Methylenetetrahydrofolate reductase genotypes and predisposition to atherothrombotic disease

Evidence that all three MTHFR C677T genotypes confer different levels of risk

L. A. J. Kluijtmans* and A. S. Whitehead

Department of Pharmacology and Center for Pharmacogenetics, University of Pennsylvania School of Medicine, Philadelphia, PA, U.S.A.

Aims Elevated plasma homocysteine is an independent risk factor for atherothrombotic disease. Individuals homozygous for the methylenetetrahydrofolate reductase (MTHFR) 677C allele exclusively accumulate 5-methyltetrahydrofolate, the methyl donor for homocysteine remethylation, in their red blood cells; this contrasts with 677 TT homozygotes who also accumulate significant levels of non-methylated folate derivatives. Those with the MTHFR 677 TT, CT and CC genotypes may therefore differ qualitatively with respect to folate utilization and hence their capacity to remethylate homocysteine. This study was consequently designed to establish whether all three genotypes confer different levels of atherothrombotic risk.

Methods and Results The risk of atherothrombotic disease conferred by the MTHFR 677 CT and 677 CC genotypes was assessed using a ‘restricted’ meta-analysis approach applied to subjects from the first ten studies reporting a significantly increased risk conferred by the 677 TT genotype. The defined risk of the TT genotype in each of these ten studies was judged by us to denote ‘genetic vulnerability’ in the populations from which subjects were drawn. After proportional adjustment for the greater number of case TT homozygotes, the CT and CC frequencies observed in cases were compared with expectations based on the frequencies of these genotypes in controls. The observed CT frequency among cases was higher than expected in eight of the ten studies. In the meta-analysis, which included 1857 cases and 2942 controls, 847 (45·6%) cases, instead of the 777 (41·8%) expected, had the MTHFR CT genotype (P=0·010).

Conclusions Our findings suggest that the three MTHFR C677T genotypes confer different levels of atherothrombotic risk in ‘genetically vulnerable’ populations: CT heterozygotes have an elevated risk over CC homozygotes. One explanation is that the CT genotype actively confers atherothrombotic risk. An alternative interpretation however, for which a biologically plausible mechanism is proposed, is that CC is a protective genotype.

Key Words: Methylenetetrahydrofolate reductase polymorphism, atherothrombotic risk, folate derivatives, homocysteine, heterozygote risk.

Introduction

Hyperhomocysteinaemia is a major independent and graded risk factor for atherothrombotic diseases. It has been estimated that up to 10% of all coronary artery disease deaths, i.e. more than 50 000 a year in the U.S.A., can be attributed to mild hyperhomocysteinaemia[1]. Homocysteine elevations of only 5 μmol.1⁻¹ can increase the risk of coronary artery disease by 60% in men and by 80% in women[1], which is quantitatively comparable to the risk enhancement conferred by established lipid factors[1].

Hyperhomocysteinaemia can be caused by dysfunction of the folate-dependent enzyme methylenetetrahydrofolate reductase (MTHFR), which reduces 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate,
the methyl donor in homocysteine remethylation (see Fig. 1 for an overview). Frosst and colleagues\(^2\) identified a common C\(\rightarrow\)T transition at nucleotide 677 that mandates an alanine to valine substitution in the MTHFR sequence and results in a thermolabile isoform. The observation that TT homozygotes are predisposed to hyperhomocysteinaemia, especially in those with a relatively low folate status\(^3,4\), prompted many investigators to assess whether the TT genotype is associated with an increased risk of atherothrombotic diseases. Some studies have found such an association, while others have not\(^5\).

Recently, Bagley and Selhub\(^6\) reported that the in vivo effects mandated by the 677C\(\rightarrow\)T mutation include a differential distribution of folate derivatives in red blood cells; TT homozygotes accumulate both formylated tetrahydrofolate and 5-methyltetrahydrofolate polyglutamates, whereas CC homozygotes exclusively accumulate the latter. This establishes that there are qualitative, in addition to quantitative, differences between these MTHFR genotypes.

