosis, is associated with future risk of myocardial infarction and stroke, and is highly heritable. Studies of genetic factors influencing CIMT may identify genes that alter risk of ischemic heart disease and stroke, in particular large artery ischemic stroke, thereby helping us to better predict the risk of developing these clinical conditions and better understand their aetiology. We systematically identified and performed meta-analyses of all studies of the association of CIMT with variation in any gene that had been studied in >2 studies with a total of >5000 individuals. We found only 1 gene (apolipoprotein E, which affects cholesterol levels) that had a robust association with CIMT, but the magnitude of the association was quite small, limiting its utility in clinical risk prediction. Genome-wide studies and large confirmatory candidate gene studies are needed for reliable identification of genes associated with CIMT.

SUPPLEMENTAL MATERIAL

Supplemental methods

Stage 1 Medline search strategy*

- 1 exp carotid artery diseases/ge
- 2 exp carotid arteries/
- 3 (carotid adj8 (atherosclero\$ or stenosis\$ or plaque\$ or intima media\$ or intimal media\$ or ultrasound or sclero\$ or atheroma\$ or wall or thick\$)).tw.
- 4 2 or 3
- 5 exp genetics/ or exp genotype/ or exp inheritance patterns/ or exp "linkage (genetics)"/ or exp phenotype/ or exp "variation (genetics)"/ or chromosomes/ or exp genes/ or exp genome/
- 6 (polymorphi\$ or genotyp\$ or gene or genes or genetic\$ or allel\$ or mutat\$).tw.
- 7 5 or 6
- 8 4 and 7
- 9 1 or 8
- 10 limit 9 to humans

* we used a similar, appropriately adapted strategy for Embase

Stage 2 Medline search strategy for MTHFR*

- 1 exp carotid artery diseases/ge
- 2 exp carotid arteries/
- 3 (carotid adj8 (atherosclero\$ or stenosis\$ or plaque\$ or intima media\$ or intimal media\$ or ultrasound or sclero\$ or atheroma\$ or wall or thick\$)).tw.
- 4 1 or 2 or 3
- 5 exp "Methylenetetrahydrofolate Reductase (NADPH2)"/ge [Genetics]
- 6 (MTHFR or methylenetetrahydrofolate or c677t or nadph2).tw.
- 7 methylene tetrahydrofolate.tw.
- 8 5 or 6 or 7
- 9 4 and 8

* we used a similar, appropriately adapted strategy for the other genes and for Embase

Details of method of genetic model selection

We describe the process of genetic model selection by denoting the genotypes for a particular polymorphism with two possible alleles as aa , ab , and bb , where a is the common allele and b is the rare allele. For each study, i , we calculated $MD1_i$, the difference in mean CIMT for subjects with ab versus aa genotypes, and $MD2_i$, the difference in mean CIMT for subjects with bb versus aa genotypes. We performed a weighted linear regression of $MD1_i$ on $MD2_i$, weighting each study by the inverse of the square of the mean of the standard errors of $MD1_i$ and $MD2_i$ for that study, and forcing the regression line to pass through the origin $(0,0)$. The gradient of the regression line estimated the value λ (and its confidence interval, CI), allowing selection of the most appropriate genetic model for meta-analysis,

depending on whether λ was closest to 0 (recessive, bb versus ab/aa), 0.5 (co-dominant, bb versus ab versus aa, giving mean CIMT per single allele change), or 1 (dominant, bb/ab versus aa).

Where there was only one study for a particular selected gene, meta-ANOVA in step one was replaced by ANOVA, and λ was simply calculated as MD1/MD2 for that study.

Supplemental tables

Supplemental table 1. Characteristics of studies included for each of the 5 selected genes (as shown in Table 1)

