Polymorphisms of glutathione S-transferase and methylenetetrahydrofolate reductase genes in Moldavian patients with ulcerative colitis: Genotype-phenotype correlation

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A B S T R A C T
Background: Glutathione S-transferases (GSTM1, GSTT1, and GSTP1) and methylenetetrahydrofolate reductase (MTHFR) are important enzymes for protection against oxidative stress. In addition, MTHFR has an essential role in DNA synthesis, repair, and methylation. Their polymorphisms have been implicated in the pathogenesis of ulcerative colitis (UC). The aim of the present study was to investigate the role of selected polymorphisms in these genes in the development of UC in the Moldavian population.

Methods: In a case-control study including 128 UC patients and 136 healthy individuals, GSTM1 and GSTT1 genotypes (polymorphic deletions) were determined using multiplex polymerase chain reaction (PCR). The GSTP1 rs1695 (ile105Val), MTHFR rs1801133 (C677T), and MTHFR rs1801131 (A1298C) polymorphisms were studied with restriction fragment length polymorphism (RFLP) analysis. Genotype-phenotype correlations were examined using logistic regression analysis.

Results: None of the genotypes, either alone or in combination, showed a strong association with UC. The case-only sub-phenotypic association analysis showed an association of the MTHFR rs1801133 polymorphism with the extent of UC under co-dominant (p correct = 0.040) and recessive (p correct = 0.020; OR = 0.15; CI = 0.04–0.62) genetic models. Also, an association between the MTHFR rs1801131 polymorphism and the severity of UC was reported for the over-dominant model (p correct = 0.023; coefficient = 0.32; 95% CI = 0.10–0.54).

Conclusion: The GST and MTHFR genotypes do not seem to be a relevant risk factor for UC in our sample. There was, however, evidence that variants in MTHFR may influence the clinical features in UC patients. Additional larger studies investigating the relationship between GST and MTHFR polymorphisms and UC are required.

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1. Introduction

Ulcerative colitis (UC) is a chronic inflammatory condition of the large intestine, which along with Crohn’s disease comprises the major part of the inflammatory bowel diseases (Abraham and Cho, 2009). Inflammatory bowel diseases (IBD) and specifically UC are not evenly distributed throughout the world, with North America and western/northern Europe having the highest rate (100–250 per 100,000 population for UC) (Burisch and Munkholm, 2015). In the Republic of Moldova, as in most other eastern/southeastern European countries, UC appears to be much less common (20–25 per 100,000 population in Moldavia, unpublished data), although continuous increase in the disease incidence over the past years has been reported (Burisch and Munkholm, 2015). The precise etiology of UC remains unclear. Several mechanisms related to immunological, genetic, toxic, and infection abnormalities are implicated in the pathogenesis of UC (Ananthakrishnan, 2015).

Oxidative stress due to overproduction of reactive oxygen species (ROS) and decreased efficiency of antioxidant defenses has been considered to be a common pathogenic factor in UC and its complications. ROS overproduction has been shown in the inflamed mucosa of UC patients and in experimental animal models of IBD (reviewed by Rezaie et al., 2007). Excessive amounts of ROS can destroy biomolecules such as lipids, proteins, and DNA, leading to cellular stress and endothelial dysfunction in UC patients (Valko et al., 2007; Piechota-Polanczyk and Fichna, 2014). Production of oxidants and free radicals can also facilitate activation of signaling events that mediate expression of inflammatory
genes as well as genes regulating cell division, differentiation, and apoptosis (Valko et al., 2007). It has been suggested that the impaired prooxidant and antioxidant system in UC patients may contribute to the disease process (Pravda, 2005). The human glutathione S-transferases (GSTs) are well-known oxidative stress-related detoxification enzymes. Located mainly in the cytosol, GST enzymes catalyze the conjugation of electrophilic substrates to glutathione, thus facilitating detoxification and further metabolism and excretion (Hayes et al., 2005). They also play an important role in peroxidase and isomerase activities (Sheehan et al., 2001). Several classes of GSTs have been identified, with GSTT1, M1, and P1 being the most well-characterized forms. Polymorphisms within these genes either decrease or abolish GST enzyme activity (Strange et al., 2000). Thus, a functionally significant A to G transition in exon 5 of the GSTP1 gene (A313G, rs1695), which results in replacing isoleucine with valine (Ile105Val), substantially diminishes GSTP1 enzyme activity. By contrast, homozygous whole gene deletions of GSTT1 or GSTM1 cause a lack of the respective enzyme function. These GST genes polymorphisms have been linked to inflammation and immune processes in a number of reports (Bekris et al., 2005; Aguilera et al., 2004; Babushok et al., 2013; Liang et al., 2013; Živković et al., 2013; Ding et al., 2014).

