Int J Clin Exp Med 2017;10(5):7431-7442
www.ijcem.com /ISSN:1940-5901/IJCEM0047536

Review Article

Risk of inflammatory bowel disease associated with MTHFRC677T and prothrombin G20210A mutation: a meta-analysis

Lili Lu[1*], Wei Wang[2*], Li Li[1], Xunlei Pang[1], Sujuan Fei[1]

[1]*Department of Gastroenterology, The Affiliated Hospital of Xuzhou Medical University, Xuzhou 221000, Jiangsu, China;* [2]*School of Health Sciences, Wuhan University, Wuhan 430000, Hubei, China.* [*]*Equal contributors and co-first authors.*

Received December 28, 2016; Accepted March 16, 2017; Epub May 15, 2017; Published May 30, 2017

Abstract: It has been demonstrated that inflammatory bowel disease (IBD) patients had a higher risk of thromboembolism, numerous observational studies have reported the prevalence of MTHFR C677T mutation and G20210A prothrombin mutation in patients with IBD. Whereas the magnitude of association between these two inherited thrombophilic abnormalities and IBD remains unknown. We conduct a meta-analysis to examine these two associations. The literature search was conducted using PubMed database, Odd ratios (ORs) with 95% confidence interval (CI) were calculated using random- or fixed-model. Finally, nineteen studies were included for MTHFR C677T mutation and fourteen studies were included for prothrombin G20210A mutation. No significant heterogeneity was found in the present analysis. As regards MTHFR C677T mutation, although no significantly increased IBD risk was found in the overall population (OR=1.06, 95% CI 1.07-1.17), subgroup analyses based on ethnicity suggested that MTHFR C677T mutation was associated with a slight increased risk of IBD in the Caucasian (OR=1.17, 95% CI 1.01-1.35) and Turkish population (OR=2.01, 95% CI 1.10-3.69). With respect to prothrombin G20210A mutation, not only the overall results (OR=0.66, 95% CI 0.65-1.50) but also the subgroup analyses showed no significant association was found between IBD and G20210A mutation. This meta-analysis indicated that MTHFR C677T mutation was significantly associated with the risk of IBD in Caucasian and Turkish population. No significant association was found between IBD and prothrombin G20210A mutation.

Keywords: MTHFR, prothrombin, inflammatory bowel diseases, meta-analysis

Introduction

Inflammatory bowel disease (IBD) is defined as a chronic or relapsing inflammatory disorder of the gastrointestinal tract, and it encompasses two major forms which are ulcerative colitis (UC) and crohn's disease (CD). Increasing IBD incidence and prevalence have been observed in different regions of the world, especially in the developing countries [1]. Due to the reduction of the life quality and the ability to work and increases disability, IBD places a heavy burden on patients [2]. At present, the exact etiologies and pathogenesis of IBD are not completely elucidated, various factors contributed to it including genetic predisposition, environmental, aberrant immunological factors and their interaction [3]. Previous data have demonstrated that IBD is associated with an increased risk of vascular complications [4-6], suggest that thromboembolism is a disease-specific extraintestinal manifestation of IBD [7]. Owing to the established association between IBD and thrombosis, it may hence be reasonable to hypothesize that these conditions may share some common inherited abnormalities. The main frequent hereditary thrombophilic abnormalities are factor V Leiden (FVL), prothrombin (FII) gene G20210A mutation, and methylenetetrahydrofolate reductase (MTHFR) gene C677T mutation.

FVL is a single point mutation of the coagulation factor V gene at position 506 [8], leading to replacement of Arg506 with Gln, in the factor V molecule (FVR506Q). Many studies reported it

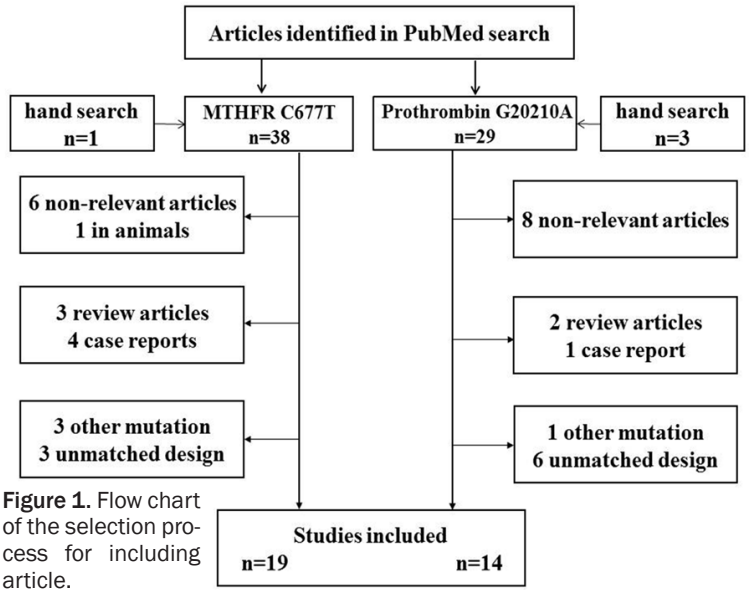

Figure 1. Flow chart of the selection process for including article.

prominently contributed to thromboembolism [9, 10]. Two meta-analysis have confirmed FVL mutation was significantly associated with an increased risk of thromboembolism in IBD patients, but not significantly associated with the risk of IBD [11, 12].

MTHFR is a major enzyme participated in the remethylation pathway of homocysteine metabolism. A common mutation (C677T) located on the exon 4 has been identified in the MTHFR gene lead to the amino acid replacement from alanine to valine [13]. Several meta-analysis have found similar results that C677T mutation was associated with a higher risk of Venous thromboembolism [14, 15]. So far, numerous observational studies have reported the prevalence of MTHFR C677T mutation in patients with IBD. A meta-analysis on genetic variants of homocysteine metabolism pathway in IBD did not find a relationship between MTHFR C677T polymorphism and IBD risk [16]. Another meta-analysis found deficient folate status is associated with a higher impact of MTHFR C677T polymorphism on IBD risk [17]. However, with the publication of additional studies related to this polymorphism, sufficient data were available to perform a more comprehensive meta-analysis.

Prothrombin is a robust enzyme usually produced in the liver in an inactive form of thrombin. It plays an important role in the coagulation system by activating many clotting factors and other elements like the blood platelets. Prothrombin G20210A mutation is a genetic variant at position 20210 in the 3'-untranslated region of the prothrombin gene, single nucleotide substitution (G to A) and was first found by Poort et al in 1996 [18]. A recent meta-analysis concluded that carriers of A allele had a substantial higher risk of thrombosis [19]. The effect of prothrombin G20210A mutation on the risk of IBD varies among studies, but the relationship between this mutation and IBD remains controversial. Furthermore, there is no firm conclusions made by a meta-analysis up to now.

