Interactions of 5'-UTR Thymidylate Synthase Polymorphism with 677C→T Methylene Tetrahydrofolate Reductase and 66A→G Methyltetrahydrofolate Homocysteine Methyl-Transferase Reductase Polymorphisms Determine Susceptibility to Coronary Artery Disease

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Aim: The current study aimed to address the inconsistencies in association studies, specifically with reference to methylene tetrahydrofolate reductase (MTHFR) C677T polymorphism in the light of gene-gene and gene-nutrient interactions.

Methods: A case-control study was conducted to analyze four genetic polymorphisms i.e. thymidylate synthase (TYMS) 5'-UTR 28 bp tandem repeat, MTHFR C677T, methyltetrahydrofolate homocysteine methyltransferase (MTR) A2756G, methyltetrahydrofolate homocysteine methyltransferase reductase (MTRR) A66G using PCR-AFLP and PCR-RFLP methods; plasma folate and B12 using AxSYM kits; plasma homocysteine by reverse phase HPLC and nitric oxide using Griess reaction. Fisher’s exact test, logistic regression analysis and multifactor dimensionality reduction analysis were used for statistical analysis of genetic parameters. Student’s t-test was used for biochemical parameters.

Results: MTHFR C677T and MTRR A66G were found to increase the risk for CAD by 1.61-fold (95%CI: 1.04-2.50) and 1.92-fold (95% CI: 1.29-2.87) whereas TYMS 2R allele was found to reduce the risk for CAD (OR: 0.66, 95%CI: 0.49-0.88) by counteracting MTHFR and MTRR variant alleles. Significant gene-gene interactions were observed among TYMS/MTRR (P<0.0001), MTR/TYMS/MTRR (P<0.0001), and MTHFR/MTR/TYMS/MTRR (P<0.0001). MTHFR was found to increase the risk (OR: 2.36, 95% CI: 1.28-4.37) only in the absence of the TYMS 2R allele, with marked impairment of the remethylation process (P=0.007). This impairment was predominant when the dietary folate was in the lowest tertile. In subjects with dietary folate intake in the highest tertile, no such impairment was observed.

Conclusion: Dietary folate status and TYMS 5'-UTR 28bp tandem repeat polymorphism are important effect modifiers of CAD risk associated with genetic variants in remethylating genes.

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Key words: Coronary artery disease, methylene tetrahydrofolate reductase, methyltetrahydrofolate homocysteine methyltransferase, methyltetrahydrofolate homocysteine methyltransferase reductase, thymidylate synthase
CAD in all cases, triggering research into novel risk factors. Perturbations in folate metabolism can lead to elevated homocysteine and decreased functional folate, S-adenosylmethionine, glutathione (1). Elevated homocysteine acts as pro-oxidant, generating free radicals by auto-oxidation, inducing lipid peroxidation (2), causing endothelial cell damage and also perturbing hemostasis (3). Decreased functional folate is associated with the defective synthesis of purines and uracil misincorporation in DNA (4). Decreased S-adenosyl methionine (SAM) with a subsequent increase in S-adenosyl homocysteine methyltransferase (MTR, MIM: 156570) A2756G (rs1805087) and methyl tetrahydrofolate homocysteine methyltransferase reductase (MTRR, MIM: 602568) A66G (rs1801394). These polymorphisms interfere with the synthesis of methyltetrahydrofolate, remethylation of homocysteine, reductive methylation of cobalamin respectively (Fig. 1); however, there have been no studies on functional polymorphism in thymidylate synthase (TYMS) i.e., 28bp tandem repeat in 5'-UTR (transcription enhancer element). Presence of the 2R allele was found to affect the transcription process compared to 3R allele and might modulate folate flux. Several studies have shown that MTHFR C677T is an independent risk factor for CAD, especially in subjects with low folate status (9). Although polymorphisms in MTR and MTRR genes together

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**Fig. 1.** One Carbon metabolism
have been reported to be associated with hyperhomocysteinemia\(^\text{10}\), their association with CAD is still debated.

The current study was conducted on CAD subjects to address the underlying causes of inconsistencies in association studies reported world-wide, specifically by studying interactions between genes and micronutrients regulating one-carbon metabolism.