It is known that homocysteine (Hcy) induces oxidative stress (Lloca, 1996). Hyperhomocysteinemia is common among UC patients, and elevated Hcy levels are associated with increased risk of UC compared with current smokers, as reported previously (Lakatos et al., 2013). The demographics and clinical features of the study population are depicted in Table 1. EDTA anti-coagulated venous blood samples were collected from all participants, and genomic DNA was extracted from peripheral blood leukocytes using a standard salting out method (Miller et al., 1988). The local ethics committee approved the study, and informed written consent was received from all subjects.

### 2. Materials and methods

#### 2.1. Samples

This case-control study comprises 128 unrelated UC patients, recruited at the Department of Gastroenterology, Republican Clinical Hospital, Moldova. The diagnosis of UC was based on standard clinical, endoscopic, and histological criteria (Lennard-Jones, 1989). The distribution of UC lesions was defined according to the Montreal classification (Satsangi et al., 2006). The subjects with UC were also classified using three additional clinical categories: (i) disease severity as assessed by the modified Truelove–Witts disease activity index (Digvass et al., 2012); (ii) UC-related outcomes—i.e., strictures, lead pipe colon, malignancy, steroid-dependency, and colectomy: absent versus present; and (iii) disease relapse rate: infrequent (≤1/year) versus frequent (≥2/year). All patients were Caucasians of European descent. The control population consisted of 136 unrelated and ethnically matched healthy individuals who had no history of autoimmune or oncological disease. Information on smoking habits was collected from both patients and healthy controls. According to smoking habit, cases were divided into 3 groups: current smokers, ex-smokers, and never smokers, and controls were classified as current smokers and current non-smokers. The lack of correspondence between cases and controls in smoking definitions is due to the lack of information on former smoking status in controls. Therefore, ex-smoker and never-smoker UC patients were combined in one group to make them comparable with healthy controls in the case-control study. The combination is further justified by findings that both the never- and ex-smokers have an increased risk of UC compared with current smokers, as reported previously (Miller et al., 1988). The local ethics committee approved the study, and informed written consent was received from all subjects.

### 2.2. Genotyping

GSTM1 and GSTT1 genotypes were determined using multiplex polymerase chain reaction (PCR). Three sets of primers were used to amplify a 434-bp sequence of the GSTT1 gene (Zheng et al., 2001), a 267-bp fragment of the GSTM1 gene (Tujague et al., 2006), and a 212-bp segment of the human albumin gene as an internal amplification control (Tujague et al., 2006). The PCR reactions were carried out in 20 μl containing 5 pmol of each primer, 100 ng genomic DNA, 1.5 mmol/L MgCl2, 200 μmol/L dNTPs, and 0.5 unit of Taq DNA polymerase in the buffer provided by the manufacturer. Amplification was performed in a Bio-Rad T100 Thermal Cycler (Bio-Rad, Hercules, California) for the PCR reaction. The amplification conditions consisted of an initial melting step 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 40 s; and a final elongation step of 72 °C for 5 min. Resulting fragments were visualized using ethidium bromide staining and 3% agarose gel electrophoresis (Fig. 1). MTHFR rs1801133 (C677T, Ala222Val) and GSTP1 rs1801131 (A1298C, Glu429Ala), two single nucleotide polymorphisms (SNPs) in the MTHFR gene (A313G, rs1695), which results in replacing isoleucine with valine (Ile105Val), substantially diminishes GSTP1 enzyme activity. By contrast, homozgyous whole gene deletions of GSTT1 or GSTM1 cause a lack of the respective enzyme function. These GST genes polymorphisms have been linked to inflammation and immune processes in a number of reports (Bekris et al., 2005; Aguilera et al., 2004; Babushok et al., 2013; Liang et al., 2013; Živković et al., 2013; Ding et al., 2014).

