The association of the MTHFR C677T polymorphism with inflammatory bowel diseases in the Israeli Jewish population

An example of genetic heterogeneity

Amir Karban, MD\textsuperscript{a,b,c}, Tzah Feldman, BSc\textsuperscript{a,c}, Matti Waterman, MD\textsuperscript{b,d}, Ronit Leiba, MA\textsuperscript{e}, Edna Efrati, PhD\textsuperscript{c}

Abstract

MTHFR C677T is a common gene polymorphism that has been shown to be associated with hyperhomocysteinemia. Studies on the role of MTHFR in inflammatory bowel diseases (IBD) have yielded conflicting results, perhaps due in part to genetic heterogeneity. The prevalence of the MTHFR C677T variant allele varies according to Jewish subpopulations: Ashkenazi vs non-Ashkenazi. The aim of this study was to examine the association between MTHFR C677T genotype and IBD in the different Jewish populations.

DNA samples were assessed for the presence of the MTHFR C677T variant allele in 445 Jewish Israeli IBD patients: 338 with Crohn’s disease (CD) [214 Ashkenazi and 124 non-Ashkenazi Jews] and 107 with ulcerative colitis (UC) [73 Ashkenazi and 34 non-Ashkenazi Jews], and in 347 healthy controls: 173 Ashkenazi and 174 Non-Ashkenazi Jews. Possible genotype-phenotype associations were investigated.

We showed a significantly higher frequency of MTHFR 677T variant genotypes in non-Ashkenazi CD patients: Odds ratio of 1.86 for heterozygotes (CT) and 2.89 for homozygotes (TT) compared to non-Ashkenazi healthy controls. No significant association was found for UC in non-Ashkenazi patients or for CD or UC in Ashkenazi patients.

Our findings suggest that the MTHFR 677T variant may contribute to the risk of CD in non-Ashkenazi but not Ashkenazi Jews. This may result from genetic heterogeneity and highlights the complexity of the genetic etiology of IBD.

Abbreviations: CARD 15 = Caspase recruitment domain-containing protein 15, CC = wild type for MTHFR C677T polymorphism, CD = Crohn’s disease, CI = confidence interval, CT = heterozygosity for MTHFR C677T polymorphism, ID = inflammatory bowel diseases, MTHFR = methylenetetrahydrofolate reductase, NOD2 = nucleotide-binding oligomerization domain-containing protein 2, OR = odds ratio, SD = standard deviation, TT = homoyzgotes for MTHFR C677T polymorphism, UC = ulcerative colitis.

Keywords: Ashkenazi Jews, Crohn’s disease, inflammatory bowel diseases, MTHFR gene, ulcerative colitis

1. Introduction

5,10-Methylenetetrahydrofolate reductase (MTHFR) plays a major role in the metabolism of homocysteine and folate, and may thus modulate homocysteine levels. A common thermolabile variant of MTHFR was characterized and found to correspond to the MTHFR C677T gene polymorphism (rs1801133). This polymorphism is associated with hyperhomocysteinemia, as well as with various cancers, cardiovascular diseases, and thromboembolism. Increased levels of homocysteine and increased risk of thromboembolism have been described in inflammatory bowel diseases (IBD). Although the MTHFR gene is located on chromosome 1 (1p36.3), a locus found to be associated with IBD risk, other genome-wide association studies (GWAS) did not identify the MTHFR C677T polymorphism in individuals with IBD.

Association studies examining MTHFR polymorphisms and IBD show conflicting results. The first published study in 1999 showed an association of MTHFR C677T to IBD (Odds ratio (OR) 2.64, 95% confidence interval (CI) 1.39–5.08). A meta-analysis, including 13 studies from populations of different ethnic descent, altogether 1849 and 2249 IBD cases and controls for MTHFR C677T, respectively, found no statistically significant association between the MTHFR C677T polymorphism and the risk of Crohn’s disease (CD) or Ulcerative colitis (UC). Failure of the meta-analysis to corroborate the association between IBD and MTHFR C677T may be due to the heterogeneity of the populations studied. Indeed, heterogeneity in the risk of IBD-associated loci among different populations has been described for several important IBD loci. For example, NOD2/CARD15 (nucleotide-binding oligomerization domain-containing protein 2) gene polymorphisms are associated with CD; however, the 3
risk alleles R702W, G908R, and 1007fsInsC in NOD2 that are associated with susceptibility to CD, have demonstrated remarkable heterogeneity across ethnicities and populations, with regional variation even across Europe. In the Japanese, Korean, Chinese and Indian populations, the NOD2/CARD15 variants are not associated with CD.\[10\]