Study (first author & publication year)	No. of subjects	Country	Ethnicity of subjects	Type of subjects	Mean age ± SD	% male	Vascular risk status	HWE	Carotid segment	Near/far carotid wall	Right/left carotid	Mean/max
APOE												
Terry 1996 ^{W1}	254	US	White	Coronary angiography referrals	59±9	50	High	✓	CCA	Both	Both	Mean of max of 4 sites
Cattin 1997 ^{W2}	254	Italy	White	Population sample	53±7	46	Low	✓	CCA	Both	Both	Mean of right and left
Kogawa 1997a ^{W3}	349	Japan	E.Asian	NIDDM patients	60±11	58	High	✓	CCA/BIF	*	*	Mean of 3 sites
Kogawa 1997b ^{W3}	231	Japan	E.Asian	Non-diabetic subjects	51±11	37	Low	✓	CCA/BIF	*	*	Mean of 3 sites
Olmer 1997 ^{W4 ‡}	66	France	White	Hemodialysis patients	50±15	50	High	✓	CCA	Far	Both	Mean of 3 each side
Vauhkonen 1997a ^{W5 §}	83	Finland	White	NIDDM patients	56±7	52	High	✓	CCA/BIF	Far	Both	Mean of max of 4 sites
Vauhkonen 1997b ^{W5 §}	123	Finland	White	Population sample	54±5	46	Low	✓	CCA/BIF	Far	Both	Mean of max of 4 sites
Sass 1998 ^{W6}	144	France	White	Population sample	41±4	52	Low	*	CCA	*	Both	Mean of 2 each side
Zhang 1998 ^{W7}	52	China	E.Asian	CHD patients	57±8	100	High	✓	CCA/BIF/ICA	Far	Both	Mean of 8 sites
Guz 2000 ^{W8}	261	Turkey	White	Hemodialysis patients	46±15	57	High	✓	CCA	*	Both	Mean of 3 each side
Hanon 2000 ^{W9}	312	France	White	Patients with vascular risk factors/disease	49±12	53	High	*	CCA	Far	Right	One measurement
Horejsi 2000 ^{W10}	112	Czech Republic	White	Lipoprotein disorder patients	53±*	45	High	*	CCA	Far	*	Mean of max of 3 sites
Ilveskoski 2000 ^{W11}	189	Finland	White	Population sample	54±3	100	Low	✓	CCA	Both	Both	Max of 4 sites
Slooter 2001 ^{W12}	5264	Netherlands	White	Population sample	69±9	41	Low	✓	CCA	Far	Both	Mean of left and right
Tabara 2001 ^{W13}	202	Japan	E.Asian	Population sample	70±9	32	Low	✓	CCA	Far	Right	Mean of 3 sites
Haraki 2002 ^{W14}	95	Japan	E.Asian	Healthy subjects	50±8	100	Low	✓	CCA	Far	Right	Mean of 9 sites
Karvonen 2002a ^{W15 ‡}	258	Finland	White	Hypertensive patients	51±6	100	High	✓	CCA/BIF/ICA	Far	Both	Mean of 20 sites
Karvonen 2002b ^{W15 ‡}	253	Finland	White	Population sample	51±6	100	Low	✓	CCA/BIF/ICA	Far	Both	Mean of 10 sites
Asakimori 2003 ^{W16 ‡}	162	Japan	E.Asian	Hemodialysis patients	55±11	52	High	✓	CCA	Far	Both	Maximum
Beilby 2003 ^{W17}	1079	Australia	White	Population sample	53±13	50	Low	✓	CCA	Far	Both	Mean of 3 each side
Li 2003 ^{W18}	92	China	E.Asian	Hypertensive patients	64±11	55	High	✓	CCA	*	*	*
Xiang 2003a ^{W19}	253	China	E.Asian	NIDDM patients	*	*	High	✓	CCA/BIF/ICA	Far	Both	Mean of 3 each side
Xiang 2003b ^{W19}	106	China	E.Asian	Healthy controls	*	*	Low	✓	CCA/BIF/ICA	Far	Both	Mean of 3 each side
Elosua 2004 ^{W20}	2723	US	White	Population sample	59±10	48	Low	✓	CCA	Both	Both	Mean of max each side
Fernandez 2004 ^{W21}	225	Spain	White	CHD patients	61±8	85	High	*	CCA	Far	Both	Mean of 3 each side
Kahraman 2004 ^{W22}	118	Turkey	White	Renal transplant recipients	40±8	68	High	*	CCA	*	Both	Mean of left and right
Bednarska 2005 ^{W23}	127	Poland	White	Alcoholics	49±6	100	High	✓	CCA	Far	Both	Mean of 3 each side
Bleil 2006 ^{W24}	182	US	White	Hypertensive patients	56±9	100	High	✓	CCA/BIF/ICA	Both	Both	Mean of all sites
Brenner 2006 ^{W25 ‡}	470	France	White	Ischemic stroke patients	range 18-85	*	High	*	CCA	Far	Both	Mean of right and left
Debette 2006 ^{W26}	5764	France	White	Population sample	74±5	40	Low	✓	CCA	Far	Both	Mean of right and left
Junyent 2006 ^{W27 ‡}	163	Spain	White	Familial hypercholesterolemia patients	47±*	*	High	✓	CCA	Far	Both	Mean of right and left
Volcik 2006a ^{W28}	3187	US	Black	Population sample	range 45-64 [†]	*	Low	✓	CCA/BIF/ICA	*	Both	Mean of 6 sites