In order to obtain the most convinced estimates of the prevalence of MTHFR C677T and prothrombin G20210A mutation, and to evaluate the risk of IBD associated with these two inherited mutations therefore we conducted the present meta-analysis.

Materials and methods

Search strategy

We searched PubMed for articles, with the last search performed on Aug 30, 2016. Different combinations of search terms: (Ulcerative colitis OR UC OR Crohn's disease OR CD OR Inflammatory bowel diseases OR IBD) AND (MTHFR OR C677T OR Factor II OR Prothrombin OR G20210A). We reviewed the reference lists of the original publications by manual search and review articles were also examined for additional eligible studies. If data were published in more than one article, only the study with the largest sample size was included. The searched studies were no language restrictions.

Inclusion and exclusion criteria

Eligible studies were selected based on the following criteria: (1) Evaluating the association between the two mutations and IBD; (2) Case-control or cohort or cross-sectional design for human beings which included a comparison group; (3) Supplying data like sample size, distribution of alleles, etc. (4) The control groups were healthy subjects or patients without a his-

Table 1. Characteristics of the studies on MTHFR C677T mutation and inflammatory bowel disease (IBD)

Author	Year	Country	Ethnicity	Disease	Study design	Genotyping methods	Case/Control				Strength of the evidence
							CT/TT	CC	Females (%)	mean age (years)	
Mahmud	1999	Ireland	Caucasian	IBD	Case-control	PCR	102/145	72/128	56/56	39/35	7
Vecchi	2000	Italy	Caucasian	CD, UC	Case-control	PCR	66/139	36/56	48/48	42/41	8
Gue 'don a	2001	France	Caucasian	IBD	Case-control	PCR	37/43	21/41	Nr/60	31/36	6
Gue 'don b	2001	France	Caucasian	IBD	Case-control	PCR	6/52	8/58	Nr/71	41/49	6
Papa	2001	Italy	Caucasian	IBD	Case-control	PCR	43/108	21/87	30/38	40/30	8
Larsen	2002	Denmark	Caucasian	IBD	Cross-sectional	PCR	64/539	42/545	65/Nr	41/Nr	7
Nakano	2003	UK	Caucasian	IBD	Cross-sectional	PCR	27/23	16/23	44/46	14/10	6
Toruner	2004	Turkey	Turkish	CD, UC	Case-control	PCR	7/6	55/74	52/Nr	37/Nr	6
Herrlinger	2005	UK	Caucasian	CD, UC	Case-control	PCR	248/109	159/80	54/Nr	29/Nr	8
Stocco	2006	Italy	Caucasian	CD, UC	Case-control	PCR	68/92	24/38	53/Nr	15/Nr	7
Yilmaz	2006	Turkey	Turkish	IBD	Case-control	PCR	17/7	16/20	48/37	34/34	7
Yasa	2007	Turkey	Turkish	CD, UC	Case-control	PCR	14/18	13/29	26/25	34/49	7
Bernstein	2007	Canada	Mixed	CD, UC	Case-control	Taqman	262/243	224/166	56/73	36/40	7
Koutroubakis a	2007	Greece	Caucasian	IBD	Case-control	PCR	29/28	31/26	37/Nr	49/47	7
Koutroubakis b	2007	Greece	Caucasian	IBD	Case-control	PCR	16/15	14/15	37/Nr	52/51	7
Banait	2008	India	Asian	UC	Case-control	PCR	5/67	23/431	32/Nr	37/Nr	7
Chen	2008	China	Asian	UC	Case-control	PCR	104/127	64/88	58/62	41/41	6
Biroulet	2008	France	Caucasian	CD	Case-control	PCR	79/142	61/106	62/Nr	39/35	7
Xu	2011	China	Asian	UC	Case-control	RFLP-PCR	148/394	126/332	40/45	43/45	7
Jiang	2012	China	Asian	UC	Case-control	RFLP-PCR	161/506	149/430	41/40	40/42	7
Senhaji	2013	Morocco	Caucasian	CD, UC	Case-control	RFLP-PCR	46/88	50/94	41/Nr	Nr/Nr	6

IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; CT/TT, heterozygous or homozygous; CC, normal genotype; Nr, not reported. PCR, polymerase chain reaction; RFLP-PCR, restriction fragment length polymorphism-polymerase chain reaction.