The Jewish population in Israel is heterogeneous and is historically divided into 2 major groups: Ashkenazi Jews from Central and Eastern Europe and non-Ashkenazi Jews from the Mediterranean, North Africa, and Asia. The incidence and prevalence of inflammatory bowel diseases has been reported to be 2- to 4-fold higher in Ashkenazi Jews than in non-Jewish Caucasians.\[11\]

We previously showed that the carrier rate of the 3 NOD2 polymorphisms R702W, G908R, and 1007fsInsC is higher in Ashkenazi Jewish CD patients than in non-Ashkenazi Jews (47.4% vs 27.5%, \( P = 0.034 \)).\[12\] We also showed that Ashkenazi Jews display a 1.9-fold higher frequency of variant MTHFR 677T than do non-Ashkenazi Jews (\( P < 0.001 \)).\[13\] However, no study to date has focused on the association of the MTHFR C677T polymorphism with IBD in the Jewish population. The aim of the present study was to examine the association of the MTHFR C677T polymorphism with IBD in Ashkenazi versus non-Ashkenazi Jews in order to elucidate controversial findings of associations between the MTHFR C677T polymorphism and IBD. We assumed that an association between MTHFR C677T polymorphism and IBD can be found in the heterogeneous Jewish population in Israel.

2. Materials and methods

2.1. Study design

In order to examine the association of the MTHFR C677T polymorphism with IBD in the Jewish population, DNA samples were assessed for the presence of the MTHFR C677T variant in Jewish Israeli individuals with IBD and healthy Jewish controls. The subjects and the controls were divided to 2 subgroups by ethnicity (Ashkenazi and non-Ashkenazi Jews). Several comparisons were conducted between subjects and controls genotype frequencies according to ethnicity.

2.2. Study subjects

Starting from 1996, we recruited 445 Jewish Israeli individuals with IBD (338 with CD and 107 with UC) from Rambam Medical Center in Haifa, Israel. The diagnosis of UC and CD was confirmed all diagnoses. Confirmation required first-hand review of endoscopic, pathology and radiology reports, and operative notes.

2.3. Healthy controls

We used for comparison, DNA samples of 347 healthy Jewish Israeli controls, 173 Ashkenazi Jews, and 174 non-Ashkenazi Jews, that were collected by the National Laboratory for the Genetics of Israeli Populations, Department of Human Molecular Genetics & Biochemistry, Sackler Faculty of Medicine, Tel-Aviv University.\[13\]

2.4. Phenotypic evaluation

Age at diagnosis, tobacco use, ethnicity, and phenotypic parameters (extent of disease, perianal disease in CD, extra-intestinal manifestations) were determined from medical records, questionnaires, and interviews. Ashkenazi and non-Ashkenazi ethnicities were carefully assigned on the basis of the birthplace of the 4 grandparents. Non-Ashkenazi ethnicity includes all individuals who had at least 3 Sephardic grandparents (originating from Spain, Portugal, Iraq, and North Africa). Georgian, Indian, and Ethiopian Jews were not included. Patients were considered smokers if they smoked a minimum of 7 cigarettes per week for at least 1-year anytime during their life. Family history of IBD was the occurrence of IBD in a first cousin or more closely related relative.

2.5. DNA samples

Genomic DNA was extracted from peripheral blood samples using the Qiaamp DNA mini kit (Qiaegen, Germany) according to the manufacturer’s protocol.

2.6. MTHFR genotyping

DNA samples were assessed for the presence of polymorphism C677T in MTHFR using the PCR followed by probe-free high-resolution melting technology (HRM), a genotyping technique based on the effect of DNA changes on amplicon melting.

DNA samples were amplified using HRM-enabled real-time PCR, Rotor-Gene 6000 (Corbett Research, UK) with Fast Plus EvaGreen qPCR master mix (Biotium, USA) and 2 primers: 5’-CTTTGAGGCTGACCTGAAGC-3’ and 5’-AGAAAAGCTGC GTGATGATGA-3’.