**Materials and Methods**

**Subjects**

Subjects were recruited from January 2009 to June 2010. Sample sizes were calculated based on the estimated proportion of the MTHFR 677T-allele in CAD cases and controls (0.18 vs. 0.11) from the existing literature\(^\text{11}\). With a type I error of 0.05 and 80% power, the required sample size was estimated to be 425 alleles (~213 subjects).

A total of 352 cases (281 males and 71 females, age: 20-80 yrs) with angiographically documented CAD with 50% coronary artery stenosis or a history of coronary angioplasty or surgical revascularization were included in the study. A positive family history was recorded when CAD was present in a first degree relative prior to 65 yrs old. Diabetes was diagnosed when fasting plasma glucose was >6.6 mmol/L or when the subject was taking any anti-diabetic agents. Hypertension was diagnosed when the average of three ambulatory blood pressure measurements was >140 mmHg (systolic) or >90 mmHg (diastolic) or when the subject was taking anti-hypertensive agents. Hyperlipidemia was considered when total cholesterol was >5.2 mmol/L or LDL cholesterol was >3.1 mmol/L or when the subject was taking hypolipemic agents. Smokers were defined as subjects who had been smoking cigarettes regularly from at least one year prior to the onset of symptoms or who had given up smoking after inclusion in the study. Alcohol intake was considered when the subject had been consuming alcohol from at least one year prior to the onset of symptoms or who stopped drinking alcohol after inclusion in the study.

The control group was selected by carefully matching each case for age, gender and ethnic background. The total of 284 controls (228 males and 56 females, age: 20-80 yrs) consisted of healthy blood donors and laboratory personnel, with no clinical symptoms of CAD. All subjects were asked to answer the food frequency questionnaire of 60 items based on Indian foods and food habits in order to assess the dietary intake of folate. Daily folate intake was calculated as grams of food multiplied by the amount of folate in the food and the frequency of consumption, summed for all foods. The composition of raw and cooked food items was determined from the 2007 reprint of the Nutritive value of Indian foods\(^\text{12}\). In certain cases, where information was not available on the composition, McCance and Widdowson’s The Composition of Foods\(^\text{13}\) and the US Department of Agriculture’s National Nutrient Database for Standard Reference release 19 (USDA, Washington, DC, USA) were consulted. During the study period, none of the subjects were on vitamin supplementation. Subjects with co-morbid conditions, such as acute or chronic inflammatory disease, immunological disease or neoplastic disease, were excluded from the study. The institutional ethics committee approved the study protocol.

**Methods**

**Biochemical analysis**

Whole blood samples were collected in EDTA and heparin from all subjects after overnight fasting. Plasma was separated immediately and stored at −20°C until analysis.

**Micronutrient analysis**

Plasma folate and plasma B12 levels were estimated on an AxSYM (Abbott Laboratories, USA) auto analyzer using commercial kits (Abbott Laboratories) as per the manufacturer’s instructions.

**Plasma nitric oxide analysis**

Plasma nitrite as a measure of nitric oxide levels was estimated using the Griess reaction.

**Total plasma homocysteine estimation**

Plasma samples were treated with tributyl-N-phosphine to reduce the thiols and deproteinization was performed using 10% trichloroacetic acid. The reduced thiols were derivatized with SBD-F (ammonium 7-fluorobenzo-2-oxa-1, 3-diazole-4-sulphonate), a thiol-specific fluorogenic probe. Isocratic high-performance liquid chromatography using Hichrom C18, 250 × 4.6 mm column (Hichrom Ltd., Berckshire, UK) as the stationary phase and 0.1 M KH:PO4 buffer (pH 2.1 adjusted with orthophosphoric acid): acetoniitrile mixture in the ratio of 96:4 as mobile phase was employed for the resolution of thiols. The fluorescence detector was set at Ex 385 nm/ Em 515 nm to detect the peaks of interest\(^\text{14}\).

**Genetic analysis**

Genomic DNA was isolated from the whole
blood using the method described by Luis A et al.\(^5\).