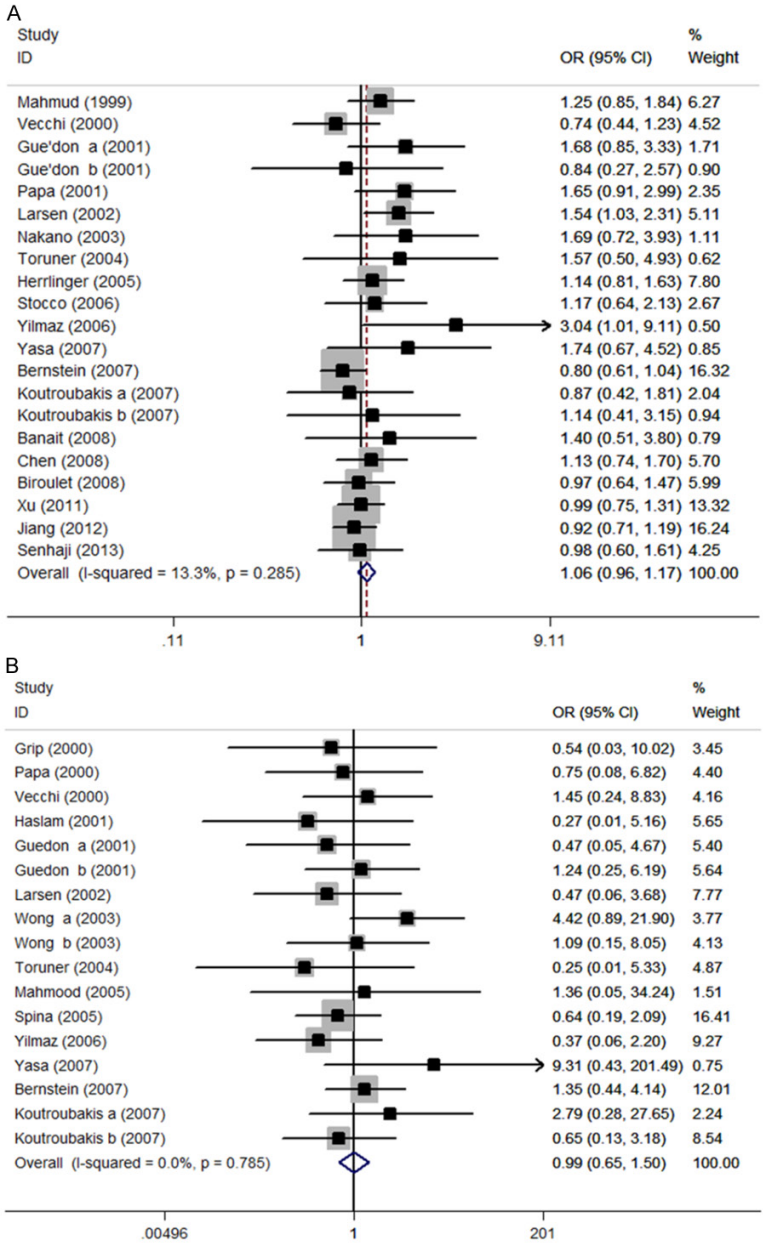

Figure 2. Forest plot of the association between IBD and MTHFR C667T (A) and prothrombin G20210A mutation (B). MTHFR indicates methylenetetrahydrofolate reductase; IBD, inflammatory bowel disease.

tory of IBD. Exclusion criteria: (1) no controls; (2) duplicate publications; (3) reviews, comments or animal studies; (4) studies that did not provide usable data.

Data extraction

The following data were independently extracted by two researchers (Lili Lu and Wei Wang) from all eligible studies: last name of first author, year of publication, original country, ethnicity, gender, mean age, number of cases and controls for each C677T and G20210A genotype.

Evaluation of study quality

Two researchers (Lili Lu and Wei Wang) independently completed the assessment of study equality, according to the Newcastle Ottawa Scale (NOS) for Case-control or cohort study [20] and Agency for Healthcare Research and Quality (AHRQ) for cross-sectional study [21]. Selection, comparability and exposure as the three main items were assessed in NOS whichaquality score giving a range from 0 (the lowest) to 11 (the highest) was obtained by summing each component, and studies with scores equal to or higher than five were considered to be of high quality. An 11-item checklist which was recommended by Agency for Healthcare Research and Quality (AHRQ) to assess the cross-sectional study. An item would be scored '0' if it was answered 'NO' or 'UNCLEAR'; if it was answered 'YES', then the item scored '1'. Article quality was assessed as follows: low quality =0-3; moderate quality =4-7; high quality =8-11. If there was discrepancies then it would be resolved by discussion.

Statistical analysis

Data were entered into the STATA version 12.0 (Stata Corp, College Station, Texas). We combined the heterozygous and homozygous patients as 'polymorphism-positive'. The association between the two mutations and the risk of IBD was estimated by the odds ratio (OR) and its 95% confidence interval (95% CI). The Chi-square based Q test and I^2 statistics were used

Table 2. Characteristics of the studies on prothrombin G20210A mutation and inflammatory bowel disease (IBD)

Author	Year	Country	Ethnicity	Disease	Study design	Genotyping methods	Case/Control				Strength of the evidence
							GA/AA	GG	Females (%)	Mean age (years)	
Grip	2000	Sweden	Caucasian	IBD	Case-control	Nr	0/7	11/92	50/59	53/64	6
Papa	2000	Italy	Caucasian	CD, UC	Case-control	PCR	1/4	51/152	31/31	42/37	8
Vecchi	2000	Italy	Caucasian	CD, UC	Case-control	PCR	2/3	91/198	48/48	42/41	8
Haslam	2001	UK	Caucasian	CD, UC	Cohort	PCR	0/4	39/96	Nr/Nr	Nr/Nr	6
Gue 'don a	2001	France	Caucasian	IBD	Case-control	PCR	1/3	57/81	Nr/60	31/36	6
Gue 'don b	2001	France	Caucasian	IBD	Case-control	PCR	2/13	12/97	Nr/49	41/48	6
Larsen	2002	Denmark	Caucasian	IBD	Cross-sectional	PCR	1/10	105/490	65/Nr	41/Nr	7
Wong a	2003	UK	Caucasian	IBD	Case-control	Nr	7/2	76/96	Nr/Nr	Nr/Nr	5
Wong b	2003	UK	Caucasian	IBD	Case-control	Nr	2/2	53/58	Nr/Nr	Nr/Nr	5
Toruner	2004	Turkey	Turkish	CD, UC	Case-control	PCR	0/2	62/78	52/Nr	37/Nr	6
Spina	2005	Italy	Caucasian	CD, UC	Case-control	PCR	1/0	67/30	32/32	42/41	6
Mahmood	2005	UK	Caucasian	CD, UC	Case-control	PCR	4/12	43/82	52/47	42/38	7
Yilmaz	2006	Turkey	Turkish	IBD	Case-control	PCR	2/4	31/23	48/37	34/34	7
Yasa	2007	Turkey	Turkish	CD, UC	Case-control	PCR	2/0	25/47	26/25	34/49	7
Bernstein	2007	Canada	Mixed	CD, UC	Case-control	Taqman	8/5	484/407	56/73	36/40	7
Koutroubakis a	2007	Greece	Caucasian	IBD	Case-control	PCR	3/1	57/53	37/Nr	49/47	7
Koutroubakis b	2007	Greece	Caucasian	IBD	Case-control	PCR	3/4	30/26	37/Nr	51/51	7

IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; GA/AA, heterozygous or homozygous ; GG, normal genotype; Nr, not reported. PCR, polymerase chain reaction.

to test the heterogeneity among studies [22]. When P<0.10, or I^2>50 % indicated evidence of heterogeneity, a random effect model was used to estimate the summary ORs; whereas the fixed-effects model was used [23, 24]. The meta-analysis consisted of the overall analysis which includes all available data concerning IBD. Then, meta-analyses for CD and UC were performed separately. Subgroup analyses were conducted based on ethnicity. Potential publication bias was evaluated by the Begg test for funnel plot [25] and Egger regression test asymmetry [26], P<0.05 was considered statistically significant. Sensitivity analysis was used for assessing the stability of the results by deleting one single study each time to examine the influence of single data.

