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. Hyperhomocysteinaemia (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 B_{12} 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 B_{12} therapy to protect against the thromboembolic complications of raised tHcy.**

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Keywords: methylenetetrahydrofolate reductase; C677T variant; inflammatory bowel disease

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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 B_{12} 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),[3] especially in patients with low folate levels.[4] Furthermore, it is associated with neural tube defects[5] and with coronary artery disease in some[6] but not in other studies.[7] 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 mutation in 36% of patients with IBD who had had a previous thromboembolic event.[15] Over *et al* have shown a similar mutation in 45% of Crohn's disease but not in ulcerative colitis patients.[16] Mild hyperhomo-

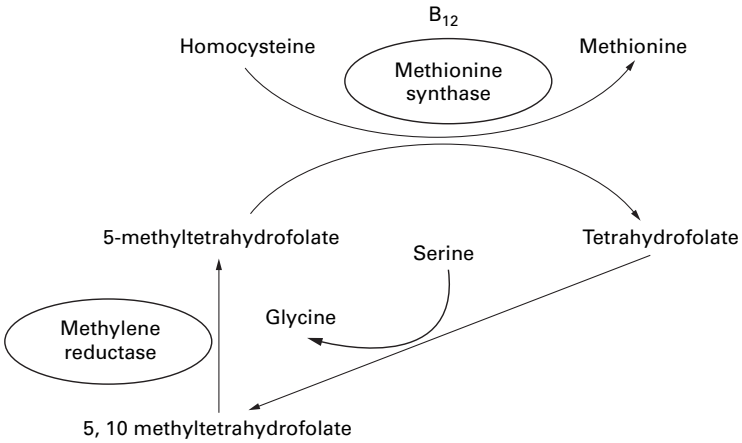

Figure 1 Metabolic pathways of homocysteine and methionine.

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

Table 1 Characteristics of the patients with inflammatory bowel disease (IBD) and controls

	IBD	*Controls*
Number	174	89
Sex		
Male	77	39
Female	97	50
Median age (range) (y)		
Male	40.5 (20–77)	36 (21–64)
Female	37 (17–69)	35 (20–61)
Ulcerative colitis	91	
Crohn's disease	83	
Duration of disease (range) (y)	9.01 (0.3–40)	
Receiving oral 5-ASA treatment	144	
Sulphasalazine (Salazopyrin)	40	
pH dependent release mesalazine (Asacol)	8	
Slow release mesalazine (Pentasa)	28	
Olsalazine (Dipentum)	62	
Concurrent use of oral steroids	13	
Concurrent use of azathioprine with/without steroids	13	
Enema therapy only (5-ASA or steroid)	6	
Receiving no maintenance therapy (5-ASA)	18	
History of intestinal resection	18	
Proctocolectomy	12	
Terminal ileum or a part of small bowel	6	
Disease parameters—median (range)		
Haemoglobin (12–17 g/dl)	12.5 (8.5–16.2)	
Platelet count (150–350 × 10^3/mm^3)	162 (158–678)	
Harvey Bradshaw index	2 (0–11)	
Serum C-reactive protein	3.27 (3.27–54.40)	

5-ASA, 5-aminosalicylic acid.

cysteinaemia is now recognised as an independent risk factor for thromboembolism.[17] It is also associated with atheromatous vascular disease including coronary artery disease,[18 19] cerebrovascular,[20] and peripheral vascular disease.[21] Recently, a high prevalence of hyperhomocysteinaemia (hyper-tHcy) has been described in patients with IBD,[22] which might imply that they may also be at increased risk of developing the thromboembolic complications associated with hyper-tHcy.[23]

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[24] was 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 homocysteine profile was obtained (n=89). Table 1 gives further demographic details. Each sample was collected into potassium/edetic acid and was logged and divided into three labelled polypropylene tubes. The first contained a sample of whole blood; the second an aliquot of whole blood diluted (1/10) into 1% ascorbic for the analysis of red cell folate; and the third contained the plasma sample. Plasma tHcy, folate, red cell folate, and vitamin B_{12} were measured in all patients with IBD and healthy hospital controls. Plasma folate,[25] red cell folate,[25] and vitamin B_{12}[26] were measured by microbiological methods. Plasma homocysteine levels were measured by an HPLC method.[27] DNA samples were genotyped for the MTHFR C677T variant by polymerase chain reaction (PCR) and restriction analysis as described previously.[28] Patients with IBD and controls were categorised according to whether they were homozygous for the thermolabile (TT), heterozygous for wild type and thermolabile (CT), or homozygous for the wild type (CC).