High-resolution melting technology (HRM) is a genotyping technique that incorporates an intercalating dye into a double-stranded PCR amplicon. The dye fluoresces brightly when it is bound to double-stranded amplicon. Thus, incrementing the temperature of an amplicon to melting point while measuring the fluorescence of the dye results in the generation of a sequence specific melting curve. Normalization and comparison between different shapes of melting curves allows sensitive discrimination between different genotypes and identification of heterozygotes, homozygotes, and wild type variants.

2.7. NOD2/CARD15 mutation analysis

Patients were genotyped for the following 3 gene polymorphisms in NOD2: Arg702Trp, Gly908Arg, and Leu1007fsInsC using the restriction enzyme digestion assay as described elsewhere.\[14\]

2.8. Statistical analysis

Statistical analysis was performed using SPSS version 21. Individuals with any missing data were excluded from the statistical analysis regarding the data that were missing. However, they were included in other analyses regarding their available data.

The Fisher exact test and the chi square test were used to analyze differences between groups (example: CD Ashkenazi patients and CD non-Ashkenazi patients), in the categorical parameters (gender, smoking, family history of IBD, disease extent, CD-type, CD-perianal, extra-intestinal manifestations, and NOD2 carrier). T-test analysis was used to analyze data according to differences in patient age (a continuous parameter).

Odds ratio with 95% confidence interval for MTHFR C677T was calculated for measuring the strength of the association for Jewish healthy controls (Ashkenazi vs non-Ashkenazi) and for...
IBD patients versus healthy controls (among Ashkenazi and non-Ashkenazi Jews).

Adjustment for multiple comparisons was made by the Bonferroni method.

$P < 0.05$ was considered as significant.

2.9. Ethical considerations

Informed consent for participation in molecular genetic studies was obtained from all study subjects and ethical approval from the local Helsinki Committee.

3. Results

All recruited individuals were included in the study. Individuals with any missing data were excluded from the statistical analysis regarding the data that were missing. However, they were included in other analyses regarding their available data.

The study included 338 Jewish CD patients, (Table 1): 214 Ashkenazi and 124 non-Ashkenazi. In total, 53% of the patients were males and the mean age at diagnosis was $24.8 \pm 13$ years. Phenotypic characteristics were similar in Ashkenazi and non-Ashkenazi patients except for a higher prevalence of smokers among non-Ashkenazi patients, 31% versus 18%, $P = 0.009$; a slightly higher proportion with family history of IBD, 31% versus 21%; and a significantly lower carrier rate of NOD2 mutations, 24% versus 41%.

The study included 107 UC patients (Table 2): 73 of Ashkenazi and 34 of non-Ashkenazi descent; 54% of the patients were males; the mean age at diagnosis was $30.7 \pm 15.1$ years. Phenotypic characteristics were similar in Ashkenazi and non-Ashkenazi patients.

Comparing Ashkenazi with non-Ashkenazi Jews in the control groups, we found that the frequency of the MTHFR $677T$ variant genotype was significantly higher in Ashkenazi than non-Ashkenazi Jews (44.5% vs 21.3%, OR 2.96, 95% CI 2.12–4.14, $P < 0.0001$) (Table 3). Because of the difference in the genotype frequencies between control Ashkenazi and non-Ashkenazi Jews, the following comparisons were conducted according to ethnicity.

Among Ashkenazi Jewish individuals, no significant difference was found in MTHFR C677T allele frequencies, between those with and without CD, or with and without UC (Fig. 1).

Among non-Ashkenazi individuals, 45% of those with CD are heterozygotes (CT) for MTHFR polymorphism, compared to 33% of those without CD (OR 1.86, 95% CI 1.14–3.03, $P$-value = 0.0127). However, 10% of those with CD are homozygotes (TT) for MTHFR polymorphism, compared to 4.6% of those without CD (OR 2.89, 95% CI 1.11–7.48, $P$-value = 0.0286). No significant difference was found in the frequency of heterozygotes or homozygotes, between those with and without UC.