Recent studies suggest that CT heterozygotes have mean homocysteine concentrations that are modestly, but significantly, increased relative to CC homozygotes\(^4,7\). Given that homocysteine concentration is a graded risk factor for atherothrombotic disease, the above leads to the prediction that CT heterozygotes and CC homozygotes should, respectively, be over-represented and under-represented among atherothrombotic patients. If so, such a frequency distortion should be most apparent in populations with the most pronounced homocysteine-modifying genetic effect. We judged the most objective criterion for such an effect to be a statistically significantly increased frequency of the TT genotype among patients. The first ten studies that defined cohorts meeting this criterion were identified. None of these reported an analysis of the relative distribution of CT and CC genotypes within the case and control groups, i.e. they were unselected with respect to deviations from expected CT to CC genotype ratios in cases. We performed a ‘restricted’ meta-analysis of the data included in these ten studies, and established that each of the three MTHFR genotypes confers a different level of risk.

Methods

Study populations

A systematic search of major medical subject headings and abstracts from January 1995 to October 1998 was undertaken using the terms ‘MTHFR’ and ‘thermolabile methylenetetrahydrofolate reductase’. All abstracts were reviewed and case-control studies that had investigated the association of the MTHFR 677C\(\rightarrow\)T mutation in atherothrombotic diseases were identified. A ‘restricted’ meta-analysis was performed, in which inclusion was limited to those studies that had reported a significantly increased frequency of the TT genotype among atherothrombotic disease patients relative to controls.

Calculation of expected MTHFR CT and CC frequencies in cases

The documented difference in TT genotype frequency (as a percentage) between cases and controls was proportionally divided between the CT and CC genotypes, according to their observed frequencies in controls ($CT_{\text{controls}}$ and $CC_{\text{controls}}$ in the following equations). The expected case CT and CC frequencies ($CT_{\text{cases}}$ and $CC_{\text{cases}}$) can therefore be calculated as follows:

![Figure 1](https://example.com/figure1.png)

**Figure 1** Overview of folate and homocysteine metabolic pathways. THF, tetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; MS, methionine synthase; CBS, cystathionine β-synthase; AdoMet, S-adenosylmethionine; AdoHcy, S-adenosylhomocysteine.
Table 1  Methylenetetrahydrofolate reductase genotype distribution and OR (95% CI) of the MTHFR CT vs CC genotype in ten case-control studies documenting an association between the TT genotype and atherothrombotic disease

<table>
<thead>
<tr>
<th>Study</th>
<th>Cases</th>
<th>Controls</th>
<th>OR CT vs CC</th>
<th>95% CI</th>
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<tr>
<td></td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>CC</td>
</tr>
<tr>
<td>Ou et al. [9]</td>
<td>69</td>
<td>84</td>
<td>61</td>
<td>110</td>
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<tr>
<td>Morita et al. [10]</td>
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<td>188</td>
<td>57</td>
<td>338</td>
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<td>Gallagher et al. [11]</td>
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<td>53</td>
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<tr>
<td>Izumi et al. [12]</td>
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<td>110</td>
<td>50</td>
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<td>Kluijtmans et al. [13]</td>
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<tr>
<td>Ferrer-Antunes et al.</td>
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<td>71</td>
</tr>
<tr>
<td>Morita et al. [17]</td>
<td>50</td>
<td>121</td>
<td>55</td>
<td>153</td>
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<tr>
<td>Combined</td>
<td>643</td>
<td>847</td>
<td>367</td>
<td>1252</td>
</tr>
</tbody>
</table>

The reference number of each study is given as a superscript.

*The pooled estimate and its 95% CI were calculated by a Mantel–Haenszel method.

\[
\begin{align*}
CT_{\text{cases}} &= CT_{\text{controls}} - \left[ \frac{CT_{\text{controls}}}{CT_{\text{controls}} + CC_{\text{controls}}} \right] \times (TT_{\text{cases}} - TT_{\text{controls}}) \\
CC_{\text{cases}} &= CC_{\text{controls}} - \left[ \frac{CC_{\text{controls}}}{CT_{\text{controls}} + CC_{\text{controls}}} \right] \times (TT_{\text{cases}} - TT_{\text{controls}})
\end{align*}
\]

**Statistics**

Differences between the expected and observed CT and CC frequencies in cases, and possible genotype distortions from Hardy–Weinberg equilibrium in control groups, were assessed by chi-squared analysis. Odds ratios (OR) and 95% Confidence Intervals (95% CI) were calculated as estimates of relative risk conferred by the different genotypes. The summary OR was calculated using a Mantel–Haenszel method [8]. P values are two-tailed, and P < 0.05 was considered statistically significant.