### Table 1

| Characteristic                                  | UC (n = 128) n (%) | Healthy controls (n = 136) n (%) |
|------------------------------------------------|--------------------|---------------------------------|
| Sex                                            |                    |                                 |
| Female                                         | 59 (46.1)          | 52 (38.2)                       |
| Male                                           | 60 (53.9)          | 84 (61.8)                       |
| Smoking                                        |                    |                                 |
| Never                                          | 98 (76.6)          | NA                              |
| Former                                         | 16 (12.5)          | NA                              |
| Current                                        | 14 (10.9)          | 48 (35.3)                       |
| Age at recruitment, median ± S.D. (years)      | 41.4 ± 13.7        | 45.9 ± 10.8                     |
| Age at diagnosis, median ± S.D. (years)        | 36.7 ± 13.4        |                                 |
| Extent of disease                              |                    |                                 |
| Distal colitis                                 | 53 (41.4)          |                                 |
| Left-sided colitis                             | 39 (30.5)          |                                 |
| Pancolitis                                     | 36 (28.1)          |                                 |
| Severity                                       |                    |                                 |
| Mild                                           | 41 (32.0)          |                                 |
| Intermediate                                   | 60 (46.9)          |                                 |
| Severe                                         | 27 (21.1)          |                                 |
| Negative UC-related outcomes                   |                    |                                 |
| Absent                                         | 73 (57.9)          |                                 |
| Present                                        | 53 (42.1)          |                                 |
| Relapse rate                                   |                    |                                 |
| Infrequent                                     | 67 (53.2)          |                                 |
| Frequent                                       | 59 (46.8)          |                                 |

NA, not available.
2.3. Statistical analysis

by random re-genotyping of the respective loci and therefore, all fragments (222, 107, and 104 bp). Genotyping errors were excluded (329 bp and 104 bp), AG heterozygotes demonstrated four DNA bands GSTP1 the fragments) for AC, and 84, 31, 30 and 18 bp (4 fragments) for CC. For 28, and 18 bp (5 fragments) for AA; 84, 56, 31, 30, 28, and 18 bp (6 fragments) for TT. For rs1801131 genotypes, the fragments were 56, 31, 30, 28, and 18 bp (5 fragments) for AA; 84, 56, 31, 30, 28, and 18 bp (6 fragments) for AC, and 84, 31, 30 and 18 bp (4 fragments) for CC. For the GSTP1 rs1695 polymorphism, AA homozygotes had two fragments (329 bp and 104 bp), AG heterozygotes demonstrated four DNA bands (329, 222, 107, and 104 bp) and GG homozygotes showed three fragments (222, 107, and 104 bp). Genotyping errors were excluded by random re-genotyping of the respective loci and therefore, all polymorphisms were included into the association analysis.

2.3. Statistical analysis

Comparison of demographical parameters between cases and controls was performed using Student’s t-test for continuous variables and the χ² test for categorical data. Deviation from Hardy–Weinberg equilibrium (HWE) was assessed by the Fisher exact test. The linkage disequilibrium between the two polymorphisms in MTHFR gene was examined using D’ and r² coefficients. Genotype frequencies in the case and control groups were compared by logistic regression with adjustment for sex, age at investigation, and current smoking status under the additive, dominant, recessive, co-dominant, and over-dominant genetic models. Logistic regression model was also constructed to determine the effect of genetic polymorphisms on the clinical phenotypes of UC (disease extent, relapse, severity, complications, and age at onset). The odds ratios (OR) with their corresponding 95% confidence intervals (CI) and p-values were calculated as measures of associations. p-values <0.05 were considered as significant. The Bonferroni correction for multiple comparisons was applied where appropriate, and the threshold was calculated as 0.05/5 = 0.01. For significant p-values, Fisher exact test was additionally calculated as suggested in (Lettre et al., 2007). The Fisher test is particularly relevant when one of the alleles is rare, and together with pooling the genotypes according to the genetic model, the statistical significance can be estimated more adequately (Lewis, 2002). Combined effects of polymorphisms on the disease risk were analyzed using logistic regression for all possible combinations of loci under three genetic models: dominant, recessive, and over-dominant. In the dominant model, the rare homozygous variant was combined with the heterozygous, while in the recessive model, the rare homozygous genotype was considered alone. In the over-dominant model, the heterozygous and both homozygous genotypes together were encoded using two dummy variables. Therefore, tests for genotype combinations had nine degrees of freedom for pairwise combinations of MTHFR rs1801133, rs1801131, and GSTP1 rs1695 polymorphisms (three for each SNP), six degrees of freedom for combinations of MTHFR rs1801133, rs1801131, and GSTP1 rs1695 polymorphisms with GSTT1 and GSTM1 loci, and a four-degree of freedom for combined GSTT1 and GSTM1 genotypes. Accordingly, significant p-values were corrected using the Bonferroni method by multiplying by factors 9, 6, or 4. Power of the study was calculated post-hoc assuming the following variables: significance (type 1 error) 0.05, genetic effects (odds ratio [OR]) 1.5 and 2.0, and disease prevalence of 0.00025; allele frequencies were those found in control population. The statistical tests were performed with SNPsStats program and E-Views software (IHS Global Inc.).