A total of 32% of the alleles of those with CD are $T$ allele of MTHFR, compared to 21% for those without CD (OR 1.76, 95% CI 1.21–2.55, $P$-value = 0.0027) (Fig. 1). No significant

---

Table 1

| Crohn’s disease patients cohort characteristics. | CD total (n = 338) | CD Ashkenazi patients (n = 214) | CD non-Ashkenazi patients (n = 124) | $P$ |
|-----------------------------------------------|-------------------|---------------------------------|-------------------------------------|-----|
| Gender, males, % | 179 (53%) | 122 (57%) | 57 (46%) | 0.054 |
| Age at diagnosis years, mean ± SD | 24.8 ± 13 | 24.6 ± 13.1 | 25.1 ± 12.6 | 0.74 |
| Smoking | | | | |
| Current | 75 (23%) | 37 (18%) | 38 (31%) | 0.009 |
| Past | 43 (13%) | 23 (11%) | 20 (16%) | 0.18 |
| Never | 207 (64%) | 144 (70%) | 63 (52%) | 0.001 |
| Family history of IBD | | | | |
| First degree | 44 (13%) | 23 (11%) | 21 (17%) | 0.13 |
| 2nd or 3rd degree | 39 (11%) | 21 (10%) | 18 (14%) | 0.22 |
| None | 251 (75%) | 166 (79%) | 85 (68%) | 0.03 |
| Disease extent, Vienna | | | | |
| Terminal ileum | 133 (40%) | 86 (41%) | 47 (38%) | 0.72 |
| Colon only: | 44 (13.3%) | 27 (13%) | 17 (14%) | 0.86 |
| Ileo-colon | 133 (40%) | 84 (40%) | 49 (40%) | 1.00 |
| Upper GI | 21 (6.3%) | 12 (6%) | 9 (7%) | 0.64 |
| CD-type | | | | |
| Inflammatory | 173 (52%) | 110 (53%) | 63 (51%) | 0.82 |
| Strictureing | 68 (21%) | 40 (19%) | 29 (24%) | 0.40 |
| Penetrating | 89 (27%) | 59 (28%) | 31 (25%) | 0.61 |
| CD-perianal | | | | |
| Yes | 82 (26%) | 54 (28%) | 28 (23%) | 0.43 |
| No | 231 (74%) | 140 (72%) | 91 (77%) | |
| Extra-intestinal manifestations | | | | |
| At least 1 | 97 (30%) | 62 (30%) | 35 (28%) | 0.71 |
| No | 232 (70%) | 143 (70%) | 89 (72%) | |
| NOD2 carrier* | | | | |
| Wt | 214 (66%) | 122 (59%) | 92 (77%) | $P = 0.016$ |
| Heterozygotes | 77 (24%) | 52 (25%) | 25 (21%) | $P = 0.41$ |
| Homozygotes | 35 (10%) | 32 (16%) | 3 (2.5%) | $P < 0.001$ |

CD = Crohn’s disease, GI = gastrointestinal tract, IBD = inflammatory bowel diseases, NOD2 = nucleotide-binding oligomerization domain-containing protein 2, SD = standard deviation, Wt = wild type for the 3 NOD2 mutations tested, heterozygotes = heterozygotes for 1 of the 3 NOD2 mutations tested, homozygotes = compound heterozygote or homozygote for 1 of the 3 NOD2 mutations tested.
association was found in the frequency of T allele, between those with and without UC.

Further phenotype-genotype correlations were conducted in non-Ashkenazi CD patients (Table 4). No MTHFR C677T phenotype–genotype correlations were found in this subgroup of patients. No interaction (epistasis) was found between NOD2 genotypes and MTHFR C677T genotypes in this population as well.

4. Discussion

This study compared frequencies of the MTHFR 677T variant allele in 445 Jewish Israeli individuals with IBD (338 with CD and 107 with UC) to 347 Jewish healthy controls. No study to date has focused on the association of the MTHFR C677T polymorphism with IBD in the Jewish population. We showed a significantly higher frequency of MTHFR 677T variant in non-Ashkenazi CD patients: odds ratio of 1.86 for heterozygotes (CT) and 2.89 for homozygotes (TT), compared to 174 non-Ashkenazi healthy controls. We did not find any correlation between MTHFR C677T genotype and age at diagnosis, smoking status or disease phenotype, or with NOD2 gene polymorphism status among the non-Ashkenazi CD patients.