Table 2 Median (range) plasma homocysteine (tHcy), folate (PF), red cell folate (RCF) and vitamin B_{12} concentrations in patients with inflammatory bowel disease (IBD) and healthy controls

	IBD (n=174)		*Controls (n=89)*		*p Value*
tHcy (μmol/l)	10.85 (3.25–39.24)	n=169	8.99 (4.13–16.16)	n=87	0.0001
PF (μg/l)	4.82 (1.15–18.5)	n=169	5.80 (1.90–17.75)	n=87	0.03
RCF (μg/l)	381 (97–1342)	n=167	417 (159–1092)	n=83	0.5
Vitamin B_{12} (ng/l)	378 (171–956)	n=164	452 (165–801)	n=87	0.001

On the day of the visit, clinical disease activity was quantified using the simple Harvey Bradshaw index (HBI)[29] and all patients also had routine biochemistry and estimation of C-reactive protein by nephelometry. Disease was considered active if the HBI was 4 or greater.

Of the 174 patients with IBD, 39 also had blood samples taken following six weeks' treatment with folic acid (400 μg per day) for the measurement of plasma homocysteine, folate, and red cell folate levels.

STATISTICAL ANALYSIS.

The data were not normally distributed, therefore the results are expressed as median (range). A probability value of less than 0.05 was considered to be statistically significant. Mann-Whitney U and Fisher's exact tests were used for the analysis of data. A regression model was used to identify the predictors influencing homocysteine levels. Starting from the full model with all variables included, non-significant variables were progressively deleted with a step down procedure based on a likelihood ratio test.

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).

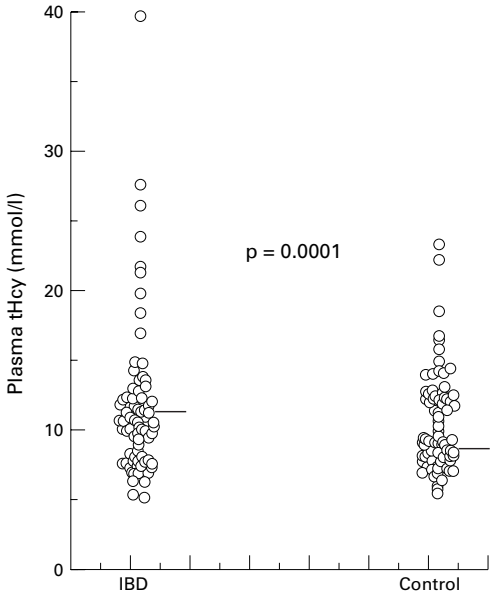

Figure 2 Plasma homocysteine (tHcy) concentrations in patients with inflammatory bowel disease (IBD) and healthy controls. Median is shown by a horizontal line.

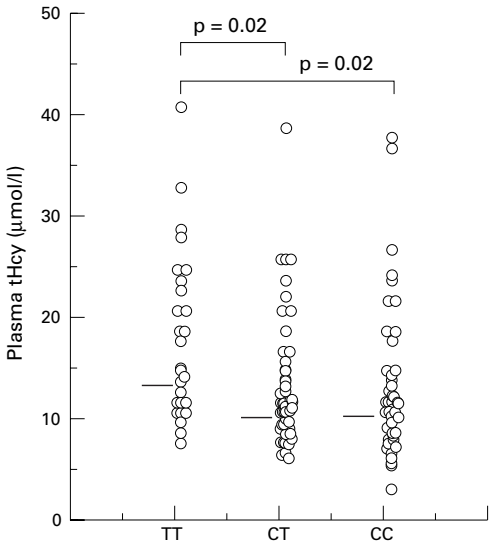

Figure 3 Plasma homocysteine (tHcy) concentrations according to the MTHFR C677T variant genotype in patients with IBD. Median is shown by a horizontal line.