3. Results

There were significant differences in age and smoking status (current smokers vs. non-smokers) distributions between the case and control groups (p = 0.00322 and p = 3.0E-6, respectively; Table 1). Although there was no significant difference in sex ratio between the two groups (p = 0.168462), their matching on gender was imperfect: the cases had a higher percentage of female (46.1%) than the controls (38.2%) (Table 1). All three of these demographic variables were included as covariates in all subsequent multivariate regression analyses.

Table 2 summarizes the distribution of MTHFR and GST genotypes and allele frequencies in the cohort of 128 UC patients and in 136 healthy controls. The observed frequencies of the studied polymorphisms were in a range of values observed in other Caucasian–European populations. Allelic distributions of the investigated SNPs were in accordance with Hardy–Weinberg equilibrium (HWE) in both groups except for GSTP1 rs1695, which showed a slight deviation from HWE in controls (p = 0.026). Linkage disequilibrium analysis showed strong LD (pairwise D’ = 0.969) between the two MTHFR loci at nucleotide positions 677 (rs1801133) and 1298 (rs1801131). However, due to a weak correlation (r² = 0.205), the two SNPs cannot substitute each other and were analyzed in an independent manner.

No significant differences were observed in the frequencies of the MTHFR and GST genotypes and alleles between UC subjects and controls (Table 2). The lack of association persisted after stratification by gender or smoking status (data not shown). In pairwise combined analyses of MTHFR rs1801133 and rs1801131, GSTP1 rs1695, GSTM1, and GSTT1 gene polymorphisms, only five different genotype combinations were significantly associated with UC (Table 3). However, the significance was eliminated after applying a Bonferroni correction (p corrected > 0.05). No other combinatorial genotypes reached a nominal significance (data not shown).

In addition, we also performed a detailed genotype–phenotype analysis of GST and MTHFR variants in UC patients. There was no association of these polymorphisms with age of onset, complications, and relapse rate (data not shown). Also, no association was observed between GSTM1 and GSTT1 genes and disease spread or severity (data not shown). We showed that the AC genotype of MTHFR rs1801131 was significantly associated with increased severity in the over-dominant genetic model (coefficient = 0.32; 95% CI = 0.10–0.54; p = 0.0046; Table 4). Both the co-dominant genetic model (p = 0.018) and the

![Fig. 1. Electrophoresis of the products of the multiple PCR. Presence of 267 and 434-bp fragments indicates GSTM1 and GSTT1 wild-type (non-null) genotypes, respectively. The 212-bp band corresponding to a fragment of the human albumin gene, which serves as a positive control for the PCR. Lane 5: molecular weight marker (GeneRuler 50 bp DNA Ladder, Thermo Fisher Scientific); lane 1: GSTM1 and GSTT1 non-null genotype; lane 2: GSTM1 null/GSTT1 non-null genotype; lane 3: GSTM1 non-null/GSTT1 null genotype; lane 4: negative PCR control.](image-url)
dominant genetic model (coefficient = 0.26; 95% CI = 0.04–0.48; p = 0.022) also obtained significant results (Table 4). However, only the p-value under the over-dominant model remained significant after Bonferroni correction for multiple tests was applied (p corrected = 0.023). We also report a significant association between MTHFR rs1801133 polymorphism and disease extent (co-dominant model: p = 0.008; Table 5). This was primarily due to a significantly higher frequency of the TT genotype in patients with distal colitis than in patients with more extensive UC types (recessive model: OR = 0.15; CI = 0.04–0.63; p = 0.0041). The values were still significant after Bonferroni correction (p corrected = 0.040 and p corrected = 0.020 for co-dominant and recessive models, respectively; Table 5). Furthermore, the associations were validated by Fisher exact test (p corrected = 0.0375 and p corrected = 0.0185 for co-dominant and recessive models, respectively; Table 5).