Results

Study characteristics

After a comprehensive literature searched in PubMed, 38 articles about IBD and MTHFR C677T mutation along with 29 articles about IBD and prothrombin G20210A mutation potentially met inclusion criteria and were retrieved for detailed review. Additionally, one study for MTHFR C677T mutation and three studies for prothrombin G20210A mutation were identified by manually reviewing the references of the eligible articles retrieved. The detailed selection process are described in **Figure 1**. Finally, 19 articles [27-45] about IBD and MTHFR C677T mutation, and 14 articles [28-35, 46-51] about IBD and Prothrombin G20210A mutation met our eligibility criteria were included in the meta-analysis. Among the 19 articles about IBD and MTHFR C677T mutation, two [33, 37] were cross-sectional studies and 17 were case-control studies. The articles about IBD and Prothrombin G20210A mutation were one [33] cross-sectional study and onecohort study [50], the remaining were all case-control studies. All included studies were published in English, except one [43] in Chinese. Strength of the evidence scores for the each study ranged from 5 to 8, all the studies being classified as moderate quality.

MTHFR C677T gene mutation

The 19 studies involving 2774 cases and 5758 controls were assessed for the association between MTHFR C677T gene mutation and IBD risk. The main baseline characteristics of eligible studies were summarized in **Table 1**. There was no significant heterogeneity between the studies (I^2=13.3%, P=0.285), thus fixed-effects

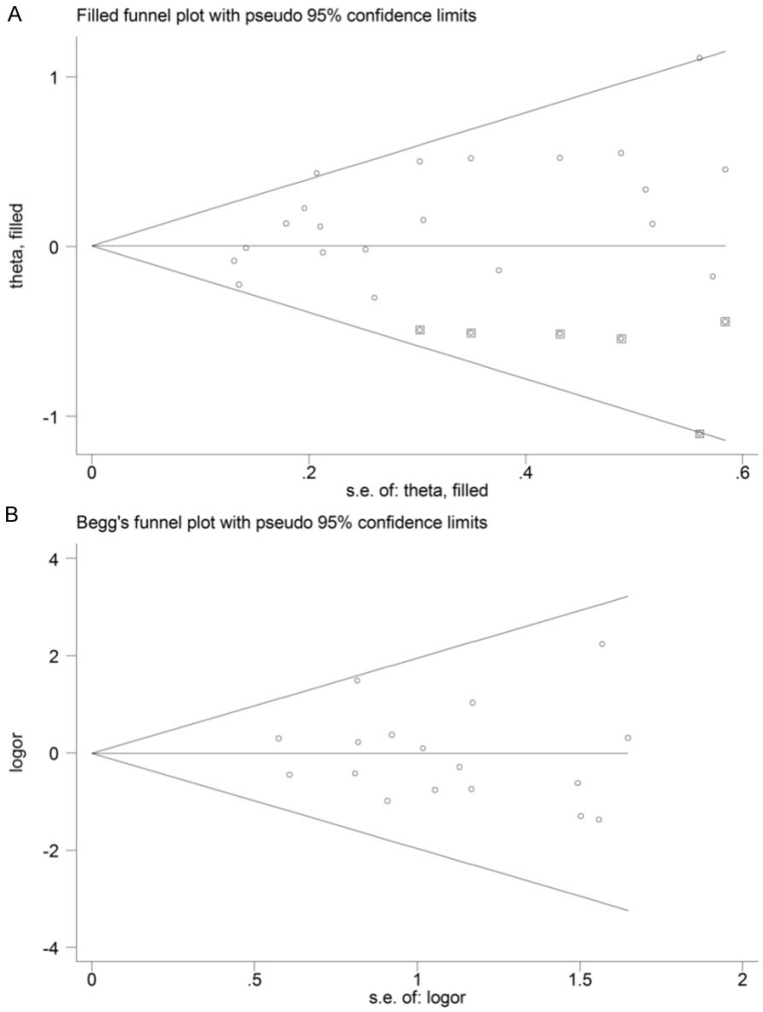

Figure 3. Funnel plots with and without trim and fill to detect publication bias for the association between MTHFR C677T (A) and prothrombin G20210A mutation (B) and the risk of IBD. MTHFR indicates methylenetetrahydrofolate reductase; IBD, inflammatory bowel disease.

model was used. In the overall analysis, the MTHFR C677T mutation was not significantly associated with increased risk of IBD (OR=1.06, 95% CI 1.07-1.17). More specifically, in two studies [33, 34] the prevalence of the MTHFR 677T allele was was marginally higher in cases than in controls, and in the remaining study the prevalence was similar between cases and controls (**Figure 2A**). Only two studies [35, 39] involved children, the remaining were performed in adult populations (**Table 2**). Begg funnel plot and Egger test were performed to assess publication bias. The results of Begg's funnel plot (P=0.139) suggested no statistical evidence for publication bias, but based on the results of Egger's regression test, statistical significance for funnel plot asymmetry was provided (P=0.010). After a trim and fill method was implemented under fixed effects models (**Figure 3A**) we found there was not sharp asymmetry in funnel plot. In addition, pooled estimates were unchanged, indicating the results were statistically valid.

Taking into account the differences of races, we performed subgroup analyses based on ethnicity. Eleven studies contained thirteen researches from Caucasian populations were further analyzed, no significant heterogeneity was found in the results of the nine studies (I^2=0.0%, P=0.587). The summary OR showed that MTHFR C677T gene mutation was significantly associated with increased risk of IBD in Caucasians (OR=1.17, 95% CI 1.01-1.35) (**Figure 4**). This analysis was conducted using the fixed effects model. Similarly, three studies from Turkish populations showed there was a statistical significance between MTHFR C677T mutation and the risk of IBD (OR=2.01, 95% CI 1.10-3.69). There was also no significant heterogeneity exist in the three studies (I^2=0.0%, P=0.667). However, no significant association was found in other populations.

As two types of IBD, UC and CD have their own characteristics, and their genetic information might differ [52]. Among the 19 studies we analyzed, eight studies [28-31, 38, 39, 42, 45] detected the frequency of the MTHFR C677T mutation in CD and eleven studies [28-31, 38-41, 43-45] in UC separately. Regarding the relationship between the C677T polymorphism and CD, overall, the allele contrast showed lack of heterogeneity between studies (I^2=19.4%, P=0.276) with the association being non-significant (OR=0.79, 95% CI 0.81-1.15). In investigating the association between C677T and UC, overall, the allele contrast showed non-signifi-