**MTHFR C677T polymorphism**

A 173-bp band from exon 4 of MTHFR was amplified using specific primers i.e. 5'-TTT GAG GCT GAC CTG AAA CAC CTT GAG GAG-3 and 5'-GAG TGG TAG CCC TGG ATG GGA AAG ATC CCG-3'. Each 25 μL of PCR mixture was composed of 100 ng genomic DNA, 2.5 μL 10x PCR buffer [50 mM KCl, 10 mM Tris-HCl (pH: 9.0), 1.5mM MgCl2], 0.2 mM of deoxynucleoside triphosphate, 10 pmol of each primer and 1 unit of Taq DNA polymerase. PCR conditions were: initial denaturation, 95°C for 10 min; denaturation, 95°C for 1 min; annealing, 60°C for 1 min; extension, 72°C for 1 min; number of cycles, 30. To test for the polymorphism, 20 μL of PCR product was digested with 1 unit of HaeIII restriction enzyme in 1X NEB buffer 2. Presence of the MTHFR 677T variant allele created a HaeIII restriction site, causing cleavage of the 173-bp product into 125-bp and 48-bp fragments.\(^{16}\)

**MTR A2756G polymorphism**

A 211-bp band was amplified using specific primers i.e. 5'-TGT TCC CAG TGT TTA GAT GAA CTC CTC-3' and 5'-GAT CCA AAG CCT TTT ACA AATC-3 and 5'-GAG TGG TAG CCC TGG ATG GGA AAG ATC CCG-3'. Each 25 μL of PCR mixture was composed of 100 ng genomic DNA, 2.5 μL 10x PCR buffer, and 0.2 mM deoxynucleoside triphosphate, 10 pmol of each primer and 1 unit of Taq DNA polymerase. PCR conditions were: initial denaturation, 95°C for 10 min; denaturation, 95°C for 30 s; annealing, 60°C for 30 s; extension, 72°C for 30 s; number of cycles, 40. To test for the polymorphism, 20 μL of PCR product was digested with 1 unit of HaeIII restriction enzyme in 1X NEB buffer 2. Presence of the MTR A2756G variant allele created a HaeIII restriction site, causing cleavage of the 211-bp product into 131-bp and 80-bp fragments\(^{15}\).

**TYMS 2R 3R**

PCR using specific primers, i.e. forward: 5' GTG GCT CCT GCG TTT CCC CC-3' and reverse: 5' CCA AGC TTT GCT CCG AGC CAG GCA TGG CGC GG-3' resulted in 220-bp and 250-bp fragments for 2R and 3R alleles, respectively. PCR conditions were: initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing at 62°C for 30 s, extension at 72°C for 45 s, final extension at 72°C for 5 min, total number of cycles: 35.

**Statistical analysis**

Unpaired Student's t-test and Fisher's exact test were performed for continuous and categorical variables, respectively. Independent genetic effects were evaluated by controlling for confounding factors using multivariate logistic regression. The statistical significance of individual genetic polymorphisms was checked using two approaches: I) by observing the P\(_{\text{trend}}\) across different genotypes based on the number of variant alleles (0, 1 and 2), and II) by considering the allele frequency distribution between cases and controls after controlling for confounding effects. A predominantly recessive model was used to ascertain statistical significance. Multifactor Dimensionality Reduction analysis (version: 2.0, beta 6) was used to explore gene-gene interactions. For all other statistical analysis, www.statpages.org was used. A p value of < 0.05/n was considered significant where 'n' indicates the number of variables analyzed.

**Results**

Among the personal habits, smoking was found to be associated with a 1.51-fold risk for CAD (95%CI: 1.07-2.09), whereas alcohol intake showed no significant association. Diabetes, hypertension and hyperlipidemia were found to be associated with 6.41- (95%CI: 3.88-10.58), 3.94- (95%CI: 2.65-5.84) and 2.78 (95%CI: 1.58-4.89) -fold risks for CAD, respectively (Table 1). Despite the gender differences in total plasma homocysteine levels in both groups, CAD cases showed a statistically significant elevation in homocysteine levels. Plasma nitric oxide levels were significantly lower in CAD cases than controls (Table 2).
Among the four genetic polymorphisms, MTHFR C677T and MTRR A66G were found to increase the risk for CAD by 1.61-fold (95% CI: 1.04-2.50) and 1.92-fold (95% CI: 1.29-2.87), whereas TYMS 2R allele was found to reduce the risk for CAD (OR: 0.66, 95% CI: 0.49-0.88) (Table 3). With reference to wild genotype combination at all three loci, co-segregation of MTHFR and MTRR variant alleles in the absence of TYMS 2R allele was associated with a 3.44-fold risk (95% CI: 1.52-7.73, \( P = 0.004 \)) for CAD.