Study (first author & publication year)	No. of subjects	Country	Ethnicity of subjects	Type of subjects	Mean age \pm SD	% male	Vascular risk status	HWE	Carotid segment	Near/far carotid wall	Right/left carotid	Mean/max
Volcik 2006b ^{W28}	9304	US	White	Population sample	range 45-64 [†]	*	Low	✓	CCA/BIF/ICA	*	Both	Mean of 6 sites
Altamura 2007a ^{W29 ‡}	68	Italy	White	Alzheimer disease patients	75 \pm 8	31	High	*	CCA	*	Both	Mean of right and left
Altamura 2007b ^{W29 ‡}	33	Italy	White	Vascular dementia patients	77 \pm 8	51	High	*	CCA	*	Both	Mean of right and left
Wohlin 2007 ^{W30}	437	Sweden	White	Population sample	all 75	100	Low	✓	CCA	Far	Both	Mean of 3 each side
ACE												
Castellano 1995 ^{W31}	187	Italy	White	Population sample	58 \pm 3	52	Low	✓	CCA/BIF/ICA		Both	Mean of all sites
Dessi 1995 ^{W32}	240	Italy	White	Outpatients without vascular risk factors	53 \pm 7	57	Low	✓	CCA/BIF/ICA	Both	Both	Mean
Markus 1994 ^{W33}	101	UK	White	Ischemic CVD patients	65 \pm 9	68	High	✓	CCA	Far	*	Maximum
Kauma 1996 ^{W34}	515	Finland	White	Hypertensive patients	51 \pm 6	49	High	✓	CCA	Far	Both	Mean of max at each site
Puija 1996 ^{W35}	132	Italy	White	NIDDM patients	50 \pm 10	100	High	✓	CCA	Far	Both	Mean of 6 sites
Kogawa 1997a ^{W3}	356	Japan	E.Asian	NIDDM patients	60 \pm 11	58	High	✓	CCA/BIF	*	*	Mean of 3 sites
Kogawa 1997b ^{W3}	235	Japan	E.Asian	Non-diabetic subjects	51 \pm 11	37	Low	✓	CCA/BIF	*	*	Mean of 3 sites
Watanabe 1997 ^{W36 §}	169	Japan	E.Asian	Healthy volunteers	59 \pm 6	51	Low	✓	CCA/BIF/ICA	Both	Both	Mean
Arnett 1998 ^{W37}	495	US	White	Population sample	59 \pm 6	42	Low	✓	CCA/BIF/ICA	Far	Both	Mean of 6 sites
Frost 1998 ^{W38}	148	Germany	White	IDDM patients	30 \pm 7	38	High	✓	CCA	Far	Both	Maximum
Girerd 1998 ^{W39}	340	France	White	Patients with vascular risk factors/disease	49 \pm 12	53	High	✓	CCA	Far	Right	One measurement
Sass 1998 ^{W40}	150	France	White	Population sample	41 \pm 4	52	Low	✓	CCA	Far	Both	Mean of all
Ferrieres 1999 ^{W41}	355	France	White	Population sample	54 \pm 7	100	Low	✓	CCA	Far	Both	Mean of 12 sites
Huang 1999 ^{W42}	219	Finland	White	Population sample	54 \pm 3	100	Low	✓	CCA	Far	Both	Maximum
Hung 1999 ^{W43}	1106	Australia	White	Population sample	53 \pm 12	50	Low	✓	CCA	Far	Both	Mean of 6 sites
Nergizoglu 1999 ^{W44}	51	Turkey	White	Hemodialysis patients	36 \pm 9	69	High	✓	CCA	Far	Both	Mean of 6 sites
Pit'ha 1999 ^{W45}	47	Czech Republic	White	Hypertensive patients	62 \pm 3	100	High	✓	CCA	Far	Both	Mean of 10 sites
Jeng 2000 ^{W46}	175	China	E.Asian	Hypertensive patients	57 \pm 10	52	High	X	CCA	Far	Both	Mean of right and left
Pontremoli 2000 ^{W47 ‡}	215	Italy	White	Hypertensive patients	48 \pm 9	62	High	✓	CCA	Far	Both	Mean of 3 sites
Taute 2000 ^{W48}	98	Germany	White	PAD patients	61 \pm 9	79	High	✓	CCA	Far	Both	Maximum
Mannami 2001 ^{W49}	3657	Japan	E.Asian	Population sample	60 \pm 12	46	Low	✓	CCA	Both	Both	Mean of 4 sites
Markus 2001a ^{W50 ‡}	199	UK	White	Population sample	60 \pm 8	100	Low	✓	CCA	Far	Both	Mean
Markus 2001b ^{W50 ‡}	88	UK	Black	Population sample	64 \pm 8							
Tabara 2001 ^{W13}	205	Japan	E.Asian	Healthy population sample	70 \pm 9	32	Low	✓	CCA	Far	Right	Mean
Balkestein 2002 ^{W51}	380	Belgium	White	Population sample	40 \pm 16	50	Low	✓	CCA	Far	Right	Mean of 3 sites
Diamantopoulos 2002 ^{W52}	184	Greece	White	NIDDM patients	62 \pm 8	41	High	✓	CCA	Far	Both	Max of mean from each side
Kawamoto 2002 ^{W53 §}	184	Japan	E.Asian	In-patients being evaluated for possible atherosclerosis	67 \pm 14	47	High	✓	CCA	Far	Both	Mean of right and left
Piao 2002 ^{W54 §}	262	Japan	E.Asian	NIDDM patients	58 \pm 10	66	High	*	CCA/BIF/ICA	*	Both	Mean of 6 sites
Czarnecka 2004a ^{W55}	127	Poland	White	Population sample – parents	51 \pm 5	40	Low	✓	CCA	Both	Both	*
Czarnecka 2004b ^{W55}	157	Poland	White	Population sample – offspring	24 \pm 5	50	Low	✓	CCA	Both	Both	*
Li 2004 ^{W56}	102	China	E.Asian	Hypertensive patients	54 \pm 9	*	High	X	CCA	Both	Both	Mean of 12 sites
Pall 2004a ^{W57}	120	Hungary	White	Hypertensive students	16 \pm 1	53	High	✓	CCA	*	*	Mean of 3 sites