No significant association was found between the MTHFR C677T polymorphism and UC in non-Ashkenazi Jewish patients, though a small sample size limited the statistical power.

Unlike Ashkenazi Jews who are a definite population isolate, non-Ashkenazi Jews show significant genetic heterogeneity. In broad terms, they could be a mixture of Spanish Moroccan, North-African, Iraqi, Yemenite, Georgian, Indian, Ethiopian Jews, and so on. We included in the study individuals that at least 3 of their grandparents originated from Spain, Portugal North Africa, and Iraq. It has been described in the literature how population substructure can result in false-positive association results. This is particularly liable to be a problem in candidate gene studies in which corrections for genome-wide population differences are not undertaken.

The association found between MTHFR C677T and CD is in agreement with some previous studies. In a Caucasian population in Ireland, 17.5% of UC and 16.8% of CD patients were homozygous for the C677T variant compared with 7.3% of controls. Xu et al reported higher prevalence of the MTHFR C677T polymorphism in Chinese UC compared to healthy patients. However, most studies that compared IBD patients and healthy controls did not find a difference in the frequency of MTHFR C677T variant. Some of the studies that showed “negative result” were carried out on populations from Turkey and North Africa. These populations are close in ethnicity to non-Ashkenazi Jews, whose ancestors migrated from Spain and Portugal to North Africa and Turkey during the fifteenth century.

Yasa et al studied a very small cohort of 27 Turkish IBD patients. Heterozygosity of MTHFR C677T polymorphism was

### Table 2

| UC total (n = 107) | UC Ashkenazi patients (n = 73) | UC non-Ashkenazi patients (n = 34) | P |
|-------------------|-------------------------------|-----------------------------------|---|
| Gender, males, %  | 58 (54%)                      | 43 (59%)                          | 15 (44%) | 0.21 |
| Age at diagnosis years, mean ± SD | 30.7 ± 15.1 | 31 ± 15.7 | 30.1 ± 14 | 0.77 |
| Smoking          |                               |                                   |         |     |
| Current          | 10 (10%)                      | 7 (10%)                           | 3 (10%) | 1.00 |
| Past             | 20 (20%)                      | 14 (21%)                          | 6 (19%) | 1.00 |
| Never            | 69 (70%)                      | 47 (69%)                          | 22 (71%) | 1.00 |
| Family history of IBD |               |                                   |         |     |
| First degree     | 13 (12.5%)                    | 8 (11%)                           | 5 (15%) | 0.75 |
| Distant degree   | 9 (8.6%)                      | 8 (11%)                           | 1 (3%)  | 0.26 |
| None             | 82 (79%)                      | 54 (77%)                          | 28 (82%) | 0.62 |
| Disease extent   |                               |                                   |         |     |
| Proctitis        | 15 (14.7%)                    | 13 (19%)                          | 2 (6%)  | 0.14 |
| Lt. colon        | 42 (41%)                      | 27 (36%)                          | 15 (47%) | 0.52 |
| Pancolitis       | 45 (44%)                      | 30 (43%)                          | 15 (47%) | 0.83 |
| Extra-intestinal manifestations | | | |     |
| At least 1       | 14 (13.5%)                    | 8 (11%)                           | 6 (19%) | 0.35 |
| No               | 90 (86.5%)                    | 64 (89%)                          | 26 (81%) | 0.81 |

IBD = inflammatory bowel diseases, SD = standard deviation, UC = ulcerative colitis.

### Table 3

| Jewish controls (n = 347) | Ashkenazi controls (n = 173) | Non-Ashkenazi controls (n = 174) | OR | CI | P |
|---------------------------|-------------------------------|----------------------------------|----|----|---|
| CC                        | 166 (47.8%)                   | 58 (33.5%)                       | 108 (62.1%) | ref |
| CT                        | 134 (38.6%)                   | 76 (44%)                         | 58 (33.3%) | 2.44 | 1.5287 to 3.8943 | 0.0002 |
| TT                        | 47 (13.6%)                    | 39 (22.5%)                       | 8 (4.6%)  | 9.0776 | 3.9784 to 20.7125 | <0.0001 |
| C                         | 466 (67.1%)                   | 192 (55.5%)                      | 274 (78.7%) | ref |
| T                         | 228 (32.9%)                   | 154 (44.5%)                      | 74 (21.3%) | 2.9699 | 2.1287 to 4.1434 | <0.0001 |