**Results**

Data from the first ten studies [9–18] that have reported a significantly increased frequency of the MTHFR 677 TT genotype among atherothrombotic disease patients compared with controls were combined. These comprise a total of 1857 cases (643 CC; 847 CT; 367 TT) and 2942 controls (1252 CC; 1364 CT; 326 TT) (Table 1) which is sufficiently large for meta-analysis of the association of CT and CC genotypes with disease.

The combined control group is in Hardy–Weinberg equilibrium (\(\chi^2 = 1.25; P = 0.54\)), as is each of the control groups from the component studies. The summary OR and 95% CI for the CT genotype relative to the CC genotype is 1.27 (95% CI, 1.11–1.44), indicating that CT individuals are at increased risk. Two large Japanese studies, which included completely independent study groups (H. Kurihara, personal communication), showed a significant elevation of the CT genotype frequency among those with atherothrombotic disease, raising the concern that inclusion of data from these studies may have biased the overall outcome of the meta-analysis reported here. However, after their exclusion, a nearly significant and comparable odds ratio of 1.12 (95% CI, 0.95–1.32) could still be calculated, indicating that the remaining eight studies showed a similar trend in genotype distortion favouring an elevated CT frequency.

Each of the ten studies was re-analysed separately to test whether the observed case CT and CC frequencies are substantially different from those expected from the frequencies of these genotypes in controls, after adjustment for the documented increase in case TT frequency. In eight of the studies, the observed case CT genotype frequency was higher than expected (Fig. 2).

This analysis was extended to the combined case group in which there were 847 (45.6%) individuals with the CT genotype, whereas only 777 (41.8%) would have been expected. Conversely, at 643 (34.6%) the number of patients with the CC genotype was lower than the 713 (38.4%) expected (\(\chi^2 = 6.63; P = 0.010\)). Therefore, in

atherothrombotic disease patients selected from ‘genetically vulnerable’ populations, there is a significant distortion of genotype frequency in favour of CT heterozygotes and against CC homozygotes that supports the increased summary OR for the CT vs CC genotype. This level of risk is modest in individual terms; however, as 10% of all coronary artery disease deaths are attributable to hyperhomocysteinaemia[1], the MTHFR 677 CT genotype is potentially an important contributor to the population risk of atherothrombotic disease and may have considerable public health impact, especially where suboptimal nutrition and/or other genetic risk factors are prevalent.

Discussion

There is compelling evidence that an elevated plasma homocysteine concentration is an independent and graded risk factor for atherothrombotic disease[13]. One of the major contributors to the elevation in circulating homocysteine is the thermolabile variant of MTHFR[19], genetically defined by the MTHFR 677 TT genotype.

In recent studies, homocysteine concentrations in CT individuals were shown to be increased relative to those of their CC peers[4,7]. These observations led us to hypothesize that, in ‘genetically vulnerable’ populations, the three MTHFR genotypes will confer different levels of atherothrombotic risk, i.e. CT heterozygotes will be measurably over-represented among patients with atherothrombotic disease, relative to the number predicted from the frequency of this genotype among controls, and after proportional adjustment for the increase in TT genotype frequency among cases.

In most of the component studies (i.e. in eight out of ten), we observed a higher than expected CT genotype frequency that was balanced by a corresponding decrease in CC genotype frequency. In the analysis of subjects pooled from all ten studies, the differences between the observed and expected case CT and CC genotype frequencies were statistically significant, i.e. the number of CT heterozygotes among cases was significantly higher than expected whereas the number of CC homozygotes was significantly lower. Evidence that the CT genotype is over-represented, and the CC genotype under-represented in patients supports the involvement of MTHFR genetic variants in predisposition to atherothrombotic disease, and in particular strengthens the hypothesis that the TT genotype is an important contributor to disease in ‘genetically vulnerable’ populations.