Study (first author & publication year)	No. of subjects	Country	Ethnicity of subjects	Type of subjects	Mean age ± SD	% male	Vascular risk status	HWE	Carotid segment	Near/far carotid wall	Right/left carotid	Mean/max
Pall 2004b ^{W57}	58	Hungary	White	Non-hypertensive students	*	*	Low	✓	CCA	*	*	Mean of 3 sites
Bednarska 2005 ^{W23}	130	Poland	White	Alcoholics	48±6	100	High	✓	CCA	Far	Both	Mean of each side
Sleegers 2005 ^{W58}	6488	Netherlands	White	Population sample	69±9	41	Low	✓	CCA	Both	Both	*
Varda 2005a ^{W59 †}	56	Slovenia	White	Offspring of CVD patients	18±6	52	High	✓	CCA/ICA	*	Both	Mean of 4 sites
Varda 2005b ^{W59 †}	48	Slovenia	White	Subjects without parental history of CVD	18±6	52	Low	✓	CCA/ICA	*	Both	Mean of 4 sites
Bilici 2006 ^{W60}	64	Turkey	White	Memory impaired patients	57±13	83	High	✓	CCA	Far	Both	Mean of right and left
Brenner 2006 ^{W25 †}	470	France	White	Ischemic stroke patients	range 18-85	*	High	*	CCA	Far	Both	Mean of right and left
Burdon 2006 ^{W61 †}	737	US	White	NIDDM patients & their siblings	61±10	43	High	✓	CCA	Both	Both	Mean of 20 sites
Islam 2006 ^{W62}	224	Finland	White	Population sample	34±2	54	Low	✓	CCA	Far	Left	Mean of 4 sites
Yamasaki 2006 ^{W63 †}	690	Japan	E.Asian	NIDDM patients	63±7	52	High	*	CCA/BIF/ICA	*	Both	Mean of max
Bartoli 2007 ^{W64 §}	53	Italy	White	Systemic sclerosis patients	60±11	11	High	✓	CCA	Far	Both	Mean of right and left
Tanriverdi 2007 ^{W65}	88	Japan	E.Asian	Coronary angiography patients	55±11	55	High	X	CCA	*	Both	Mean of 8 sites
MTHFR												
Arai 1997 ^{W66}	222	Japan	E.Asian	NIDDM patients	60±8	73	High	✓	BIF	Both	Both	Maximum
Demuth 1998 ^{W67 †}	144	France	White	Patients with vascular risk factors/disease	48±13	46	High	✓	CCA	Far	Right	*
Mazza 1999 ^{W68}	95	Italy	White	NIDDM patients	53±10	35	High	✓	CCA	Far	Both	Mean of 6 sites
McQuillan 1999 ^{W69 †}	1111	Australia	White	Population sample	53±13	50	Low	✓	CCA	Far	Both	Mean of 6 sites
Kawamoto 2001 ^{W70 †}	136	Japan	E.Asian	Patients with vascular risk factors	74±12	45	High	✓	CCA	Far	Both	Mean
Lim 2001 ^{W71}	151	Taiwan	E.Asian	End stage renal disease patients	55±14	42	High	✓	CCA	Both	*	Mean
Markus 2001a ^{W50 †}	195	UK	White	Population sample	60±8	100	Low	✓	CCA	Far	Both	Mean
Markus 2001b ^{W50 †}	84	UK	Black	Population sample	64±8							
Pallaud 2001 ^{W72 §}	121	France	White	Population sample	43±5	64	Low	✓	CCA	Far	Both	Mean
Passaro 2001 ^{W73}	120	Italy	White	Healthy post-menopausal women	62±4	0	Low	✓	CCA	Both	Both	Mean of max
Ravera 2001 ^{W74}	206	Italy	White	Hypertensive patients	48±9	*	High	✓	CCA	Far	Both	Mean of 3 sites
Scaglione 2002 ^{W75}	124	Italy	White	NIDDM patients	65±8	76	High	✓	CCA	Far	Both	Mean of 6 sites
de Maat 2003 ^{W76 †}	691	Denmark	White	Population sample	All 60	47	Low	✓	CCA/BIF/ICA	Both	Right	Mean of 3 sites
Inamoto 2003 ^{W77}	3247	Japan	E.Asian	Population sample	59±13	48	Low	✓	CCA	Both	Both	Mean
Kelemen 2004a ^{W78 †}	260	Canada	White	Population sample	49±*	49	Low	✓	CCA/BIF/ICA	Both	Both	Mean of max
Kelemen 2004b ^{W78 †}	275	Canada	E.Asian	Population sample	47±*	53	Low	✓	CCA/BIF/ICA	Both	Both	Mean of max
Kelemen 2004c ^{W78 †}	283	Canada	S.Asian	Population sample	48±*	54	Low	✓	CCA/BIF/ICA	Both	Both	Mean of max
Durga 2005 ^{W79}	815	Netherlands	White	Patients with high homocysteine	60±6	72	High	✓	CCA	Both	Both	Mean of max
McDonald 2005 ^{W80 †}	201	Australia	White	Population sample	37±*	44	Low	X	CCA	Both	*	Mean of 6 sites
Linnebank 2006 ^{W81}	714	Germany	White	Vascular event patients	64±9	49	High	✓	CCA	Far	*	Mean
Yamasaki 2006 ^{W63 †}	690	Japan	E.Asian	NIDDM patients	63±7	52	High	*	CCA/BIF/ICA	*	Both	Mean of max
Fernandez 2007 ^{W82 §}	61	Spain	White	Patients with coronary disease	68±7	82	High	*	CCA	Far	Both	Mean of 6 sites
Liu 2007 ^{W83}	541	Taiwan	E.Asian	Healthy volunteers	53±15	50	Low	✓	CCA	Far	Either	Mean of 4 sites