Using Multifactor Dimensionality Reduction analysis, we found significant gene-gene interactions, i.e., TYMS/MTRR (\( p < 0.0001 \)), MTR/TYMS/MTRR
In the presence of TYMS 2R, MTHFR T allele showed no association with CAD. In the absence of TYMS 2R, MTHFR T allele was found to increase the risk for CAD by 2.36-fold (95% CI: 1.28-4.37) with marked impairment in the remethylation process, as evidenced by the significant elevation in plasma homocysteine levels ($P = 0.007$) (Table 4), indicating counteracting interactions between two variant alleles. In subjects with the MTR 2756 G allele, alcohol intake was found to increase the risk for CAD by 1.97-fold (95% CI: 1.08-3.59, $P = 0.04$) (data not shown).

Mean dietary intake of folate in cases and controls was found to be $349.33 \pm 102.82 \text{ } \mu g/\text{day}$ and $342.69 \pm 114.77 \text{ } \mu g/\text{day}$, respectively ($P = 0.48$). To validate the food frequency questionnaire, dietary folate values were correlated with plasma folate ($r = 0.32$) and significant correlation was observed ($P = 0.002$).

Segregation of TYMS/MTHFR data based on tertiles of dietary folate indicated inflated CAD risk with the MTHFR 677T allele in the absence of TYMS 2R allele with a low dietary intake of folate. With the increase in the dietary intake of folate, this risk was found to reduce in a dose-dependent manner. Across all tertiles of dietary folate, TYMS 2R was found to be protective. Co-segregation of variant alleles from both loci was found to negate their effect (Table 5).

### Table 3. Genotype and allele frequency distribution in cases and controls:

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotypes</th>
<th>Minor allele frequency</th>
<th>Adjusted OR (95% CI)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR C677T</td>
<td>CC</td>
<td>100/700 (14.28%)</td>
<td>1.61 (1.04-2.50)</td>
<td>0.04*</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>49/560 (8.75%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTR A2756G</td>
<td>AA</td>
<td>211/700 (30.14%)</td>
<td>1.00 (0.73-1.36)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTRR A66G</td>
<td>GG</td>
<td>256/702 (36.46%)</td>
<td>1.92 (1.29-2.87)</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYMS 2R</td>
<td>3R3R</td>
<td>187/692 (27.02%)</td>
<td>0.66 (0.49-0.88)</td>
<td>0.005*</td>
</tr>
<tr>
<td></td>
<td>2R3R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2R2R</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Adjusted OR: Odds ratio adjusted for conventional risk factors, CI: Confidence interval *: $p$ value statistically significant; $p_{\text{trend}}$ values for MTHFR, MTR, MTRR and TYMS polymorphisms (based on number of variant alleles) were 0.003, 0.87, 0.0001, 0.0006, respectively.

### Table 4. Counteracting interactions between TYMS and MTHFR and their impact on remethylation processes

<table>
<thead>
<tr>
<th>TYMS/MTHFR</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>Hcy(µM) Mean ± SD</th>
<th>$P$ value for Hcy</th>
</tr>
</thead>
<tbody>
<tr>
<td>−/−</td>
<td>127</td>
<td>96</td>
<td>1.00 (Ref)</td>
<td>13.5 ± 8.0</td>
<td>Ref</td>
</tr>
<tr>
<td>−/+</td>
<td>50</td>
<td>16</td>
<td>2.36 (1.28-4.37)*</td>
<td>19.9 ± 17.4</td>
<td>0.007*</td>
</tr>
<tr>
<td>+/−</td>
<td>125</td>
<td>133</td>
<td>0.71 (0.50-1.02)</td>
<td>16.5 ± 13.3</td>
<td>0.07</td>
</tr>
<tr>
<td>+/+</td>
<td>40</td>
<td>32</td>
<td>0.95 (0.56-1.61)</td>
<td>12.4 ± 6.1</td>
<td>0.50</td>
</tr>
</tbody>
</table>

For TYMS locus, ‘−’ indicates 3R3R genotype and ‘+’ indicates 2R3R and 2R2R genotypes; For MTHFR locus, ‘−’ indicates CC genotype and ‘+’ indicates CT and TT genotypes; OR: Odds ratio; CI: Confidence interval; Ref: Reference genotype; Hcy: Total plasma homocysteine (fasting); SD: standard deviation. *: $p=0.008$

($p<0.0001$), MTHFR/MTR/TYMS/MTRR ($p<0.0001$).