BLOOD VITAMIN LEVELS IN PATIENTS WITH IBD AND HEALTHY CONTROLS

Table 2 presents a comparison of plasma folate, red cell folate, and vitamin B_{12} concentrations between patients with IBD and controls. Plasma folate and vitamin B_{12} levels were significantly lower in patients with IBD. The plasma folate and vitamin B_{12} 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, RED CELL FOLATE, AND VITAMIN B_{12} 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).

Table 3 Relation of plasma homocysteine (tHcy) with plasma folate (PF), red cell folate (RCF), and vitamin B_{12} in patients with inflammatory bowel disease (IBD) and controls

	TT	*p Value*	*CT*	*p Value*	*CC*	*p Value*
Patients with IBD						
PF	*r*=−0.47	0.01	*r*=−0.38	0.001	*r*=−0.47	0.001
RCF	*r*=−0.41	0.03	*r*=0.15	0.20	*r*=−0.13	0.30
Vitamin B_{12}	*r*=0.02	0.90	*r*=0.12	0.30	*r*=−0.14	0.27
Controls						
PF	*r*=−0.21	0.62	*r*=−0.47	0.02	*r*=−0.20	0.10
RCF	*r*=−0.50	0.20	*r*=−0.09	0.70	*r*=−0.10	0.70
Vitamin B_{12}	*r*=−0.50	0.26	*r*=−0.02	0.51	*r*=−0.25	0.37

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 B_{12} (*r*=0.1; p=0.5; table 3).

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 TTs 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 B_{12} 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

	tHcy (μmol/l)				
Genotype	IBD		Controls		p Value
TT	12.91 (3.20–39.24)	n=30	11.56 (8.70–14)	n=7	0.16
CT	10.47 (5.54–38)	n=70	9.07 (6.33–14.56)	n=38	0.06
CC	10.50 (1.56–36.40)	n=72	8.96 (5.41–13.75)	n=42	0.01

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

	PF (μg/l)				
Genotype	IBD		Controls		p Value
TT	4.40 (1.15–7.65)	n=27	6.0 (3.96–10.40)	n=7	0.01
CT	5.56 (1.55–22.50)	n=70	6.15 (2.20–16.10)	n=38	0.90
CC	6.05 (1.25–23.60)	n=72	5.6 (1.90–17.75)	n=42	0.50

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

	RCF (μg/l)				
Genotype	IBD		Controls		p Value
TT	337 (97–737)	n=27	327 (158–485)	n=7	0.7
CT	407 (113–786)	n=73	420 (227–686)	n=30	0.3
CC	395 (113–624)	n=67	477 (205–848)	n=42	0.9

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

Table 7 Median (range) vitamin B_{12} (ng/l) concentrations in patients with inflammatory bowel disease (IBD) and normal healthy controls stratified by MTHFR C677T variant

	Vitamin B_{12} (ng/l)				
Genotype	IBD		Controls		p Value
TT	364 (96–596)	n=24	497 (344–673)	n=7	0.02
CT	390 (171–875)	n=70	470 (265–748)	n=31	0.02
CC	397 (212–801)	n=70	428 (165–800)	n=44	0.02

IBD: TT versus CT, p=0.1; TT versus CC, p=0.06.
Controls: TT versus CT, p=0.9; TT versus CC, p=0.9.

Table 8 Variable selected from stepwise procedure

	Estimated coefficient	t Value	p Value
Age	0.008	3.15	0.002
Disease type (UC/CD)	−0.042	−0.56	0.57
Medication (5-ASA with/without steroid or azathioprine)	−0.25	−2.23	0.02

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)

	Pretreatment	Post-treatment	p Value
tHcy	13.6 (5.40–37.95)	9.01 (4.96–17.16)	0.0001
PF	4.15 (1.85–13.50)	25.10 (4.10–150)	0.0001
RCF	381 (113–1063)	703 (184–2070)	0.0001
Vitamin B_{12}	365 (219–694)	393 (188–716)	0.70

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

EFFECT OF PATIENT'S AGE, DISEASE SEVERITY, EXTENT, LONGEVITY, TYPE OF IBD, AND MEDICATION ON PLASMA HOMOCYSTEINE

Increase in the age of the patient was an independent predictor of hyperhomocysteinaemia. Furthermore, 5-aminosalicylic with or without steroid or azathioprine therapy had a homocysteine lowering effect. Table 8 presents 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.