NOS3

Study (first author & publication year)	No. of subjects	Country	Ethnicity of subjects	Type of subjects	Mean age \pm SD	% male	Vascular risk status	HWE	Carotid segment	Near/far carotid wall	Right/left carotid	Mean/max
Leumbo 2001 ^{W84 †}	375	Italy	White	Hypertensive patients	54 \pm *	55	High	✓	CCA/BIF/ICA	Both	Both	Maximum
Karvonen 2002 a ^{W85}	505	Finland	White	Hypertensive patients	51 \pm 6	49	High	✓	CCA	Far	Both	Mean of 10 sites
Karvonen 2002b ^{W85}	519	Finland	White	Population sample	51 \pm 7	50	Low	✓	CCA	Far	Both	Mean of 10 sites
Asakimori 2003 ^{W16 †}	163	Japan	E.Asian	Hemodialysis patients	55 \pm 11	52	High	✓	CCA	Far	Both	Maximum
Schmoelzer 2003 ^{W86 §}	932	Italy	White	Population sample	53 \pm 6	55	Low	✓	CCA/BIF/ICA	Both	Both	Mean of 12 sites
Paradossi 2004 ^{W87}	118	Italy	White	Population sample	30 \pm 5	39	Low	✓	CCA	*	Both	Mean of max
Czarnecka 2005a ^{W88}	127	Poland	White	Population sample – parents	51 \pm 5	40	Low	✓	CCA	Both	Both	*
Czarnecka 2005b ^{W88}	167	Poland	White	Population sample – offspring	24 \pm 5	50	Low	✓	CCA	Both	Both	*
Spoto 2005 ^{W89}	131	Italy	White	Hemodialysis patients	61 \pm 13	60	High	✓	CCA/BIF/ICA	Far	Both	Mean of 12 sites
Wolff 2005 ^{W90}	2448	Germany	White	Population sample	62 \pm 10	51	Low	✓	CCA	Far	Both	Mean of 20 sites
Brenner 2006 ^{W25 †}	470	France	White	Ischemic stroke patients	range 18-85	*	High	*	CCA	Far	Both	Mean of right and left
Burdon 2006 ^{W61 †}	737	US	White	NIDDM patients & their siblings	61 \pm 10	43	High	✓	CCA	Both	Both	Mean of 20 sites
Lekakis 2006 ^{W91 §}	122	Greece	White	Coronary angiography patients	61 \pm 10	84	High	*	CCA/BIF/ICA	Far	Both	Mean of max of 6 sites
Bhuiyan 2007 ^{W92 §}	661	US	White	Population sample	37 \pm 4	40	Low	✓	CCA	Far	Both	Mean of max of 6 sites
ADD1												
Castellano 1997 ^{W93}	173	Italy	White	Population sample	57 \pm 5	50	Low	✓	CCA	Far	Both	Mean
Balkestein 2002 ^{W51}	380	Belgium	White	Population sample	40 \pm 16	49	Low	✓	CCA	Far	Right	Mean of 3 sites
Sarzani 2006 ^{W94 §}	420	Italy	White	Medical student volunteers	23 \pm 2	52	Low	X	CCA/BIF	Both	Both	Mean of max of 8 sites
Yazdanpanah 2006 ^{W95}	5083	Netherlands	White	Population sample	69 \pm 9	40	Low	✓	CCA	Both	Both	Mean of 6 sites