Our conclusions, based on the ‘restricted’ meta-analysis reported here, contrast with those of Brattström and colleagues[20], who summarized 23 heterogeneous case-control studies and calculated a non-significant OR of 1·12 (95% CI, 0·92–1·37) for the MTHFR 677 TT genotype. The latter can be interpreted in two ways: (1) hyperhomocysteinaemia, with which the TT genotype is associated, is not causally involved in atherothrombotic diseases but is merely a consequence of the effects of
other well-established risk factors and renal function (the interpretation favoured by Brattström et al.), or (2) all of the other genetic and environmental risk factors present in the constituent studies mask the effect of the 677C→T polymorphism. Notably, many of the component studies used in the meta-analysis of Brattström et al. were from nutritionally privileged populations, e.g. the U.S.A., Australia, and United Kingdom. In such populations, the ready availability of fresh fruit and vegetables or the implementation of effective public health measures have ensured a generally good nutritional standard, and any genetic factor potentiated by poor nutritional status would not be prominent. Furthermore, genetic factors, like the 677 TT genotype, tend to be more causally involved in cases with premature disease compared with older patients, in whom environmental factors have accumulated over time to produce late-onset disease, and in whom genotype-specific mortality may have occurred. Therefore, any single polymorphic variant may have a small impact when all populations are considered, but may confer significant risk in particular subgroups. Thus, the MTHFR 677 TT genotype will, most likely, only be an important contributor to atherothrombotic risk in ‘genetically vulnerable’ populations with nutritional profiles that elicit a genotype-specific pathogenic phenotype.

In an unconventional meta-analysis, such as that reported here, it is important to recognize the potential problem of selection bias and differences in the quality of the component studies. We selected those studies which showed a positive association between the MTHFR 677 TT genotype and atherothrombotic diseases, in order to identify patients from ‘genetically vulnerable’ populations in which genetic variants, especially those driven by a poor nutritional status, would be prominent. We concurrently hypothesized that each of the three MTHFR C677T genotypes is associated with a different level of atherothrombotic risk, and unequivocally demonstrated that this is the case. None of the component studies had been analysed for genetic distortions of the case CT and CC genotype frequencies; therefore, there is no a priori evidence that the CT:CC ratio is systematically biased in these component studies. Furthermore, in each of the component studies, atherothrombotic disease had been objectively diagnosed using clinically well-established methods, including computerized tomography (CT scans) and (coronary) angiography. Controls were derived from the same population and ethnic backgrounds, and, in most studies, had a similar mean age and male:female ratio as the cases. In two studies, in which age and sex distributions were different in cases and controls, further statistical adjustments for these and other potentially confounding factors, including hypertension, diabetes and smoking, did not materially affect the observed risk elevations for the MTHFR TT genotype.

The difference in atherothrombotic risk conferred by the CT and CC genotypes raises the interesting question of whether the CC genotype should be considered protective, rather than merely being used as the ‘baseline’ genotype from which the additional risk conferred by the TT and CT genotypes is calculated. Biochemical data recently published by Bagley and Selhub suggests a mechanism whereby the MTHFR CC genotype may actively protect an individual from homocysteine-mediated atherothrombotic disease. This genotype, unlike the other two MTHFR genotypes, favours the exclusive accumulation of 5-methylTHF in red blood cells, and probably other cell types such as endothelial cells, in which elevated homocysteine has been reported to have a pro-atherothrombotic effect. The efficient provision of methyl groups for the conversion of homocysteine to methionine would ensure that, at least with respect to this critically important reaction, the capacity of cells to clear homocysteine would be maximized. A superior clearance of homocysteine through such a mechanism, driven by the MTHFR CC genotype, may be sufficient to minimize the effects of other nutritional, environmental and genetic factors that predispose to the accumulation of homocysteine through other pathways. Thus, CC homozygotes may be actively protected against hyperhomocysteinaemia-associated atherothrombotic disease by their capacity to neutralize or reduce the impact of other homocysteine raising factors.

In conclusion, the current analysis shows that in genetically vulnerable populations each of the three MTHFR C677T genotypes is associated with a different level of atherothrombotic risk. It therefore seems likely that they will each have qualitatively and quantitatively distinct interactions with other risk factors. Future work should be directed towards exploring the differential impact of the MTHFR 677 TT, CT and CC genotypes in very large multifactorial case-control studies.

References


