

Meta-analysis of MTHFR 677C→T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate?

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Abstract

Objectives To investigate the association between the MTHFR 677C→T polymorphism and coronary heart disease, assessing small study bias and heterogeneity between studies.

Data sources Medline and Embase citation searches between January 2001 and August 2004; no language restrictions.

Study selection Case-control and prospective studies of association between MTHFR 677C→T variant and myocardial infarction, coronary artery occlusion, or both; 80 studies were included.

Data extraction Data on genotype frequency and mean homocysteine concentrations by genotype were extracted. Odds ratios were calculated for TT genotype versus CC genotype. Heterogeneity was explored, with stratification by geographical region of the study samples, and meta-regression by difference in mean serum homocysteine concentrations (CC minus TT genotypes) was carried out.

Results 26 000 cases and 31 183 controls were included. An overall random effects odds ratio of 1.14 (95% confidence intervals 1.05 to 1.24) was found for TT versus CC genotype. There was strong evidence of heterogeneity ($P < 0.001$, $I^2 = 38.4\%$), which largely disappeared after stratification by geographical region. Odds ratios in Europe, Australia, and North America attenuated towards the null, unlike those in the Middle East and Asia.

Conclusions No strong evidence exists to support an association of the MTHFR 677C→T polymorphism and coronary heart disease in Europe, North America, or Australia. Geographical variability may be due to higher folate intake in North America and Europe or to publication bias. The conclusion drawn from previous meta-analyses that folic acid, through lowering homocysteine, has a role in prevention of cardiovascular disease is in some doubt.

Introduction

Observational studies have consistently shown that higher plasma homocysteine concentrations are associated with a greater risk of coronary heart disease.¹ However, homocysteine-coronary heart disease associations may be confounded and existing atherosclerosis could itself increase homocysteine concentrations.²

Associations between the genetic variant MTHFR 677C→T and coronary heart disease have been reported.³ The genetic variant is associated with elevated homocysteine concentrations but is not subject to the reverse causation or confounding that exists in observational studies of coronary heart

disease and homocysteine concentrations.⁴ Indeed, inclusion of folate—which lowers homocysteine concentrations⁵—in the proposed Polypill is supported by reference to two meta-analyses of such genetic studies.^{6 7} However, the sample sizes needed to accurately estimate the causal influence of intermediate phenotypes such as homocysteine concentration on disease outcomes by using common genetic variants are necessarily very large.⁸ Moreover, a meta-analysis of the association between MTHFR 677C→T and coronary heart disease showed potential publication bias, in which non-publication of small negative studies could have resulted in overestimation of the strength of association between the MTHFR 677C→T variant and coronary heart disease.⁷ In another meta-analysis, Wald et al tested for small study bias, reporting an Egger test of $P = 0.55$.³ We carried out an updated meta-analysis using a comprehensive literature search and fully investigated potential publication bias.

Methods

We identified eligible studies by searching Medline and Embase for all publications between January 2001 and August 2004. We used the search terms “mthfr”, “methylentetrahydrofolate reductase”, “5,10 methylentetrahydrofolate reductase”, “677C”, and “C677T” in combination with “cardiovascular disease”, “ischemic heart disease”, “coronary”, “heart disease”, “myocardial infarction”, and “angina”. We did not exclude any studies on the basis of language. We used the outcome as defined by previous investigators of myocardial infarction or coronary artery occlusion. We based unadjusted odds ratios on published genotype frequencies or extracted them directly from publications where raw data were not available.

In line with previous work, we compared the TT genotype with the CC genotype. In our meta-analysis of all studies, we stratified by the geographical region in which the studies were done—Asia, Australia, the Middle East, North America, and Europe. We quantified the extent of heterogeneity. We estimated the difference in serum homocysteine concentrations between CC and TT genotypes by using a random effects meta-analysis. We used meta-regression analysis to assess whether differences in serum homocysteine concentration affected MTHFR-disease associations.

