Increased prevalence of methylenetetrahydrofolate reductase C677T variant in patients with inflammatory bowel disease, and its clinical implications

N Mahmud, A Molloy, J McPartlin, R Corbally, A S Whitehead, J M Scott, D G Weir

Abstract

Background—Inflammatory bowel disease (IBD) is associated with an increased incidence of thromboembolic disease. Hyperhomocystinaemia (hyper-tHcy), a condition associated with the C677T variant of 5,10-methylenetetrahydrofolate reductase (MTHFR), is linked with an increased incidence of thromboembolic disease. Hyper-tHcy has been reported in patients with IBD.

Aims—To assess the prevalence of the C677T MTHFR genotype and the contribution of this genotype to hyper-tHcy in patients with IBD.

Methods—Patients with established IBD (n=174) and healthy controls (n=273) were studied. DNA samples were genotyped for the MTHFR (C677T) mutation. Subjects were categorised as homozygous for the thermolabile variant (TT), heterozygous for wild type and variant (CT), or homozygous for the wild type (CC).

Results—Plasma homocysteine concentrations were significantly higher in patients with IBD than in healthy controls. A total of 17.5% of ulcerative colitis and 16.8% of Crohn’s disease patients were homozygous for the C677T variant compared with 7.3% of controls. Homozygosity (TT) for the variant was associated with higher plasma tHcy levels in patients with IBD and in healthy controls. When all subjects who were TT for the variant were excluded, median plasma tHcy was still significantly higher in IBD than controls. Plasma vitamin B12 levels were lower in patients with IBD irrespective of MTHFR genotype.

Conclusions—There is an association between the thermolabile MTHFR C677T variant and IBD. This accounts in part for the raised plasma tHcy found in patients with IBD and may contribute to the increased incidence of thromboembolic complications. All patients with IBD should receive low dose folic acid and vitamin B12 therapy to protect against the thromboembolic complications of raised tHcy.

Keywords: methylenetetrahydrofolate reductase; C677T variant; inflammatory bowel disease

A thermolabile variant of 5,10-methylenetetrahydrofolate reductase (MTHFR) has been described.1-2 This enzyme is responsible for synthesising 5-methyltetrahydrofolate polyglutamate whose sole function is to remethylate homocysteine via the vitamin B12 dependent enzyme methionine synthase to produce methionine (fig 1). The genetic cause of this thermolabile variant has been shown to be a C to T mutation at base pair 677 causing an amino acid change from alanine to valine.2 The presence of the MTHFR C677T variant is associated with raised levels of plasma homocysteine (tHcy), especially in patients with low folate levels.3 Furthermore, it is associated with neural tube defects4 and with coronary artery disease5 in some but not in other studies.6 This variant is relatively common in the community. A recent meta-analysis showed it to be present in 9.2% of white control groups.8

The incidence of systemic thromboembolic events is raised in inflammatory bowel disease (IBD).9,10 It is suggested that this may be related to a hypercoagulable state.11-13 Despite evidence for activation of haemostasis in IBD the mechanism of the procoagulant activity remains unclear. Recently, a detailed assessment of prothrombotic agents showed no abnormality in patients with IBD who had had a thromboembolic event.14 However, Leibman et al have shown a heterozygous mutation of factor V Leiden in 36% of patients with IBD who had had a previous thromboembolic event.13 Over et al have shown a similar mutation in 45% of Crohn’s disease but not in ulcerative colitis patients.16 Mild hyperhomocysteinaemia (hyper-tHcy) is associated with raised levels of plasma tHcy and is a feature of patients with IBD. A higher prevalence of the C677T MTHFR variant was observed in patients with IBD and may contribute to the raised plasma tHcy levels.

Abbreviations used in this paper: tHcy, homocysteine; IBD, inflammatory bowel disease; MTHFR, 5,10-methylenetetrahydrofolate reductase.

Figure 1 Metabolic pathways of homocysteine and methionine.
Table 1 Characteristics of the patients with inflammatory bowel disease (IBD) and controls

<table>
<thead>
<tr>
<th>IBD (n=174)</th>
<th>Controls (n=89)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>tHcy (µmol/l)</strong></td>
<td>10.85 (3.25–39.24)</td>
<td>8.99 (4.13–16.16)</td>
</tr>
<tr>
<td><strong>PF (µg/l)</strong></td>
<td>4.82 (1.15–18.5)</td>
<td>5.80 (1.90–17.75)</td>
</tr>
<tr>
<td><strong>RCF (µg/l)</strong></td>
<td>381 (97–1342)</td>
<td>417 (159–1092)</td>
</tr>
<tr>
<td><strong>Vitamin B12 (ng/l)</strong></td>
<td>378 (171–956)</td>
<td>452 (165–801)</td>
</tr>
</tbody>
</table>

5-ASA, 5-aminosalicylic acid.