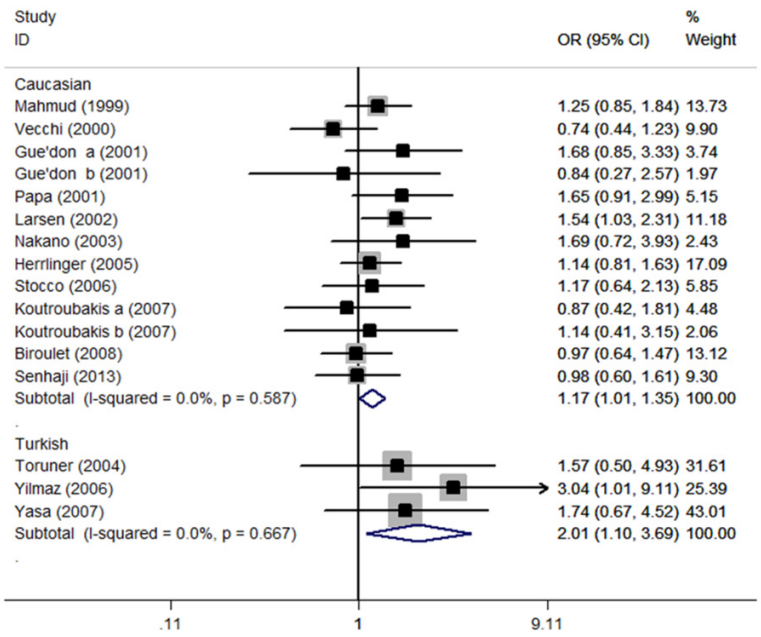

Figure 4. Association of MTHFR C677T mutation with risk of IBD in Caucasian Turkish populations. MTHFR indicates methylenetetrahydrofolate reductase; IBD, inflammatory bowel disease.

Table 3. Risk and P for heterogeneity of stratified analysis on MTHFR C677T mutation and inflammatory bowel disease (IBD)

Category of studies	No. of studies	Summary OR (95% CI)	P for heterogeneity
Risk in all population	19	1.06 (0.96, 1.17)	0.285
Risk in Caucasian	11	1.17 (1.01, 1.35)	0.587
Risk in Turkish	3	2.01 (1.10, 3.69)	0.667
Risk in Child-onset	2	1.32 (0.81, 2.16)	0.489
Risk in CD	8	0.97 (0.81, 1.15)	0.276
Risk in UC	11	0.92 (0.80, 1.05)	0.488
Risk in Thrombosis	2	0.99 (0.47, 2.10)	0.686

IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; OR, Odds ratio; CI, Confidence interval.

cant heterogeneity (I^2=0.0%, P=0.488) and the risk effect was non-significant (OR=0.92, 95% CI 0.80-1.05). Subgroup analysis for ethnicity showed similar results to the overall analysis for both CD and UC.

Prothrombin G20210A gene mutation

The 14 studies with a total of 1330 cases and 2182 controls examining the the association between prothrombin G20210A gene mutation and IBD risk. The main baseline characteristics of eligible studies were summarized in **Table 3**. No evidence of heterogeneity was observed (I^2=0%, P=0.785), so using fixed-effects model. The summary OR showed a slight but not significant increase in risk of IBD with prothrombin G20210A gene mutation (OR=0.66, 95% CI 0.65-1.50) (**Figure 2B**). More specifically, in all studies, the prevalence of the prothrombin 20210A allele was found to be similar between cases and controls. Similarly, no significant association was observed in the stratified analysis of ethnicity either (**Table 4**). The symmetric funnel plot (**Figure 3B**) suggested there was no obvious publication bias and Egger's test also showed no statistical significance for the detection of publication bias (P=0.739).

Among the 14 studies we analyzed, eight studies [28-31, 48-51] detected the frequency of the prothrombin G20210A mutation in CD or UC separately. Regarding the relationship between the G20210A mutation and CD, overall, the allele contrast showed lack of heterogeneity between studies (I^2=7.8%, P=0.369) with the association being non-significant (OR=1.24, 95% CI 0.63-2.43). In investigating the association between G20210A and UC, overall, the allele contrast showed non-significant heterogeneity (I^2=0.0%, P=0.957,) and the risk effect was non-significant (OR=1.06, 95% CI 1.01-2.31). Subgroup analysis for Caucasians showed similar results to the overall analysis for both CD and UC.

Sensitivity analyses

When we performed a sensitivity analysis through leaving out one study at a time, the overall results were not materially affected (**Figure 5**). So, results of the sensitivity analyses indicated that our results are credible and stable.

Discussion

With the first reported of thromboembolism complicating IBD in 1936 by Bargen et al [1],

Table 4. Risk and P for heterogeneity of stratified analysis on prothrombin G20210A mutation and inflammatory bowel disease (IBD)

Category of studies	No. of studies	Summary OR (95% CI)	P for heterogeneity
Risk in all population	14	0.99 (0.65, 1.50)	0.785
Risk in Caucasian	11	0.98 (0.60, 1.59)	0.849
Risk in Turkish	3	0.78 (0.25, 2.48)	0.157
Risk in CD	8	1.24 (0.63, 2.43)	0.369
Risk in UC	8	1.06 (0.49, 2.31)	0.957
Risk in Thrombosis	3	0.93 (0.35, 2.51)	0.841

IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; OR, Odds ratio; CI, Confidence interval.

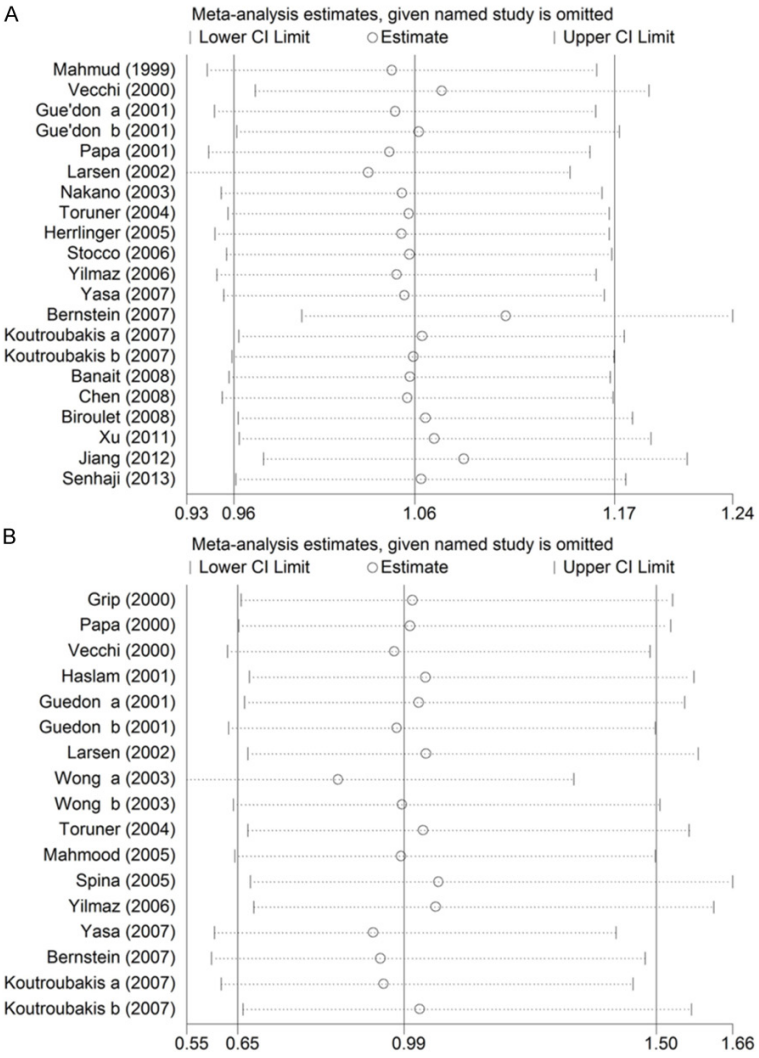

Figure 5. Sensitivity analysis of the association between IBD and MTHFR C677T (A) and prothrombin G20210A mutation (B). MTHFR indicates methylenetetrahydrofolate reductase; IBD, inflammatory bowel disease.