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References to included studies are on bmj.com



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Summary findings by geographical region: odds ratios for coronary heart disease comparing TT with CC genotypes, and difference in geometric mean serum homocysteine concentrations

Region	Odds ratio (95% CI)	No of cases:controls	No of studies	Difference in serum homocysteine CC-TT (95% CI)	Odds ratio (95% CI) for 5 $\mu\text{mol/l}$ increase in homocysteine
Europe	1.08 (0.99 to 1.18)	17 275:21 313	41	2.03 (1.15 to 2.90)	1.21 (0.98 to 1.50)
North America	0.93 (0.80 to 1.10)	3714:3969	15	1.35 (0.32 to 2.38)	0.76 (0.44 to 1.42)
Middle East	2.61 (1.81 to 3.75)	971:1316	5	8.80 (1.23 to 16.4)	1.72 (1.12 to 4.90)
Asia	1.23 (0.94 to 1.62)	2755:4735	16	3.76 (2.29 to 5.23)	1.32 (0.92 to 1.90)
Australia	1.04 (0.73 to 1.49)	1285:480	3	–	–
Overall	1.14 (1.05 to 1.24)	26 000:31 813	80	2.24 (1.55 to 2.94)	1.34 (1.12 to 1.62)

Results

In all, we included 81 data points from 80 studies in this meta-analysis (table), with a total of 26 000 cases and 31 813 controls. We identified 24 studies not included in the previous meta-analyses. We found an overall random effect odds ratio for coronary artery disease comparing TT with CC genotypes of 1.14 (95% confidence interval 1.05 to 1.24). We found evidence that effect estimates were related to study size, suggesting that small study bias, such as publication bias, may have distorted the findings (Egger test $P=0.03$).

We found strong evidence of between-study heterogeneity ($P<0.001$, $I^2=38.4\%$). Stratified analysis by geographical region removed much of the heterogeneity within Europe, North America, Australia, and the Middle East; the random effect odds ratio were 1.08 (0.99 to 1.18; $P_{\text{heterogeneity}}=0.19$, $I^2=16.1\%$) among the 42 European studies, 0.93 (0.80 to 1.10; $P_{\text{heterogeneity}}=0.64$, $I^2=0\%$) among the 15 North American studies, 1.04 (0.73 to 1.49; $P_{\text{heterogeneity}}=0.97$, $I^2=0\%$) among the three Australian studies, and 2.61 (1.81 to 3.75; $P_{\text{heterogeneity}}=0.57$, $I^2=0\%$) among the five Middle Eastern studies (figure). Evidence of heterogeneity remained between Asian studies, but this was only apparent between Japanese studies and others and within studies done in Japan. The random effects odds ratio for Japanese studies was 1.71 (1.23 to 2.37; $P_{\text{heterogeneity}}=0.01$, $I^2=62.5\%$); studies done in China, Taiwan, and Korea produced homogeneous results, with an overall odds ratio of 0.81 (0.60 to 1.10; $P_{\text{heterogeneity}}=0.41$, $I^2=3.0\%$).

We examined whether differences in homocysteine concentrations between TT and CC genotypes were associated with the size of effect of the MTHFR genotype on coronary heart disease risk. Of the 20 studies that gave mean homocysteine concentrations by genotype, a random effects meta-analysis found an overall difference between CC and TT genotypes of 2.24 $\mu\text{mol/l}$ (95% confidence interval 1.55 to 2.94). In a meta-regression analysis we found evidence to suggest that differences in serum homocysteine concentrations by genotype were associated with the effect of genotype on coronary artery disease risk ($\beta=0.103$, $P=0.02$) (see bmj.com).

Discussion

Do high homocysteine concentrations cause heart disease?

Our meta-analysis of 80 studies gives an estimate of a 14% (95% confidence interval 5% to 24%) greater risk of coronary heart disease associated with the MTHFR CC genotype. However, because of hetero-

geneity between studies, conclusions regarding a causal relation between homocysteine and coronary heart disease must be guarded. Analysis of geographical variation showed wide variation between regions, and in European, north American, and Australian studies no strong evidence existed to support any effect of homocysteine on coronary heart disease risk. Only studies carried out in the Middle East and in Asia showed evidence to support this hypothesis, and in Asia the effect was confined to Japanese studies.