In the presence of TYMS 2R, MTHFR T allele showed no association with CAD. In the absence of TYMS 2R, MTHFR T allele was found to increase the risk for CAD by 2.36-fold (95% CI: 1.28-4.37) with marked impairment in the remethylation process, as evidenced by the significant elevation in plasma homocysteine levels ($P=0.007$) (Table 4), indicating counteracting interactions between two variant alleles. In subjects with the MTR 2756 G allele, alcohol intake was found to increase the risk for CAD by 1.97-fold (95% CI: 1.08-3.59, $P=0.04$) (data not shown).

Mean dietary intake of folate in cases and controls was found to be $349.33 \pm 102.82 \mu g/\text{day}$ and $342.69 \pm 114.77 \mu g/\text{day}$, respectively ($P=0.48$). To validate the food frequency questionnaire, dietary folate values were correlated with plasma folate ($r=0.32$) and significant correlation was observed ($P=0.002$).

Segregation of TYMS/MTHFR data based on tertiles of dietary folate indicated inflated CAD risk with the MTHFR 677T allele in the absence of TYMS 2R allele with a low dietary intake of folate. With the increase in the dietary intake of folate, this risk was found to reduce in a dose-dependent manner. Across all tertiles of dietary folate, TYMS 2R was found to be protective. Co-segregation of variant alleles from both loci was found to negate their effect (Table 5).

### Discussion

The current study was planned to address the factors responsible for inconsistencies in association...
studies with reference to aberrations in one-carbon metabolism in the pathophysiology of CAD. Four genetic polymorphisms, one affecting DNA synthesis (TYMS 28bp tandem repeat) and three affecting the remethylation process (MTHFR C677T, MTR A2756G, MTRR A66G), were studied as possible candidate genetic variants to explore whether alteration in the flux of folate either in favor of DNA synthesis (5, 10-methylene tetrahydrofolate) or in favor of remethylation (5-methyl tetrahydrofolate) has any predictive role in the etiology of CAD.

Our data showed that MTHFR C677T and MTRR A66G polymorphisms increase the risk for CAD whereas TYMS polymorphism reduces the risk. Bivariate analysis showed clear evidence for these counteracting interactions between TYMS and MTHFR. Presence of the TYMS 2R allele was found to nullify the risk associated with the MTHFR 677T allele. Only in the absence of the TYMS 2R allele was the MTHFR 677T found to increase the risk for CAD by markedly impairing the remethylation process, as evidenced by elevated homocysteine levels. Multifactor Dimensionality Reduction analysis showed further evidence both for synergistic effects between genes crucial for remethylation and counteracting effects between TYMS and genes crucial for remethylation. In CAD cases, nitric oxide bioavailability was found to be significantly low compared to controls. Plasma homocysteine was found to be elevated in cases compared to controls. Among the lifestyle factors, alcohol intake was found to be associated with a 1.97-fold risk for CAD in subjects with the MTR G allele.

This is the first study, to the best of our knowledge, which has shown evidence of counteracting interactions between TYMS 2R and MTHFR 677T alleles. In subjects with low dietary folate, the MTHFR 677T allele was found to have a high risk for CAD in the absence of the TYMS 2R allele. The risk associated with the MTHFR variant allele was found to be nullified in the presence of the TYMS 2R allele or in subjects with high dietary folate. When the dietary folate was adequate (second and third tertiles), both processes, i.e. dTMP synthesis and remethylation, were not affected by the presence or absence of variant alleles at both loci, clearly indicating the risk-modifier role of folate. In the presence of the TYMS 2R allele, due to decreased transcription of TYMS, all the folate is available for the remethylation process. When the dietary folate is low or the TYMS 2R allele is absent, the folate available for the remethylation process will be significantly low and, in such conditions, the presence of the MTHFR 677T variant impairs remethylation. The counteracting interactions between TYMS and MTHFR are biologically plausible because both compete for the same substrate, i.e. 5,10-methylene tetrahydrofolate. The relationship between reduced folate levels and MTHFR in increasing the risk for CAD has been well documented in populations with a low folate status or in populations lacking strategies for fortifying food with folate. In native Indians, it was reported to be a significant risk factor whereas it showed no risk in Indians residing in the UK, probably suggesting dietary or environmental influences.