more evidence has proven that IBD is associated with venous thrombosis. The cause for this strong association has been unclear. Furthermore, it has been supposed that IBD may be a primary inflammatory vascular or thrombogenic disease and that gene mutations may underlie both the risk for venous thrombosis and the disease itself. Although the aetiology of IBD is currently unknown, it is well established that genetic predisposition plays a key role in IBD pathogenesis. Many studies examining for a gene in methionine-homocysteine metabolism and clotting factor gene mutations, which may be related to IBD.

MTHFR C677T polymorphism is the most common genetic cause of hyperhomocysteinemia. Due to the accumulation of homocysteine in the circulation, hyperhomocysteinaemia has became a well-known risk factor for venous thrombosis in the general population [53] and a potential contributor to the increased thrombotic risk in patients with IBD [54]. As a functional polymorphism linked to homocysteine metabolism, previous studies reported MTHFR 677TT carriers with lower plasma folate levels are at risk for increased plasma homocysteine levels [55, 56]. Upon Zintzaras failed in finding association of this polymorphism with IBD [16], recent meta-analysis [17] assessed the impact of MTHFR C677T polymorphism on IBD risk according to plasma folate concentration, they found the IBD risk associated with MTHFR 677TT genotype was significantly higher in patients with low folate status (OR=2.64; 95% CI, 1.44-4.81). Th-ereafter, Xu [43] and Jiang [44] continued examining the magnitude of association

between MTHFR C677T mutation and IBD respectively. Senhaji et al [45] reported that C677T mutation associated with a decrease in enzyme activity seemed not to be significantly associated with the primary risk of inflammatory bowel disease or with its phenotype in the Moroccan population. Although the overall analysis of our study showed the MTHFR C677T mutation was not significantly associated with an increased risk of IBD. Interestingly, the frequency of MTHFR C677T mutation depended on the geographical location and the ethnic background of the population. Therefore, we performed a subgroup analysis based on the different ethnicity. The nine studies enrolled participants from the Caucasians region, the pooled OR indicated that MTHFR C677T mutation was more common in patients with IBD than control subjects, suggesting a strong association between IBD and MTHFR C677T mutation in Caucasians and Turkish, so routine screening for MTHFR C677T mutation was potentially warranted in clinical practice in these populations. Limited by the data that presented the number of UC and CD subjects separately, we could not perform a stratified meta-analysis in all the 19 studies. Thus, we suggest that the risk for UC or CD separately with MTHFR C677T mutation is not equal to the risk for IBD in general.

On the other hand, as one of the most important hereditary prothrombotic factors in the general population, Presence of this mutation is found at a similar prevalence in IBD patients as well as in IBD patients with thrombosis. Our study did not result to be statistically significant associated with an elevated risk of patients with IBD, suggesting no association between IBD and prothrombin G20210A mutation. Several studies did not find prothrombin G20210A mutation in IBD cases [29, 46, 50] and healthy population [30, 51]. We speculate this difference may be caused due to environmental factors, and genetic changes can not play pathogenic roles individually in determining the risk of IBD. Result of this analysis may be due to the small number of included patients and the variation of geographic distribution. Therefore, our results should be interpreted with caution. In addition, further case-control studies should be actively performed to explore the relationship between prothrombin G20210A mutation and IBD in larger numbers.

Some investigators have tried to establish a correlation between the pathogenesis of the disease and thromboembolism (TE). Our study compared these two inherited mutations rates among two comparisons, one is IBD group without TE complications compared with control HC group, the other is IBD group with TE complications compared with control non-IBD group with TE complications. Two studies [32, 35] of MTHFR C677T mutation and three studies [32, 35, 47] of prothrombin G20210A mutation compared mutation rates among these two comparisons respectively. However, pooled estimates were all similar to the overall results.

To some extent, our meta-analysis has the following advantages: Firstly, this is the first meta-analysis to investigate the association between the prothrombin G20210A mutation and IBD susceptibility. Secondly, we had a strict selection and exclusion criteria and all the included articles possessed a high quality. Thirdly, subgroup analyses based on ethnicity and type of disease were conducted respectively to comprehend the association thoroughly. Fourthly, there was no heterogeneity exist in the present analysis. Finally, we executed sensitivity analysis to evaluate stabilization of pooled data, results suggested that the conclusion of this meta-analysis were statistically robust.

Every coin has its two sides, some limitations of our study need to be taken into consideration which resulting from the availability, quality, and heterogeneity of the published data. Firstly, due to the limited information in the included studies we were unable to obtain sufficient data to perform appropriate stratified analyses, such as gender, age, smoking status, clinical phenotype, et al. Secondly, the majority of the studies that examined the relationship between prothrombin G20210A mutation and IBD were conducted before 2007, so there might be a bias and our results should be extrapolated with caution. Thirdly, significant publication bias was detected in this meta-analysis which might be the results of null studies tend not to be easier to publish and this might had the potential to influence the results of this meta-analysis. Fourthly, there were only two studies that examined the relationship between MTHFR C677T and child-onset IBD. As a result, the small sample size available was not ideal for detecting small genetic effects. Finally, we

could not identify the gene-gene and gene-environment interactions in this study.

In conclusion, this meta-analysis demonstrated that IBD patients had higher risk of than controls. MTHFR C677T mutation was significantly associated with the risk of IBD in Caucasian and Turkish population. However, no significant association was found between IBD and prothrombin G20210A mutation. Nevertheless, large-scale well designed studies are necessary to be conducted to further confirm or refute the observed association.

Disclosure of conflict of interest

None.