Table 2 Median (range) plasma homocysteine (tHcy), folate (PF), red cell folate (RCF) and vitamin B12 concentrations in patients with inflammatory bowel disease (IBD) and healthy controls

The aim of this study was to assess the prevalence of the MTHFR C677T variant in patients with IBD and to determine whether the tHcy levels seen in these patients are related to the presence of the MTHFR C677T variant.

Methods

Blood samples were collected prospectively from 174 patients (77 men, median age 40.5 years, range 20–77; 97 women, median age 37 years, range 17–69) with established IBD (91 ulcerative colitis, 83 Crohn’s disease). The median duration of the disease was 6.6 years and ranged from three months to 40 years. The control population comprised two groups: one derived from a previous study and used to define the frequency of the MTHFR genotype frequencies in the Irish population (n=184). The second was a group of normal hospital personnel in whom a complete vitamin and health status profile was obtained (n=89).

Results

PLASMA HOMOCYSTEINE IN IBD PATIENTS AND HEALTHY CONTROLS

Patients with IBD had significantly higher levels of plasma homocysteine compared with controls (median 10.85 µmol/l (range 3.25–39.24) versus 8.99 µmol/l (4.13–16.16); p=0.0001; fig 2, table 2). Plasma homocysteine levels were similar in patients with ulcerative colitis and Crohn’s disease (median 11.09 µmol/l (range 3.25–39.24) versus 10.19 µmol/l (4.56–36.40); p=0.6).

Plasma homocysteine levels were similar in patients with IBD maintained on different 5-aminosalicylic acid therapy compared with those who were receiving no therapy or those who had had surgical treatment (data not shown).
Table 2 presents a comparison of plasma folate, red cell folate, and vitamin B₁₂ concentrations between patients with IBD and controls. Plasma folate and vitamin B₁₂ levels were significantly lower in patients with IBD. The plasma folate and vitamin B₁₂ levels were similar in patients with ulcerative colitis and Crohn’s disease; however, red cell folate levels were significantly lower in Crohn’s disease patients (median 338 ng/l, range 113–781) compared with ulcerative colitis patients (median 392 ng/l, range 97–783; p=0.03).

METHYLENETETRAHYDROFOLATE REDUCTASE STATUS AND PLASMA HOMOCYSTEINE, FOLATE, AND VITAMIN B₁₂ LEVELS

Homozygosity for the MTHFR C677T variant (TT) was present in 17.5% (n=16/91) of ulcerative colitis and 16.8% (n=14/83) of Crohn’s disease patients compared with 7.3% (n=20/273) of controls (ulcerative colitis: odds ratio 2.81; 95% confidence interval (CI) 1.28 to 6.13, p=0.01; Crohn’s disease: odds ratio 2.67; 95% CI 1.19 to 5.97, p=0.02). Plasma tHcy levels were significantly higher in patients with IBD who were TT compared with CT (median 12.91 µmol/l (range 3.20–39.24) versus 10.47 µmol/l (5.54–38), p=0.02) and CC (median 10.50 µmol/l (range 1.53–36.40), p=0.02; fig 3).

Control TT subjects also had higher tHcy levels than CT and CC groups. When TT subjects were excluded from the IBD cases and controls, the median tHcy of combined CT and CC patients with IBD was 10.5 µmol/l (range 1.15–38) compared with combined CT and CC controls (8.96 µmol/l (range 4.13–16.16); p<0.05).

A significant inverse correlation was observed between plasma tHcy and plasma folate in patients with IBD irrespective of genotype and between plasma tHcy and red cell folate in patients with IBD who were TT (r=0.41; p=0.03). There was no significant relation between tHcy and plasma vitamin B₁₂ (r=0.1; p=0.5; table 3).