Regional heterogeneity

Geographical differences may relate to nutritional habits. Differences in homocysteine concentrations by MTHFR C677T are greater at lower levels of folate intake and are reduced after folate supplementation.⁹ In our meta-analysis we found modest differences in homocysteine concentrations by genotype in the United States, where fortification of foods with folate is mandatory, but larger differences in other regions. A recent meta-analysis confirmed the lower mean difference between TT and CC MTHFR C677T genotypes in homocysteine concentration for North American but not European studies, although the numbers of participants studied were much smaller.¹⁰

We found that differences in homocysteine concentrations by genotype were positively associated with the size of the effect of genotype on risk of coronary heart disease. However, this does not provide convincing evidence of a causal relation between homocysteine concentrations and risk of coronary heart disease, because the correlation of larger mean differences and larger effect sizes could equally represent publication bias. The strongly positive studies reported from the Middle East and Japan could also represent publication bias. In support of this, although our meta-analysis included too few studies to test for small study bias, the largest Japanese study included gave a null result (odds ratio 0.99, 0.74 to 1.32),¹¹ and a further large Japanese study (445 cases) that could not be included in our meta-analysis also found a null result ($P>0.1$).¹² The largest Middle Eastern study included gave a result that was not incompatible with the null hypothesis.¹³ A similar meta-analysis of MTHFR and ischaemic stroke also found a greater increase in risk among TT homozygotes in Japan compared with other countries and regions.¹⁴ The inference that the MTHFR-disease association is greatest in Japan because folate intake is low is incongruous with the relatively low incidence of folic acid deficiency and neural tube defects in Japan.^{15 16}

What is already known on this topic

The MTHFR 677C→T polymorphism is associated with homocysteine concentration; people with TT genotype have higher concentration than those with the CC genotype

Two recent meta-analyses reported associations between MTHFR C677T genotype and coronary heart disease, concluding that high homocysteine concentrations are causally related to coronary heart disease risk

What this study adds

Since the publication of the previous meta-analyses several more studies have been published, and the total number of cases and controls has doubled

This meta-analysis provides no evidence of a causal relation between homocysteine and coronary heart disease risk in European, North American, and Australian populations

This study casts doubt on the beneficial role of lowering homocysteine concentrations, through supplementation with folic acid, to prevent heart disease

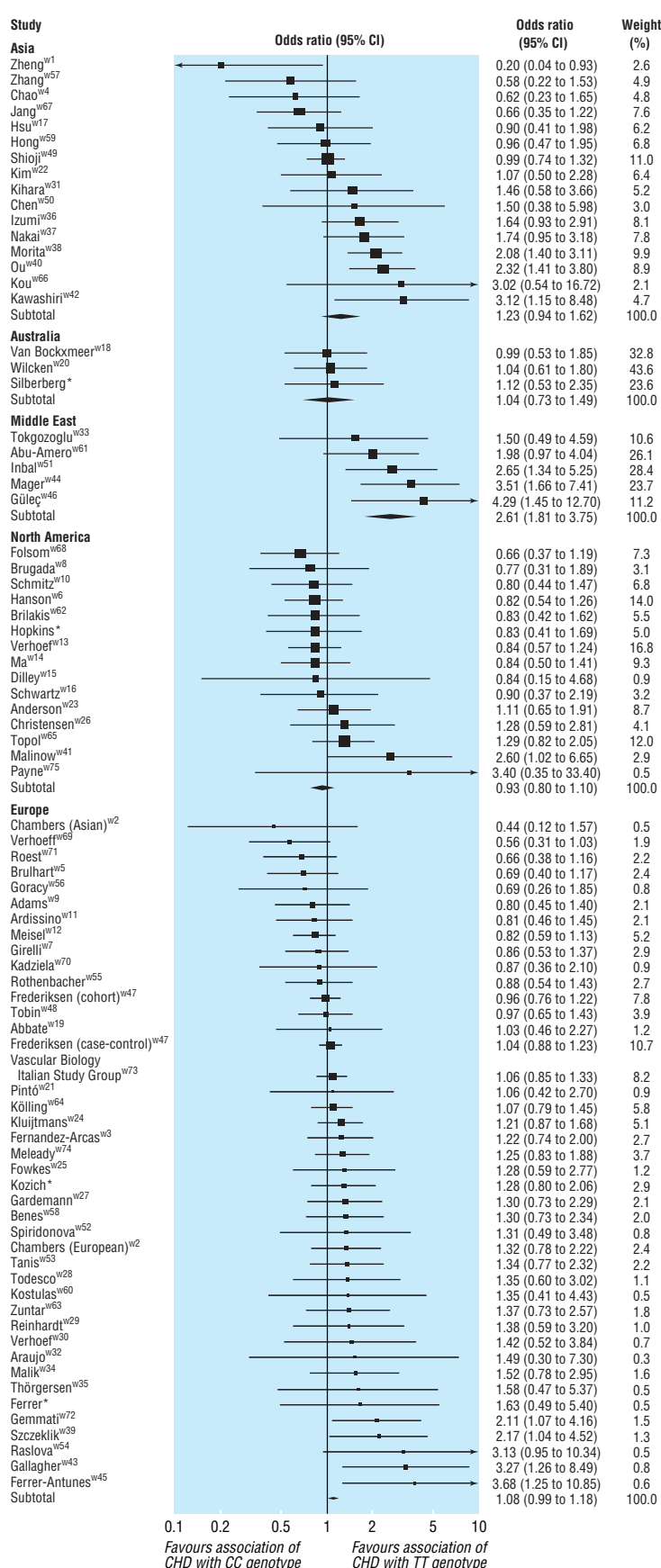
Recent trials of folate supplementation in North America, Europe, and Australia that have examined clinical outcomes and surrogates have been negative.^{17–21} These trials have largely been done in populations with high folate consumption and are unlikely to resolve the question of whether folate supplementation could be an important intervention in populations with “insufficient” folate consumption.