Address correspondence to: Sujuan Fei, Department of Gastroenterology, The Affiliated Hospital of Xuzhou Medical University, 99 West Huaihai Road, Xuzhou 221000, Jiangsu, China. Tel: 86-139522-96622; Fax: 86-051683262292; E-mail: xyfyfeisj99@163.com

References

[1] Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW and Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology 2012; 142: 46-54, e42; quiz e30.

[2] Stone CD. The economic burden of inflammatory bowel disease: clear problem, unclear solution. Dig Dis Sci 2012; 57: 3042-3044.

[3] Baumgart DC and Carding SR. Inflammatory bowel disease: cause and immunobiology. Lancet 2007; 369: 1627-1640.

[4] Solem CA, Loftus EV, Tremaine WJ and Sandborn WJ. Venous thromboembolism in inflammatory bowel disease. Am J Gastroenterol 2004; 99: 97-101.

[5] Bernstein CN, Blanchard JF, Houston DS and Wajda A. The incidence of deep venous thrombosis and pulmonary embolism among patients with inflammatory bowel disease: a population-based cohort study. Thromb Haemost 2001; 85: 430-434.

[6] Koutroubakis IE. Therapy insight: vascular complications in patients with inflammatory bowel disease. Nat Clin Pract Gastroenterol Hepatol 2005; 2: 266-272.

[7] Miehsler W, Reinisch W, Valic E, Osterode W, Tillinger W, Feichtenschlager T, Grisar J, Machold K, Scholz S, Vogelsang H and Novacek G. Is inflammatory bowel disease an independent and disease specific risk factor for thromboembolism? Gut 2004; 53: 542-548.

[8] Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA and Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369: 64-67.

[9] Segers K, Dahlback B and Nicolaes GA. Coagulation factor V and thrombophilia: background and mechanisms. Thromb Haemost 2007; 98: 530-542.

[10] Couturaud F, Kearon C, Leroyer C, Mercier B, Abgrall JF, Le Gal G, Lacut K, Oger E, Bressollette L, Ferec C, Lamure M and Mottier D. Incidence of venous thromboembolism in first-degree relatives of patients with venous thromboembolism who have factor V Leiden. Thromb Haemost 2006; 96: 744-749.

[11] Liang J, Wu S, Feng B, Lei S, Luo G, Wang J, Li K, Li X, Xie H, Zhang D, Wang X, Wu K, Miao D and Fan D. Factor V Leiden and inflammatory bowel disease: a systematic review and meta-analysis. J Gastroenterol 2011; 46: 1158-1166.

[12] Zhong M, Dong XW, Zheng Q, Tong JL and Ran ZH. Factor V Leiden and thrombosis in patients with inflammatory bowel disease (IBD): a meta-analysis. Thromb Res 2011; 128: 403-409.

[13] Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995; 10: 111-113.

[14] Den Heijer M, Lewington S and Clarke R. Homocysteine, MTHFR and risk of venous thrombosis: a meta-analysis of published epidemiological studies. J Thromb Haemost 2005; 3: 292-299.

[15] Jang MJ, Jeon YJ, Choi WI, Choi YS, Kim SY, Chong SY, Oh D and Kim NK. The 677C>T mutation of the MTHFR gene increases the risk of venous thromboembolism in Koreans and a meta-analysis from Asian population. Clin Appl Thromb Hemost 2013; 19: 309-314.

[16] 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.

[17] Oussalah A, Gueant JL and Peyrin-Biroulet L. Meta-analysis: hyperhomocysteinaemia in inflammatory bowel diseases. Aliment Pharmacol Ther 2011; 34: 1173-1184.

[18] Poort SR, Rosendaal FR, Reitsma PH and Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996; 88: 3698-3703.

[19] Simone B, De Stefano V, Leoncini E, Zacho J, Martinelli I, Emmerich J, Rossi E, Folsom AR, Almawi WY, Scarabin PY, den Heijer M, Cushman M, Penco S, Vaya A, Angchaisuksiri P, Okumus G, Gemmati D, Cima S, Akar N, Oguzulgen KI, Ducros V, Lichy C, Fernandez-Miranda C, Szczeklik A, Nieto JA, Torres JD, Le Cam-Duchez V, Ivanov P, Cantu-Brito C, Shmeleva VM, Stegnar M, Ogunyemi D, Eid SS, Nicolotti N, De Feo E, Ricciardi W and Boccia S. Risk of venous thromboembolism associated with single and combined effects of factor V leiden, prothrombin 20210A and methylenetethraydrofolate reductase C677T: a meta-analysis involving over 11,000 cases and 21,000 controls. Eur J Epidemiol 2013; 28: 621-647.

[20] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010; 25: 603-605.

[21] Hu J, Dong Y, Chen X, Liu Y, Ma D, Liu X, Zheng R, Mao X, Chen T and He W. Prevalence of suicide attempts among Chinese adolescents: a meta-analysis of cross-sectional studies. Compr Psychiatry 2015; 61: 78-89.

[22] Higgins JP and Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002; 21: 1539-1558.

[23] Mantel N and Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959; 22: 719-748.

[24] DerSimonian R and Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7: 177-188.

[25] Begg CB and Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994; 50: 1088-1101.

[26] Egger M, Davey Smith G, Schneider M and Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315: 629-634.

[27] Mahmud N, Molloy A, McPartlin J, Corbally R, Whitehead AS, Scott JM and Weir DG. Increased prevalence of methylenetetrahydrofolate reductase C677T variant in patients with inflammatory bowel disease, and its clinical implications. Gut 1999; 45: 389-394.

[28] Vecchi M, Sacchi E, Saibeni S, Meucci G, Tagliabue L, Duca F and De Franchis R. Inflammatory bowel diseases are not associated with major hereditary conditions predisposing to thrombosis. Dig Dis Sci 2000; 45: 1465-1469.

[29] Toruner M, Erkan O, Soykan I, Bozdayi M, Cetinkaya H, Yurdaydin C, Uzunalimoglu O and Ozden A. Factor V Leiden, prothrombin G20-210A and MTHFR gene mutations in inflammatory bowel disease. Turk J Gastroenterol 2004; 15: 250-252.

[30] Yasa MH, Bolaman Z, Yukselen V, Kadikoylu G, Karaoglul AO and Batun S. Factor V Leiden G1691A, prothrombin G20210A, and MTHFR C677T mutations in Turkish inflammatory bowel disease patients. Hepatogastroenterology 2007; 54: 1438-1442.

[31] Bernstein CN, Sargent M, Vos HL and Rosendaal FR. Mutations in clotting factors and inflammatory bowel disease. Am J Gastroenterol 2007; 102: 338-343.

[32] Guedon C, Le Cam-Duchez V, Lalaude O, Menard JF, Lerebours E and Borg JY. 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: 1448-1454.