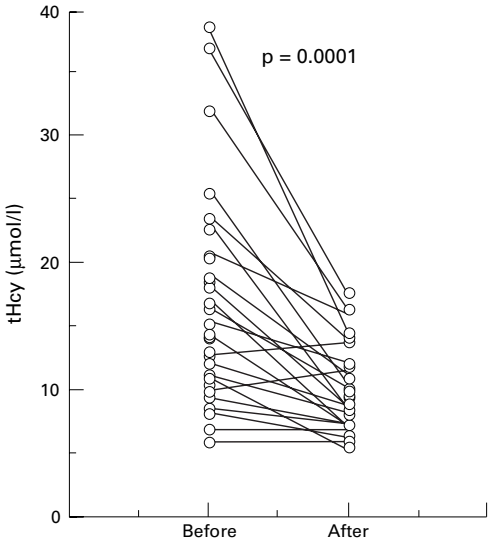

Figure 4 Effect of folic acid 400 μg/day for six weeks on plasma homocysteine (tHcy) concentrations.

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 B_{12} 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.[22 30–32] 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

C677T variant. The high tHcy levels are partly explained by the increased prevalence of homozygosity (TT) for the MTHFR C677T variant. We have recently shown in normal subjects that the MTHFR C677T variant is associated with reduced folate status[33] and others have shown that there is an increased folate requirement to maintain plasma homocysteine within normal levels.[34] This study suggests that in IBD, which is already associated with an excessive requirement for folate, the TT variant places a further burden on the homoeostatic mechanisms which might otherwise maintain a normal level. However, it must be emphasised that tHcy is still higher in patients than controls when TTs are excluded from the analysis; accordingly, hyper-tHcy and folate deficiency must be addressed in all patients with IBD. Vitamin B_{12} levels were also found to be significantly reduced in the patients with IBD (table 2), which may reflect the nutritional and malabsorption problems associated with IBD. Such deficiencies would be likely to enhance the levels of plasma tHcy (table 2). As expected low vitamin B_{12} levels were not associated with the MTHFR variant (table 3).

This study found no evidence that the prevalence of the MTHFR C677T variant was different between Crohn's disease and ulcerative colitis patients. This is known to occur in other genetically associated diseases such as diabetes types I and II.[35] The association of the C677T variant with ulcerative colitis and Crohn's disease, if confirmed, may be a marker for a common genetic association between Crohn's disease and ulcerative colitis on chromosome 1. It could also have pathogenic significance in both diseases, through its known association with vasculitis in IBD[36] and/or thromboembolic disease.[9 10 13 14]

The finding that the TT for the C677T variant is associated with a low folate status especially in patients with IBD has clinical implications. Patients with inflammatory bowel disease have an increased risk of developing colon cancer.[37 38] Evidence is also accumulating that folate deficiency predisposes to colon cancer[39] and in a case control study folate supplementation has been shown to reduce the incidence of dysplasia or cancer.[40] In this study, nearly one fifth of patients with IBD are TT for the variant which is associated with low plasma and red cell folate. This suggests that if such subjects are to be protected both from the complications associated with hyperhomocysteinaemia and potential folate deficiency induced mucosal dysplasia or cancer, they should receive extra folate taken as either tablets or fortified food staples.[41] We have shown in this study that taking a daily dose of 400 μg folate significantly reduces the levels of plasma homocysteine to a level that is likely to be maximal,[42] and elevates blood folate to levels which will protect against tHcy related thrombotic complications.[41] Even a daily dose of 100–200 μg of folic acid taken on a prolonged basis is likely to have similar effects on tHcy and folate status as in normal subjects.[43] Because of their increased requirements it would seem rational that all IBD subjects who are TT should receive long term treatment with 400 μg of folic acid daily as a prophylactic measure. As all patients with IBD are at risk of folate and vitamin B_{12} deficiency for the reasons enumerated above, it may be preferable to recommend that all patients with IBD should receive daily folate and vitamin B_{12} supplements to protect against the complications of hyperhomocysteinaemia.[41]

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