Limitations

Any meta-analysis has limitations, and although we invested considerable effort in searching for published studies and found more than previous meta-analyses, our estimates of effect are still likely to represent over-estimation through publication bias. A further limitation of our analysis is that we did not have individual level data and were not able to take account of the uncertainty in differences in mean homocysteine concentrations between people with CC and TT genotypes in our meta-regression analysis. We did not limit the estimation of our MTHFR-homocysteine effect to healthy people, and the inclusion of some people with disease may have clouded the true effect of genotype.²²

Conclusions

The results of this meta-analysis cast some doubt on the implications drawn from previous reviews that support a role for folic acid in preventing cardiovascular disease. Ironically, enthusiasm for such intervention comes from high income countries where the evidence would suggest there would be little to gain with respect to coronary heart disease risk from increasing folic acid intake. The growing observational evidence has important implications for the inclusion of folic acid in any Polypill strategy.



Association between MTHFR C677T polymorphism and coronary heart disease (TT versus CC genotype). * Unpublished data taken from Klerk et al, 2002 meta-analysis

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The hidden curriculum

When I started working as a house officer there was no curriculum. One year later, the hospital where I worked had laid out a basic outline of what senior house officers should learn. We were to learn how basic sciences applied to medicine and clinical skills. But six months later, after many no-shows by tutors and trainers, we learnt that the curriculum was a dry piece of paper and nothing more. In contrast, the hidden curriculum was living and breathing (if unwritten): it outlined how to teach (don't bother), how to learn (on your own), and the role of the patient in medical education (a useful and silent prop). There are no prizes for guessing which curriculum we followed.

Traditionally, medical curriculums have had a bad name. But Modernising Medical Careers think that they can do better.¹ They've laid out a curriculum for doctors passing through their foundation years and outlined the knowledge, skills, and attitudes that these doctors should learn. They have also outlined how trainers should assess their juniors—by using objective assessment scales such as DOPS (direct observation of procedural skills).² But the new curriculum has its critics: they say that it will take forever to do the assessments and that it is just an exercise in form filling. Modernising Medical Careers reply that the assessment tools are validated and reliable. You can argue either way, but for once there is new money to

back up this new curriculum—£73m, and it is ring fenced.

What role does BMJ Learning play in all this? We feel that any new learning initiative will live or die on the learning resources that it provides—in other words, good content is king. So we have produced learning modules to enable juniors to satisfy the generic aspects of the curriculum such as how to work in a team. However, our users have consistently asked for learning modules on the acute care aspect of the curriculum. One of these new modules is on the diagnosis and treatment of acute coronary syndrome. It concentrates on recent developments—such as the role of troponins in diagnosis and assessment of future risk, and the increasing evidence that primary coronary angioplasty (where it's available) is the treatment of choice for patients with an acute myocardial infarction. To find out more, just go to our website, www.bmjlearning.com.

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- 1 Modernising Medical Careers. www.mmc.nhs.uk/ (accessed 12 May 2005).
- 2 BMJ Learning. DOPS (direct observation of procedural skills). www.bmjlearning.com/planrecord/assessment/dops.jsp (accessed 12 May 2005).