CC = wild type genotype, CI = 95% confidence interval, CT = heterozygotes for MTHFR C677T, MTHFR = methylenetetrahydrofolate reductase, OR = odds ratio, P = P value, TT = homozygotes for MTHFR C677T.
found in 10 of 27 (37%) patients with IBD and 15 of 47 (32%) controls ($P > 0.05$). Homozygosity was detected in 4 patients (14.9%) with IBD and 3 (6.3%) controls ($P > 0.05$). Senhaji et al[20] studied Moroccan patients with IBD and concluded that the genetic risk for IBD is not modulated by the MTHFR C677T polymorphism.

It is important to mention the trans-ancestry association study of IBD, with genome-wide or immunochip genotype data from an

### Figure 1.
Odds ratio with 95% confidence interval of several MTHFR T allele comparisons conducted in this study. Each shape represents different comparisons between IBD patients and ethnically matched healthy controls, as mentioned on the left side of the graph. On the right side Odds ratio, 95% confidence interval and $P$ values for each comparison are mentioned. $P < 0.05$ was considered as significant. T allele prevalence is significantly higher among non-Ashkenazi CD patients compared to non-Ashkenazi controls. CD = Crohn’s disease, IBD = inflammatory bowel diseases, MTHFR = methylenetetrahydrofolate reductase, UC = ulcerative colitis.

### Table 4
MTHFR C677T phenotype–genotype correlations of in non-Ashkenazi Crohn’s disease patients (n = 124).

|                      | Heterozygotes (677CT) N = 56 | Homozygotes (677TT) N = 12 | Wild type (677CC) N = 56 | $P$  |
|----------------------|-----------------------------|---------------------------|--------------------------|------|
| Gender, males, %     | 23 (41%)                    | 7 (58%)                   | 27 (48%)                 | $P = 0.49$ |
| Age at diagnosis years, mean ± SD | 27.6 ± 13.1               | 25.8 ± 7.9                | 22.6 ± 12.6              | $P = 0.43$ |
| Smoking              |                             |                           |                          |      |
| Current              | 15 (28%)                    | 3 (25%)                   | 20 (36%)                 | $P = 0.80$ |
| Past                 | 8 (15%)                     | 3 (25%)                   | 9 (16%)                  |      |
| Never                | 30 (57%)                    | 6 (50%)                   | 27 (48%)                 |      |
| Family history of IBD |                             |                           |                          |      |
| First degree         | 12 (21%)                    | 1 (8%)                    | 8 (14%)                  | $P = 0.41$ |
| 2nd or 3rd degree    | 5 (9%)                      | 3 (25%)                   | 10 (18%)                 |      |
| None                 | 39 (70%)                    | 8 (67%)                   | 38 (68%)                 |      |
| Disease extent, Vienna |                          |                           |                          |      |
| Terminal ileum       | 20 (36%)                    | 6 (50%)                   | 21 (38%)                 | $P = 0.57$ |
| Colon only           | 12 (22%)                    | 1 (8%)                    | 4 (7%)                   |      |
| Ileo-colon           | 20 (36%)                    | 4 (33%)                   | 25 (45%)                 |      |
| Upper GI             | 3 (6%)                      | 1 (8%)                    | 5 (9%)                   |      |
| CD-type              |                             |                           |                          |      |
| Inflammatory         | 29 (53%)                    | 7 (58%)                   | 27 (48%)                 | $P = 0.89$ |
| Stricture            | 14 (25%)                    | 2 (17%)                   | 13 (23%)                 |      |
| Penetrating          | 12 (22%)                    | 3 (25%)                   | 16 (29%)                 |      |
| CD-pananal           |                             |                           |                          |      |
| Yes                  | 12 (23%)                    | 3 (27%)                   | 13 (24%)                 | $P = 0.95$ |
| No                   | 41 (77%)                    | 8 (73%)                   | 42 (76%)                 |      |
| Extra-intestinal man |                             |                           |                          |      |
| At least 1           | 18 (32%)                    | 3 (25%)                   | 14 (25%)                 | $P = 0.68$ |
| No                   | 39 (68%)                    | 9 (75%)                   | 42 (75%)                 |      |
| NOD2                 |                             |                           |                          |      |
| Wt                   | 37 (70%)                    | 8 (73%)                   | 47 (84%)                 |      |
| Heterozygote         | 13 (24%)                    | 3 (27%)                   | 9 (16%)                  | $P = 0.40$ |
| Homozygote           | 3 (6%)                      | 0                         | 0                        |      |