With regard to the power analysis in the combined sample, our sample set was estimated to have enough power (>89%) to detect moderate high-risk alleles (OR = 2) but limited (40–64%) for moderate low-risk alleles (OR = 1.5). The power was even smaller in the stratified and combinatorial analyses and this was a limitation of this study.

4. Discussion

Wide evidence suggests that oxidative stress is involved in UC pathogenesis (Rezaie et al., 2007; Valko et al., 2007; Piechota-Polanczyk and Fichna, 2014). The enzymes glutathione S-transferases T1, M1, and P1, and methaneetetrahydrofolate reductase (MTHFR) are implicated in the antioxidant defenses (Hayes et al., 2005; Raza, 2011; Hoffman, 2011). The analysis of their variation has been widely used in the field of cancer and inflammatory genetics. Their role in the pathogenesis of UC has been also proposed but has not been extensively studied. The present study was designed to investigate the contribution of GSTT1, GSTM1, GSTP1, and MTHFR genetic polymorphisms to the risk and pathogenesis of UC in Moldavian population. To the best of our knowledge, this is the first report on GSTT1, GSTM1, GSTP1, and MTHFR genes in UC patients from eastern–southeastern Europe.

We did not observe significant associations between any studied polymorphisms and UC. Furthermore, no Bonferroni-corrected significant associations of combined genotypes were found, implying that these combinations are probably not synergistic in their effect on UC risk in Moldavian population. These findings were not totally unexpected. Indeed, previous studies on the association between the same polymorphisms and susceptibility to UC have reported conflicting results. Thus, an association of MTHFR rs1801133 genotypes with UC has been reported in Ireland (Mahmud et al., 1999), Denmark (Nielsen et al., 2000), and Portugal (Magro et al., 2003), but not in China (Chen et al., 2005, 2008; Jiang et al., 2012), the UK (Herrlinger et al., 2005), Italy (Vecchi et al., 2000; Papa et al., 2001), Turkey (Yilmaz et al., 2006), or Morocco (Senhaji et al., 2013). For the MTHFR rs1801131 polymorphism, positive association results were obtained in studies from Southeast Asia (Jiang et al., 2012) and Turkey (Yilmaz et al., 2006) but were not confirmed on samples from Central China (Chen et al., 2005, 2008) and the UK (Herrlinger et al., 2005). Regarding GSTT1/M1 genes, both homozygous GSTT1 and GSTM1 deletion polymorphisms were shown to be associated with UC in Central China (Ye et al., 2011), northern India (Mittal et al., 2007), and in Turkish population (Buyukgoze et al., 2013). Conversely, studies in Denmark (Ernst et al., 2010) and Holland (Broekman et al., 2014) as well as in Israeli Jews (Karban et al., 2011) failed to demonstrate any relationship between GSTT1/M1 loci and the risk of UC. Likewise, the GSTP1 rs1695 polymorphism showed significant association with UC in Central China (Ye et al., 2011) and no association in Denmark (Ernst et al., 2010). The observed discrepancy between individual studies may be due to the relatively small number of patients used in different studies. In addition, the impact of these

**Table 2**

Association tests for single polymorphisms.