[33] Bjerregaard LT, Nederby NJ, Fredholm L, Brandslund I, Munkholm P and Hey H. Hyperhomocysteinaemia, coagulation pathway activation and thrombophilia in patients with inflammatory bowel disease. Scand J Gastroenterol 2002; 37: 62-67.

[34] Yilmaz S, Bayan K, Tuzun Y, Batun S and Altintas A. A comprehensive analysis of 12 thrombophilic mutations and related parameters in patients with inflammatory bowel disease: data from Turkey. J Thromb Thrombolysis 2006; 22: 205-212.

[35] Koutroubakis IE, Sfiridaki A, Tsiolakidou G, Theodoropoulou A, Livadiotaki A, Paspatis G and Kouroumalis EA. Genetic risk factors in patients with inflammatory bowel disease and vascular complications: case-control study. Inflamm Bowel Dis 2007; 13: 410-415.

[36] Papa A, De Stefano V, Danese S, Chiusolo P, Persichilli S, Casorelli I, Zappacosta B, Giardina B, Gasbarrini A, Leone G and Gasbarrini G. Hyperhomocysteinemia and prevalence of polymorphisms of homocysteine metabolism-related enzymes in patients with inflammatory bowel disease. Am J Gastroenterol 2001; 96: 2677-2682.

[37] Nakano E, Taylor CJ, Chada L, McGaw J and Powers HJ. Hyperhomocystinemia in children with inflammatory bowel disease. J Pediatr Gastroenterol Nutr 2003; 37: 586-590.

[38] Herrlinger KR, Cummings JR, Barnardo MC, Schwab M, Ahmad T and Jewell DP. The pharmacogenetics of methotrexate in inflammatory bowel disease. Pharmacogenet Genomics 2005; 15: 705-711.

[39] Stocco G, Martelossi S, Sartor F, Toffoli G, Lionetti P, Barabino A, Fontana M, Decorti G, Bartoli F, Giraldi T and Ventura A. Prevalence of methylenetetrahydrofolate reductase polymorphisms in young patients with inflammatory bowel disease. Dig Dis Sci 2006; 51: 474-479.

[40] Banait VS, Sandeep MS, Shetty S, Bapat MR, Rathi PM, Ghosh K, Mohanty D and Abraham

P. Hypercoagulable state in idiopathic ulcerative colitis: role of hyperhomocysteinemia and hyperfibrinogenemia. Indian J Gastroenterol 2008; 27: 94-98.
[41] Chen M, Peyrin-Biroulet L, Xia B, Gueant-Rodriguez RM, Bronowicki JP, Bigard MA and Gueant JL. Methionine synthase A2756G polymorphism may predict ulcerative colitis and methylenetetrahydrofolate reductase C677T pancolitis, in central China. BMC Med Genet 2008; 9: 78.
[42] Peyrin-Biroulet L, Gueant-Rodriguez RM, Chen M, Bronowicki JP, Bigard MA and Gueant JL. Association of MTRR 66A>G polymorphism with superoxide dismutase and disease activity in patients with Crohn's disease. Am J Gastroenterol 2008; 103: 399-406.
[43] Xu CL, Lin XQ, Lan DY, Wang JZ, Zheng B and Xue ZX. [The associations of methylenetetrahydrofolate reductase gene C677T and A1298C polymorphisms and ulcerative colitis]. Zhonghua Nei Ke Za Zhi 2011; 50: 374-377.
[44] Jiang Y, Xia X, Wang W, Lin L, Xu C, Cai Z, Zheng B, Pei J, Shen S and Xia B. Hyperhomocysteinemia and related genetic polymorphisms correlate with ulcerative colitis in Chinese Han population in central China [corrected]. Cell Biochem Biophys 2012; 62: 203-210.
[45] Senhaji N, Serbati N, Diakite B, Arazzakou S, Hamzi K, Badre W and Nadifi S. Methylenetetrahydrofolate reductase C677T variant in Moroccan patients with inflammatory bowel disease. Gene 2013; 521: 45-49.
[46] Grip O, Svensson PJ and Lindgren S. Inflammatory bowel disease promotes venous thrombosis earlier in life. Scand J Gastroenterol 2000; 35: 619-623.
[47] Wong T, Nightingale J, Winter M and Muller AF. Spontaneous venous thrombosis in inflammatory bowel disease: relevance of factor V Leiden and the prothrombin gene mutation. J Thromb Haemost 2003; 1: 1326-1328.
[48] Spina L, Saibeni S, Battaglioli T, Peyvandi F, de Franchis R and Vecchi M. Thrombosis in inflammatory bowel diseases: role of inherited thrombophilia. Am J Gastroenterol 2005; 100: 2036-2041.
[49] Papa A, De Stefano V, Gasbarrini A, Chiusolo P, Cianci R, Casorelli I, Paciaroni K, Cammarota G, Leone G and Gasbarrini G. Prevalence of factor V Leiden and the G20210A prothrombin-gene mutation in inflammatory bowel disease. Blood Coagul Fibrinolysis 2000; 11: 499-503.
[50] Haslam N, Standen GR and Probert CS. An investigation of the association of the prothrombin G20210A gene mutation and inflammatory bowel disease: factor II and IBD. Inflamm Bowel Dis 2001; 7: 133-135.
[51] Mahmood A, Needham J, Prosser J, Mainwaring J, Trebble T, Mahy G and Ramage J. Prevalence of hyperhomocysteinaemia, activated protein C resistance and prothrombin gene mutation in inflammatory bowel disease. Eur J Gastroenterol Hepatol 2005; 17: 739-744.
[52] Basu D, Lopez I, Kulkarni A and Sellin JH. Impact of race and ethnicity on inflammatory bowel disease. Am J Gastroenterol 2005; 100: 2254-2261.
[53] Ray JG. Meta-analysis of hyperhomocysteinemia as a risk factor for venous thromboembolic disease. Arch Intern Med 1998; 158: 2101-2106.
[54] Peyrin-Biroulet L, Rodriguez-Gueant RM, Chamaillard M, Desreumaux P, Xia B, Bronowicki JP, Bigard MA and Gueant JL. Vascular and cellular stress in inflammatory bowel disease: revisiting the role of homocysteine. Am J Gastroenterol 2007; 102: 1108-1115.
[55] Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J and Rozen R. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 1996; 93: 7-9.
[56] Gueant JL, Gueant-Rodriguez RM, Anello G, Bosco P, Brunaud L, Romano C, Ferri R, Romano A, Candito M and Namour B. Genetic determinants of folate and vitamin B12 metabolism: a common pathway in neural tube defect and down syndrome? Clin Chem Lab Med 2003; 41: 1473-1477.