CD = Crohn’s disease, BD = inflammatory bowel diseases, MTHFR = methylenetetrahydrofolate reductase, NOD2 = nucleotide-binding oligomerization domain-containing protein 2, SD = standard deviation.
found a weak effect (20% risk increase).

The effect of the MTHFR C677T variant on venous thromboembolic events and homocysteine levels among our IBD cohort and controls. Therefore, we could not study the association of MTHFR C677T with IBD and thromboembolic events.

In summary, the prevalence of the MTHFR C677T variant genotype in IBD showed discordant results, most likely due to regional and ethnic variations in the prevalence of this polymorphism between the populations studied.

The A|lele FR|quency Database (ALFRED), which is a resource of gene frequency data on human populations supported by the U.S. National Science Foundation, summarizes allele frequencies of the MTHFR C677T polymorphism in different populations (Table 5) (http://alfred.med.yale.edu). These data show low frequency of the polymorphic allele C677T in the African population and especially high frequencies in Mexico, Italy, and among Ashkenazi Jews.

In addition, the risk effect may depend on gene methylation, and thus, the gene–environment interaction between the genotypes and dietary intake. Folic acid consumption, in particular, is essential to maintain, or alter, the effect of the polymorphic variants.

Our results show that the MTHFR C677T polymorphism is a relevant genetic risk factor for IBD in certain population. It seems that in the future we will have to take into consideration the ethnicity in order to find the specific genetic polymorphisms that are relevant to IBD.

References

[1] Kono S, Chen K. Genetic polymorphisms of methylenetetrahydrofolate reductase and colorectal cancer and adenoma. Cancer Sci 2003;94:533–42.

[2] Froos P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–3.

[3] Bernstein CN, Blanchard JP, Houston DS, et al. The incidence of deep vein thrombosis and pulmonary embolism among patients with inflammatory bowel disease: a population-based cohort study. Thromb Haemost 2001;85:430–4.

[4] Nakano E, Taylor CJ, Chada L, et al. Hyperhomocysteinemia in children with inflammatory bowel disease. J Pediatr Gastroenterol Nutr 2003;37:586–90.

[5] Talbott RW, Heppell J, Dozois RR, et al. Vascular complications of inflammatory bowel disease. Mayo Clin Proc 1986;61:140–5.

[6] Cho JH, Nicolei DL, Ramos R, et al. Linkage and linkage disequilibrium in chromosome band 1p36 in American Chaldeans with inflammatory bowel disease. Hum Mol Genet 2000;9:1423–32.

[7] Silverberg MS, Cho JH, Roux JD, et al. Ulcerative colitis-risk loci on chromosomes 1p36 and 1q23 found by genome-wide association study. Nat Genet 2009;41:216–20.

[8] Mahmud N, Molloy A, McPartlin J, et al. Increased prevalence of methylenetetrahydrofolate reductase C677T variant in patients with inflammatory bowel disease, and its clinical implications. Gut 1999;45:389–94.

[9] Zintzaras E. Genetic variants of homocysteine/folate metabolism pathway and risk of inflammatory bowel disease: a synopsis and meta-analysis of genetic association studies. Biomarkers 2010;15:69–79.

[10] Lesage S, Tandon RK. Inflammatory bowel disease in the Asia-Pacific area: a comparison with developed countries and regional differences. J Dig Dis 2010;11:134–47.

[11] Yang H, McElree C, Roth MP, et al. Familial empirical risks for inflammatory bowel disease: differences between Jews and non-Jews. Gut 1993;34:517–24.

[12] Karban A, Waterman M, Panhuysen CJ, et al. NOD2/CARD15 genotype and phenotype differences between Ashkenazi and Sephardic Jews with Crohn’s disease. Am J Gastroenterol 2004;99:1134–40.