| Polymorphism | Genotype/Allele | Controls n (%) | Cases n (%) | OR (95% CI) | p-value<sup>a</sup> | p-value corrected<sup>a</sup> |
|--------------|----------------|---------------|-------------|-------------|-------------------|-----------------------------|
| MTHFR rs1801133 | CC | 70 (51.5%) | 59 (46.1%) | 1.00 | | |
| | CT | 52 (38.2%) | 55 (43%) | 1.00 | | |
| | TT | 14 (10.3%) | 14 (10.9%) | 1.00 | | |
| | C | 192 (70.6%) | 173 (67.6%) | 1.00 | | |
| | T | 80 (29.4%) | 83 (32.4%) | 1.00 | | |
| MTHFR rs1801131 | AA | 66 (48.5%) | 52 (41.3%) | 1.00 | | |
| | AC | 59 (43.4%) | 57 (45.2%) | 1.00 | | |
| | CC | 11 (8.1%) | 17 (13.5%) | 1.00 | | |
| | A | 191 (70.2%) | 161 (63.9%) | 1.00 | | |
| | C | 81 (29.8%) | 91 (36.1%) | 1.00 | | |
| GSTP1 rs1695 | AA | 60 (44.4%) | 62 (49.2%) | 1.00 | | |
| | AG | 68 (50.4%) | 53 (42.1%) | 1.00 | | |
| | GG | 7 (5.2%) | 11 (8.7%) | 1.00 | | |
| | A | 188 (69.6%) | 177 (70.2%) | 1.00 | | |
| | G | 82 (30.4%) | 75 (29.8%) | 1.00 | | |
| GSTT1 | Present | 111 (81.6%) | 108 (84.4%) | 1.00 | | |
| | Null | 25 (18.4%) | 20 (15.6%) | 1.00 | | |
| GSTM1 | Present | 61 (44.9%) | 52 (40.6%) | 1.00 | | |
| | Null | 75 (55.1%) | 76 (59.4%) | 1.00 | | |

<sup>a</sup> The p-values were obtained from logistic regression with co-dominant, dominant, recessive, over-dominant and additive models, and adjusted for sex, age and current smoking status.

**Table 3**

Pairwise genotype - genotype interaction effects on UC risk revealed by logistic regression under dominant, recessive and over-dominant genetic models. Only associations with a nominal p-value p < 0.05 are shown.

| 1st locus | Genotype | 2nd locus | Genotype | Controls n (%) | Cases n (%) | OR (95% CI)<sup>a</sup> | p-value<sup>a</sup> | p-Value corrected<sup>a</sup> |
|-----------|----------|-----------|----------|---------------|-------------|-----------------|-------------------|-----------------------------|
| MTHFR rs1801133 | CT | MTHFR rs1801131 | AC | 22 (16.2%) | 32 (25.4%) | 2.18 (1.12–4.21) | 0.019 | 0.171 |
| MTHFR rs1801133 | CT + TT | MTHFR rs1801131 | AC | 23 (16.9%) | 32 (25.4%) | 2.03 (1.06–3.89) | 0.03 | 0.27 |
| MTHFR rs1801131 | AA | GSTP1 rs1695 | AG | 32 (23.7%) | 37 (13.6%) | 0.45 (0.23–0.90) | 0.021 | 0.189 |
| MTHFR rs1801131 | AA | GSTP1 rs1695 | AG + GG | 34 (25.2%) | 21 (16.8%) | 0.51 (0.27–0.98) | 0.04 | 0.36 |
| GSTP1 rs1695 | AG | GSTM1 | Present | 34 (25.2%) | 19 (15.1%) | 0.50 (0.26–0.98) | 0.039 | 0.234 |

<sup>a</sup> Adjusted for sex, age at investigation and smoking.
polymorphisms on the risk of UC may also vary from population to population because of differences in genetic backgrounds as well as environmental and nutritional factors. For example, folate supplementation can efficiently reduce plasma Hcy level and may therefore affect UC susceptibility. A study by Chen et al. (2008) suggested a link between interethnic difference in folate consumption and association of polymorphisms in the MTHFR gene with UC. Interestingly, Moldavia, along with Kosovo, is the only European country that mandates fortification of wheat products with folic acid (Food Fortification Initiative, 2015). This background folic acid supplementation could potentially smooth possible effects of MTHFR polymorphisms on UC and partially explain the lack of association in the Moldovan population. Consideration of additional information on the dietary habits of participants and controlling for the markers of folate status (i.e., folic acid, Hcy, vitamin B12) would certainly help to clarify the role of MTHFR variants in the development of UC and, therefore, are desired for further in-depth studies of UC.

We further investigated whether GST and MTHFR polymorphisms are associated with certain phenotypic characteristics in UC patients.

In conclusion, the present study has shown that MTHFR polymorphisms in UC, it has been previously described for other human diseases (Li et al., 2014; Hubacek et al., 2015). Further research is needed to confirm the causal effect of MTHFR on the severity of UC.