The current study showed decreased levels of NO in CAD cases compared to controls. Synthesis of NO by endothelial cells is essential for vasomotor function and NO has important functions, such as...

<p>| Table 5. TYMS/MTHFR interactions according to dietary folate status |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|</p>
<table>
<thead>
<tr>
<th>TYMS-MTHFR</th>
<th>Dietary folate intake (Case/Control)</th>
<th>1st tertile</th>
<th>2nd tertile</th>
<th>3rd tertile</th>
<th>$P_{\text{trend}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>− / − genotype</td>
<td></td>
<td>61/35</td>
<td>27/28</td>
<td>39/33</td>
<td>0.19</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>1.00 (ref)</td>
<td>0.55 (0.28-1.08)</td>
<td>0.68 (0.37-1.26)</td>
<td>0.03</td>
</tr>
<tr>
<td>− / +</td>
<td></td>
<td>29/5</td>
<td>14/5</td>
<td>7/6</td>
<td>0.62</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>3.33 (1.21-9.05)</td>
<td>1.61 (0.55-4.65)</td>
<td>0.67 (0.22-2.06)</td>
<td>0.18</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>39/49</td>
<td>55/37</td>
<td>31/47</td>
<td>0.18</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>0.46 (0.25-0.82)</td>
<td>0.85 (0.48-1.53)</td>
<td>0.38 (0.21-0.70)</td>
<td>0.18</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>16/8</td>
<td>11/10</td>
<td>13/14</td>
<td>0.18</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>1.15 (0.45-2.89)</td>
<td>0.63 (0.25-1.60)</td>
<td>0.53 (0.23-1.25)</td>
<td>0.18</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>0.90</td>
<td>0.49</td>
<td>0.19</td>
<td>0.18</td>
</tr>
</tbody>
</table>

For TYMS locus: ‘−’ indicates 3R3R genotype and ‘+’ indicates 2R3R and 2R2R genotypes; For MTHFR locus: ‘−’ indicates CC genotype and ‘+’ indicates CT and TT genotypes; OR: Odds ratio; CI: Confidence interval; Ref: Reference genotype.
antiplatelet, antiproliferative, permeability-decreasing and anti-inflammatory properties\(^{24}\) and does not induce free radical generation\(^{25}\). Decrease in NO enhances leukocyte adhesion, increased expression of VCAM-1 (vascular endothelial cell adhesion molecule) and MCP-1 (monocyte chemotactic protein)\(^{29}\). Decreased bioavailability of NO is a marker for endothelial dysfunction. Elevated plasma homocysteine levels as reported in this study were found to be associated with the reduced activity of dimethyl arginine dimethylaminohydrolase (DDAH), in a dose-dependent manner, thus increasing asymmetric dimethylarginine and reduced NO synthesis\(^{30}\). Low 5-MTHF (due to MTHFR C677T) and low nitric oxide together hamper endothelium-dependent vasomotor response, increasing vascular superoxide ions due to reduced peroxynitrite scavenging and low vascular BH4\(^{27}\).

We have observed a strong and independent association of MTRR A66G polymorphism with CAD, which is in agreement with a study on French subjects\(^{28}\). Synergistic interactions of the MTRR variant allele, specifically additive interactions with MTHFR and counteracting interactions with TYMS, were evident in multifactor dimensionality reduction analysis data. MTRR is a molecular chaperone for MTR, with both forming a holoenzyme in 1:1 stoichiometric ratio to regenerate oxidized cobalamin and methylate it to methylcobalamin using SAM\(^{30}\). No association was observed for MTR A2756G individually; however, synergistic interactions were observed with other variants and in subjects with frequent alcohol intake. The gene-environment interaction between MTR-alcohol intake could be due to inhibition of the MTR enzyme\(^{30}\) or to trapping of 5-MTHF by alcohol\(^{31}\). Although numerous studies have been conducted globally, specifically on hyperhomocysteinemia and certain genetic polymorphisms in one-carbon metabolism, this current study is the first of its kind focusing on genetic polymorphisms in one-carbon metabolism, and its epigenetic mechanisms in monocytes.\(^{1}\) Finkelstein JD: Pathways and regulation of homocysteine metabolism in mammals. Semin Thromb Hemost, 2000; 26(3): 219-244


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**References**