Table 3 Relation of plasma homocysteine (tHcy) with plasma folate (PF), red cell folate (RCF), and vitamin B₁₂ in patients with inflammatory bowel disease (IBD) and controls

<table>
<thead>
<tr>
<th></th>
<th>TT</th>
<th>p Value</th>
<th>CT</th>
<th>p Value</th>
<th>CC</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with IBD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>r=-0.47</td>
<td>0.01</td>
<td>r=-0.38</td>
<td>0.001</td>
<td>r=-0.47</td>
<td>0.001</td>
</tr>
<tr>
<td>RCF</td>
<td>r=-0.41</td>
<td>0.03</td>
<td>r=0.15</td>
<td>0.20</td>
<td>r=-0.13</td>
<td>0.30</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>r=0.02</td>
<td>0.90</td>
<td>r=0.12</td>
<td>0.30</td>
<td>r=-0.14</td>
<td>0.27</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>r=-0.21</td>
<td>0.62</td>
<td>r=-0.47</td>
<td>0.02</td>
<td>r=-0.20</td>
<td>0.10</td>
</tr>
<tr>
<td>RCF</td>
<td>r=-0.50</td>
<td>0.20</td>
<td>r=-0.09</td>
<td>0.70</td>
<td>r=-0.10</td>
<td>0.70</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>r=-0.50</td>
<td>0.26</td>
<td>r=-0.02</td>
<td>0.51</td>
<td>r=-0.25</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Control TT subjects also had higher tHcy levels than CT and CC groups. When TT subjects were excluded from the IBD cases and controls, the median tHcy of combined CT and CC patients with IBD was 10.5 µmol/l (range 1.15–38) compared with combined CT and CC controls (8.96 µmol/l (range 4.13–16.16); p<0.05).

Five (2.8%) patients with IBD had experienced thromboembolic event(s) in the past (two deep venous thrombosis, two myocardial infarction with coronary artery bypass surgery, and one stroke). Two (2/5) were TT (40%) and three were CT (60%) for the MTHFR C677T variant. The tHcy levels were significantly higher in patients with IBD who had experienced (median 20 µmol/l, range 14.33–31.53) compared with those who had not experienced thromboembolic event(s) in the past (median 10.71 µmol/l, range 4.54–39.14; p=0.001).

EFFECT OF MTHFR C677T GENOTYPE ON tHcy AND BLOOD VITAMINS

Patients with IBD and control subjects who were TT for the MTHFR C677T variant had higher tHcy than CC subjects and both CC and CT subjects and patients (table 4). In patients with IBD but not in controls, plasma folate levels were also lower in TT patients (table 5). Red cell folate was lower in TT’s than CCs in both patients and controls and was lower than CTs in patients (table 6). There was no significant effect of the genotype on vitamin B₁₂ status (table 7).
TABLE 4 Median (range) plasma homocysteine (tHcy) concentrations in patients with inflammatory bowel disease (IBD) and normal healthy controls stratified by MTHFR C677T variant

<table>
<thead>
<tr>
<th>Genotype</th>
<th>IBD</th>
<th>Controls</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>12.91 (3.20–39.24)</td>
<td>n=30</td>
<td>11.56 (8.70–14)</td>
</tr>
<tr>
<td>CT</td>
<td>10.47 (5.54–38)</td>
<td>n=70</td>
<td>9.07 (6.33–14.56)</td>
</tr>
<tr>
<td>CC</td>
<td>10.50 (1.56–36.40)</td>
<td>n=72</td>
<td>8.96 (5.41–13.75)</td>
</tr>
</tbody>
</table>

IBD: TT versus CT, p=0.02; TT versus CC, p=0.03.
Controls: TT versus CT, p=0.14; TT versus CC, p=0.03.

TABLE 5 Median (range) plasma folate (PF) concentrations in patients with inflammatory bowel disease (IBD) and normal healthy controls stratified by MTHFR C677T variant

<table>
<thead>
<tr>
<th>Genotype</th>
<th>IBD</th>
<th>Controls</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>4.40 (1.15–7.65)</td>
<td>n=27</td>
<td>6.0 (3.96–10.40)</td>
</tr>
<tr>
<td>CT</td>
<td>5.56 (1.35–22.50)</td>
<td>n=70</td>
<td>6.15 (2.20–16.10)</td>
</tr>
<tr>
<td>CC</td>
<td>6.05 (1.25–23.60)</td>
<td>n=72</td>
<td>5.6 (1.90–17.75)</td>
</tr>
</tbody>
</table>

IBD: TT versus CT, p=0.03; TT versus CC, p=0.03.
Controls: TT versus CT, p=0.8; TT versus CC, p=0.9.

TABLE 6 Median (range) red cell folate (RCF, µg/l) concentrations in patients with inflammatory bowel disease (IBD) and normal healthy controls stratified by MTHFR C677T variant

<table>
<thead>
<tr>
<th>Genotype</th>
<th>IBD</th>
<th>Controls</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>337 (97–737)</td>
<td>n=27</td>
<td>327 (159–485)</td>
</tr>
<tr>
<td>CT</td>
<td>407 (113–786)</td>
<td>n=73</td>
<td>420 (227–686)</td>
</tr>
<tr>
<td>CC</td>
<td>395 (113–624)</td>
<td>n=67</td>
<td>477 (205–848)</td>
</tr>
</tbody>
</table>

IBD: TT versus CT, p=0.03; TT versus CC, p=0.03.
Controls: TT versus CT, p=0.1; TT versus CC, p=0.9.