Finally, we observed a significant association between the MTHFR rs1801133 polymorphism and the extent of inflammation. Patients with the TT genotype achieved significantly higher protection against more extensive disease (left-sided UC and pancolitis) than patients with CT or CC genotypes. This finding contradicts the oxidative stress hypothesis of UC and the previous reports on correlation between MTHFR rs1801133 and UC extension (Chen et al., 2008; Jiang et al., 2012; Senhaji et al., 2013; Vecchi et al., 2000). The differential results could be due to multifunctional properties of methylenetetrahydrofolate reductase, again modulated by population and nutritional factors. Indeed, the MTHFR rs1801133 TT genotype is known to reduce enzyme activity (~30% of normal) (Frostell et al., 1995), and therefore, its carriers may be especially susceptible to Hcy-induced diseases, particularly in the presence of folate deficiency. On the other hand, MTHFR has a crucial role in regulating cellular methylation and gene expression (Chen et al., 2001; Friso et al., 2002; Lu, 2013). A potential biochemical explanation for the inverse association between UC and the MTHFR rs1801133 TT genotype is that low MTHFR activity results in lower mean (s.e.) LR coefficient (95% CI) p-Value p-Value corrected
AA 21 (51.2%) 24 (40.7%) 7 (26.9%) 1.73 (0.1) 0 (Reference) 0.018 0.09
AC 13 (31.7%) 28 (47.5%) 16 (61.5%) 2.05 (0.1) 0.33 (0.10–0.56) 1.76 (0.18) 0.04 (0.50–0.88) 0 (Reference) 0.22 0.11
CC 7 (17.1%) 7 (11.9%) 3 (11.5%) 1.76 (0.18) 0.04 (0.50–0.88) 0 (Reference) 0.22 0.11
AC + CC 20 (48.8%) 35 (59.3%) 19 (73.1%) 1.99 (0.08) 0.26 (0.04–0.48) 1.9 (0.07) 0 (Reference) 0.43 -
CC + CT 42 (79.3%) 72 (96%) 1 (Reference) 0.00 (0.00–0.00) 0.13 (-0.46–0.19) 0 (Reference) 0.0046 0.023
AC 13 (31.7%) 28 (47.5%) 16 (61.5%) 2.05 ± 0.1 0.32 (0.10–0.54) 0.0046 0.023
- = not done.

Table 5
Effect of MTHFR polymorphism rs1801133 on severity of UC, analyzed by logistic regression.

| Genotype | Severity mean (s.e.) | LR coefficient (95% CI) | p-Value | p-Value corrected |
|----------|----------------------|--------------------------|---------|------------------|
| AA       | 1.73 (0.1)           | 0 (Reference)            | 0.018   | 0.09             |
| AC       | 2.05 (0.1)           | 0.33 (0.10–0.56)         | 0.04 (0.50–0.88) | 0 (Reference) 0.22 0.11 |
| CC       | 1.76 (0.18)          | 0.04 (0.50–0.88)         | 0 (Reference) 0.22 0.11 |
| AC + CC  | 1.99 (0.08)          | 0.26 (0.04–0.48)         | 1.9 (0.07) 0 (Reference) 0.43 - |
| CC + CT  | 1.74 ± 0.08          | 0 (Reference)            | 0.0046  0.023 |
| AC       | 2.05 ± 0.1           | 0.32 (0.10–0.54)         | 0.0046  0.023 |

1 = mild, 2 = moderate, 3 = severe.

Adjusted for sex, age at investigation and smoking.

LR, logistic regression; CI, confidence interval.

p-values above 1.0 after Bonferroni correction are not shown.

Adjusted for sex, age, at investigation and smoking.
significant influence on the primary risk of having UC in Moldavian population. However, our results suggest that MTHFR genotypes may affect the spread and severity of UC. The main limitation of our study is its small sample size, which is partly due to the low incidence of UC in Moldavia. It also does not consider the nutritional status of participants as assessed by serum Hcy and folic acid levels. Hence, further work using a larger population and studying additional folate and oxidative stress-related genes taking into account the gene–gene and gene–environment interactions is needed to refine the present results. Such knowledge may have important implications for prevention and management of UC.

Conflict of interest statement
The authors declare no conflicts of interest.

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