[13] Efrati E, Elkin H, Nahum S, et al. Population distribution of methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C risk alleles for methotrexate toxicity in Israel. Rheumatol Int 2013;33:1001–4.

[14] Lesage S, Zouali H, Gézard JP, et al. CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. Am J Hum Genet 2002;70:845–57.

[15] Need AC, Kasperaviciute D, Giruli ET, et al. A genome-wide genetic signature of Jewish ancestry perfectly separates individuals with and without full Jewish ancestry in a large random sample of European Americans. Genome Biol 2009;10:R7.

[16] Behar DM, Yunusbayev B, Metspalu M, et al. The genome-wide structure of the Jewish people. Nature 2010;466:238–42.

[17] Tian C, Gregersen PK, Seldin MF, et al. Accounting for ancestry: population substructure and genome-wide association studies. Hum Mol Genet 2008;17:R143–150.

[18] Xu CL, Lin XQ, Lan DY, et al. The associations of methylenetetrahydrofolate reductase gene C677T and A1298C polymorphisms and ulcerative colitis [article in Chinese]. Zhonghua Nei Ke Za Zhi 2011;50:374–7.

[19] Yasa MH, Bolaman Z, Yakslen V, et al. Factor V Leiden G1691A, prothrombin G20210A, and MTHFR C677T mutations in Turkish inflammatory bowel disease patients. Hepatogastroenterology 2007;54:1438–42.

[20] Senhaji N, Serbati N, Diakité B, et al. Methylenetetrahydrofolate reductase C677T variant in Moroccan patients with inflammatory bowel disease. Gene 2013;521:45–9.
[21] Guédon C, Le Cam-Duchez V, Lalaude O, et al. Prothrombotic inherited abnormalities other than factor V Leiden mutation do not play a role in venous thrombosis in inflammatory bowel disease. Am J Gastroenterol 2001;96:1448–54.
[22] Koutroubakis IE, Sfiridaki A, Tsolakidou G, et al. Genetic risk factors in patients with inflammatory bowel disease and vascular complications: case-control study. Inflamm Bowel Dis 2007;13:410–5.
[23] Bernstein CN, Sargent M, Vos HL, et al. Mutations in clotting factors and inflammatory bowel disease. Am J Gastroenterol 2007;102:338–43.
[24] Vecchi M, Sacchi E, Saibeni S, et al. Inflammatory bowel diseases are not associated with major hereditary conditions predisposing to thrombosis. Dig Dis Sci 2000;45:1465–9.
[25] Yilmaz S, Bayan K, Tüzün Y, et al. A comprehensive analysis of 12 thrombophilic mutations and related parameters in patients with inflammatory bowel disease: data from Turkey. J Thromb Thrombolysis 2006;22:205–12.
[26] Törüner M, Erkan O, Soykan I, et al. Factor V Leiden, prothrombin G20210A and MTHFR gene mutations in inflammatory bowel disease. Turk J Gastroenterol 2004;15:250–2.
[27] Liu JZ, van Sommeren S, Huang H, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nat Genet 2015;47:979–86.
[28] Magro F, Soares JB, Fernandes D, et al. Venous thrombosis and prothrombotic factors in inflammatory bowel disease. World J Gastroenterol 2014;20:4857–72.
[29] Den Heijer M, Lewington S, Clarke R, et al. Homocysteine, MTHFR and risk of venous thrombosis: a meta-analysis of published epidemiological studies. J Thromb Haemost 2005;3:292–9.
[30] Oussalah A, Güéant JL, Peyrin-Biroulet L, et al. Meta-analysis: hyperhomocysteinemia in inflammatory bowel diseases. Aliment Pharmacol Ther 2011;34:1173–84.
[31] Binia A, Contreras AV, Canizales-Quiñeros S, et al. Geographical and ethnic distribution of single nucleotide polymorphisms within genes of the folate/homocysteine pathway metabolism. Genes Nutr 2014;9:421.
[32] Mandaviya PR, Stolk L, Heil SG, et al. Homocysteine and DNA methylation: a review of animal and human literature. Mol Genet Metab 2014;113:243–52.