Grey shaded studies are those which were not included in our analyses because complete data were unavailable, either in publication or on request from authors.

* information not available from publication, † data only available for whole study so estimated to be equal for each sub-study, ‡ studies with all result data unavailable from the publication, § studies with result data only relating to a particular genetic model available in the publication.

APOE: apolipoprotein E; ACE: angiotensin I converting enzyme ; MTHFR: 5,10-methylenetetrahydrofolate reductase; NOS3: nitric oxide synthase 3; ADD1: adducin 1 (alpha); HWE: Hardy Weinberg equilibrium; CCA: common carotid artery; BIF: bifurcation; ICA: internal carotid artery; NIDDM: non-insulin-dependent diabetes mellitus; CHD: coronary heart disease; CVD: cerebrovascular disease; IDDM: insulin-dependent diabetes mellitus; PAD: peripheral artery disease.

Supplemental table 2. Sample size required to detect a CIMT difference ranging from 10 to 100 μm per genotype in a co-dominant model for a genetic polymorphism with minor allele frequency of 0.05, 0.1 or 0.2, at (a) 80% power, 2-sided $p < 0.05$, and (b) 90% power, 2-sided $p < 0.01$ *

(a) 80% power, $p < 0.05$

CIMT difference (μm)	Minor allele frequency		
	0.05	0.1	0.2
10	21147	11159	6275
25	3380	1782	1001
50	842	443	247
100	208	108	59

(b) 90% power, $p < 0.01$

CIMT difference (μm)	Minor allele frequency		
	0.05	0.1	0.2
10	40089	21154	11896
25	6408	3378	1897
50	1596	839	469
100	393	204	111

*sample size calculations performed with Quanto version 1.2 (available at <http://hydra.usc.edu/gxe/>)

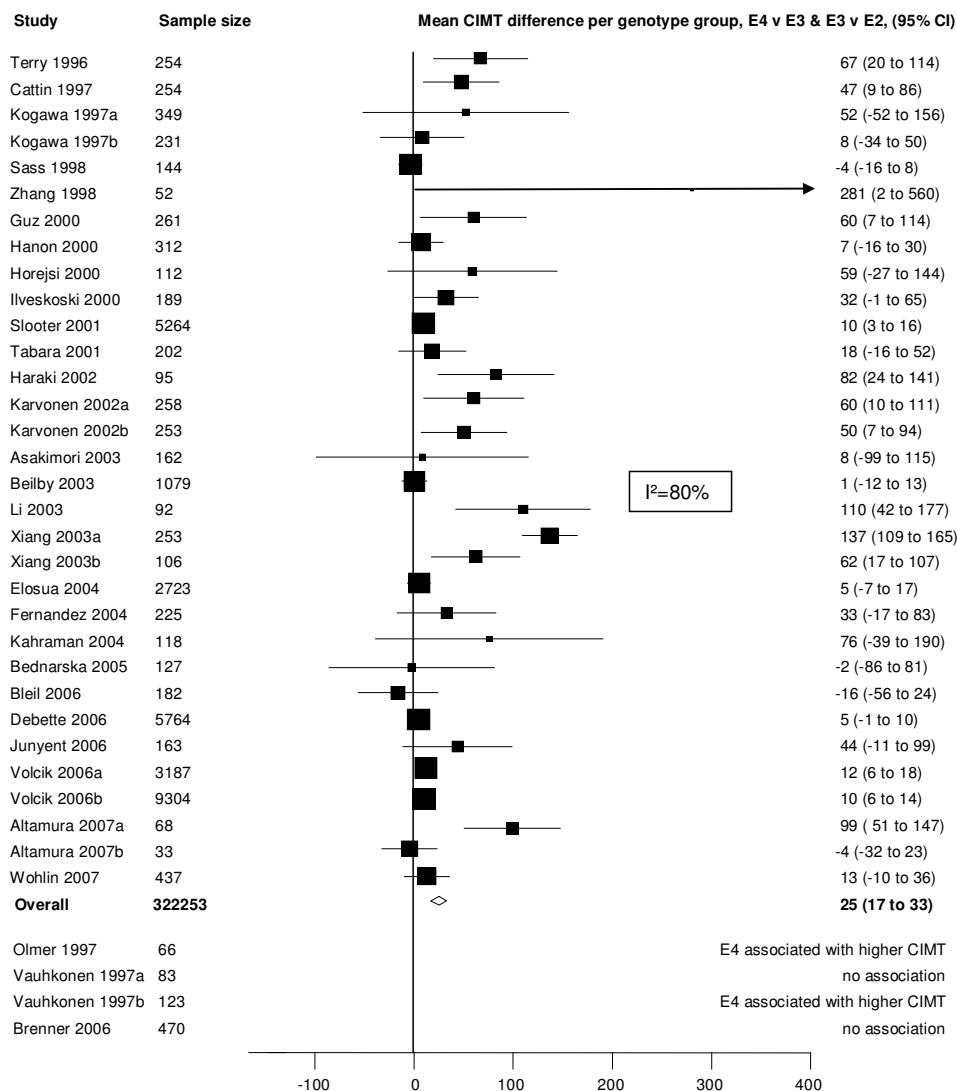
Note that required sample size:

- approximately doubles with a change from 80 % power, $p < 0.05$ to 90% power, $p < 0.01$;
- approximately halves for each doubling of minor allele frequency within the range assessed;
- falls by about 100-fold for a 10-fold increase in detectable CIMT difference per genotype from 10 to 100 μm .

Supplemental figures

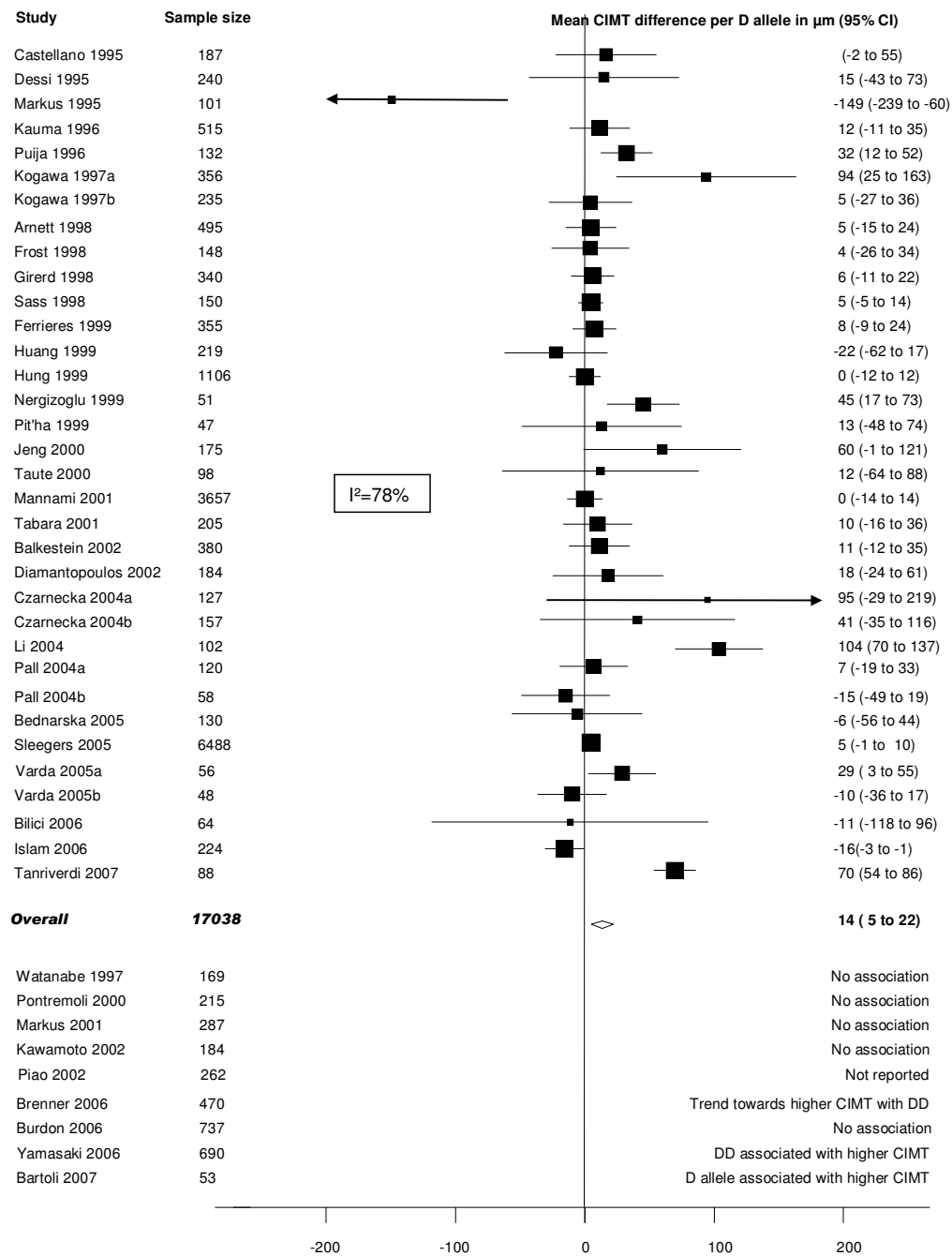
Supplemental Figure 1

SUPPLEMENTARY FIGURE 1



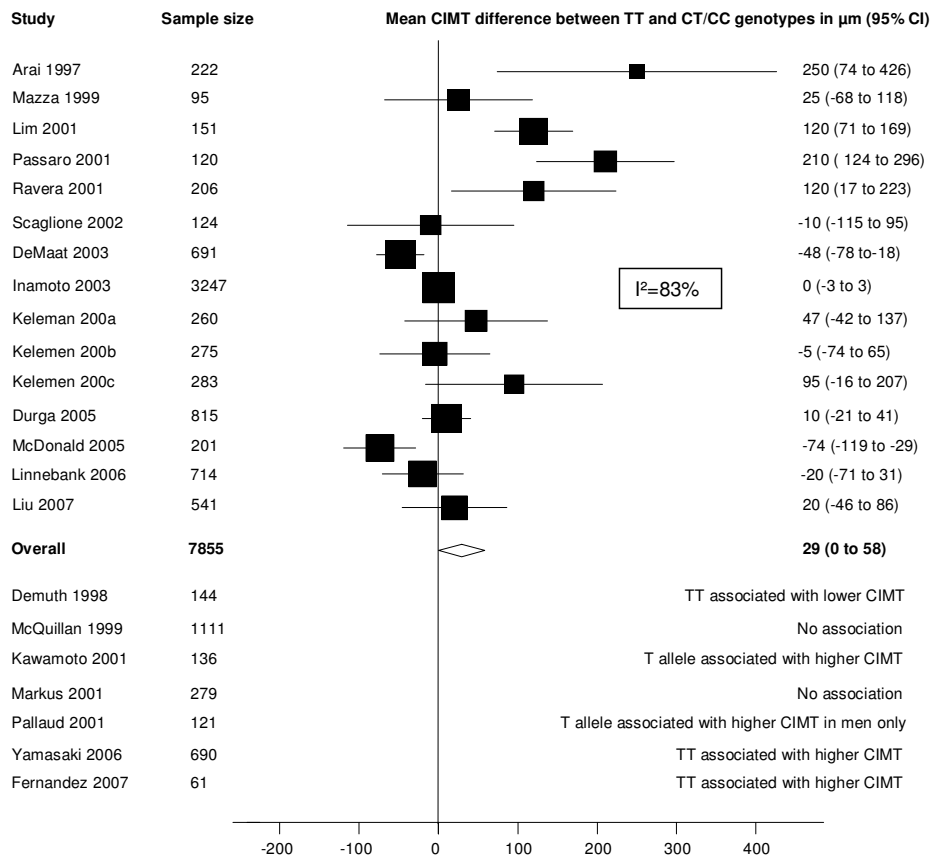
Supplemental Figure 2

SUPPLEMENTARY FIGURE 2



Supplemental Figure 3

SUPPLEMENTARY FIGURE 3



Supplemental figure legends

Supplemental Figure 1. Meta-analysis of APOE ($\epsilon 2/\epsilon 3/\epsilon 4$). The data are analysed according to a co-dominant genetic model. The size of the squares represents the weight of the study in the random effects model. The diamond represents the random effects pooled mean difference and its width represents the 95% confidence interval. No data were available for the four studies below the pooled estimate, but we show qualitative statements of the results from these studies. Heterogeneity between studies is represented by the I^2 value shown.

Supplemental Figure 2. Meta-analysis of ACE (I/D). The data are analysed according to a co-dominant genetic model. The size of the squares represents the weight of the study in the random effects model. The diamond represents the random effects pooled mean difference. No data were available for the nine studies below the pooled estimate, but we show qualitative statements of the results from these studies. Heterogeneity between studies is represented by the I^2 value shown.

Supplemental Figure 3. Meta-analysis of MTHFR (677 C/T). The data are analysed according to a recessive genetic model. The size of the squares represents the weight of the study in the random effects model. The diamond represents the random effects pooled mean difference. No data were available for the four studies below the pooled estimate, but we show qualitative statements of the results from these studies. Heterogeneity between studies is represented by the I^2 value shown.

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