TABLE 7 Median (range) vitamin B12 (ng/l) concentrations in patients with inflammatory bowel disease (IBD) and normal healthy controls stratified by MTHFR C677T variant

<table>
<thead>
<tr>
<th>Genotype</th>
<th>IBD</th>
<th>Controls</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>365 (219–694)</td>
<td>n=24</td>
<td>393 (188–716)</td>
</tr>
<tr>
<td>CT</td>
<td>390 (171–875)</td>
<td>n=70</td>
<td>470 (265–748)</td>
</tr>
<tr>
<td>CC</td>
<td>364 (96–596)</td>
<td>n=27</td>
<td>497 (344–673)</td>
</tr>
</tbody>
</table>

IBD: TT versus CT, p=0.14; TT versus CC, p=0.03.
Controls: TT versus CT, p=0.02; TT versus CC, p=0.03.

TABLE 8 Variable selected from stepwise procedure

<table>
<thead>
<tr>
<th>Estimated coefficient</th>
<th>t Value</th>
<th>p Value</th>
</tr>
</thead>
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<tr>
<td>Age</td>
<td>0.008</td>
<td>3.15</td>
</tr>
<tr>
<td>Disease type (UC/CD)</td>
<td>-0.042</td>
<td>-0.56</td>
</tr>
<tr>
<td>Medication (5-ASA with/without steroid or azathioprine)</td>
<td>-0.23</td>
<td>-2.23</td>
</tr>
</tbody>
</table>

UC, ulcerative colitis; CD, Crohn’s disease; ASA, aminosalicylic acid.

TABLE 9 Effect of folic acid treatment (400 µg/day) for six weeks in patients with inflammatory bowel disease (IBD) (n=39)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Post-treatment</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy</td>
<td>13.6 (5.40–37.95)</td>
<td>9.01 (5.96–17.16)</td>
</tr>
<tr>
<td>PF</td>
<td>4.15 (1.85–13.50)</td>
<td>25.10 (4.10–130)</td>
</tr>
<tr>
<td>RCF</td>
<td>381 (113–1063)</td>
<td>703 (184–2070)</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>369 (219–694)</td>
<td>393 (188–716)</td>
</tr>
</tbody>
</table>

tHcy, homocysteine; PF, plasma folate; RCF, red cell folate.

The regression model results. The disease severity, extent, longevity, and type of IBD were found to be not statistically significantly different in the presence of age and medication.

PLASMA HOMOCYSTEINE, FOLATE, AND RED CELL FOLATE PRE AND POST FOLIC ACID TREATMENT
Following six weeks’ treatment with 400 µg of folic acid per day plasma tHcy levels were significantly reduced in 39 patients with IBD (pretreatment tHcy: median 13.63, range 5.40–37.95 µmol/l; post-treatment tHcy: median 9.01, range 4.96–17.16 µmol/l; p=0.0001). A similar reduction in tHcy levels was observed in all three IBD patient genotypes for the MTHFR C677T (TT (n=4): pre, 21.04 (range 17–32) versus post, 12.50 (10.56–17) mmol/l, p=0.03; CT (n=12): 11 (6.50–20) versus 8 (5.22–13) mmol/l, p=0.01; CC (n=23): 14 (5–38) versus 9 (5–16) mmol/l, p=0.001). Plasma and red cell folate levels were significantly increased (table 9; fig 4).

Discussion
This study has shown that plasma homocysteine was higher in patients with IBD than in controls and this is associated with lower plasma folate and vitamin B12 levels. The nutritional reasons why folate requirements may be increased in patients with IBD are well known and include inadequate nutritional intake due to malaise and anorexia, excessive nutrient requirement due to bowel inflammation, and possible folate malabsorption induced by drug therapy such as sulphasalazine. In our study, however, sulphasalazine therapy was not associated with folate deficiency (data not shown).

In this study the MTHFR C677T genotype has also been shown to be significantly enriched in patients with IBD. The TT genotype for this variant was associated with low red cell folate and high tHcy both in patients with IBD and controls; however, low plasma folate was observed only in patients with IBD who were TT for the MTHFR...
Methylenetetrahydrofolate reductase C677T variant in IBD

The authors wish to thank Dr Alan Kelly, Senior Lecturer in Biostatistics, Trinity College, University of Dublin, for statistical advice.

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Gut 1999 45: 389-394
doi: 10.1136/gut.45.3.389

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