J Gastrointest Canc (2016) 47:55–60
DOI 10.1007/s12029-015-9794-0

ORIGINAL RESEARCH

Association of Methylenetetrahydrafolate Reductase Gene Polymorphism (MTHFR) in Patients with Gallbladder Cancer

Ruhi Dixit[1] · Gyanendra Singh[1] · Manoj Pandey[2] · Somprakas Basu[1] · Satyanam Kumar Bhartiya[1] · K. K. Singh[3] · Vijay Kumar Shukla[1]

Published online: 19 December 2015

Abstract
Purpose 5,10-Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism and plays a major role in DNA methylation. There are two popular MTHFR polymorphisms known as C677T and A1298C which are found to be involved in folate metabolism and lowering the enzyme activity, thus may be linked with cancer development. This study aims to look at the association of these polymorphisms in gallbladder cancer.
Methods Thirty patients each with gallbladder cancer, cholelithiasis, and normal gallbladder were genotyped for the above-given polymorphisms by PCR-restriction fragment length polymorphism (RFLP) method.
Results C677T MTHFR polymorphism was not associated ($\chi^2=2.44$, $p=0.85$) with an increased likelihood of having gallbladder cancer. A1298C was significantly associated ($\chi^2=28.87$, $p<0.001$) with risk of developing gallbladder cancer. A1298C was significantly correlated with grade ($r=0.337$, $p<0.001$) and histopathology ($r=0.446$, $p<0.001$).
Conclusion This study proposed that MTHFR A1298C polymorphism may be associated with risk of developing gallbladder cancer, and there is no association between C677T polymorphism and gallbladder cancer.

✉ Vijay Kumar Shukla
vkshuklabhu@gmail.com

[1] Department of General Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005, India

[2] Department of Surgical Oncology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

[3] Department of Statistics, Faculty of Science, Banaras Hindu University, Varanasi, India

Keywords 5,10-Methylenetetrahydrofolate reductase (MTHFR) · C677T · A1298C · Gallbladder cancer · Restriction fragment length polymorphism (RFLP)

Introduction

Gallbladder cancer (GBC) is usually the most prevalent malignancy in the biliary tract, which represents 80–95 % associated with biliary tract cancer around the world [1, 2]. GBC is the fifth most commonly occurring gastrointestinal cancer in the USA [3]. The worldwide rate regarding GBC malignancy exhibits variations, achieving epidemic levels for a few regions and also ethnicities. GBC carries an especially high occurrence in Chile, Japan, and also north India. Incidence of GBC is higher in north India as compared to south India [4–6]. Exact cause of etiology of GBC is still obscure. Various associated risk factors have been identified. Limited information is available related to genetic changes involved in GBC. The precise sequence associated with molecular alteration underlying GBC pathogenesis stays ambiguous. These genetic determinants within gallbladder carcinogenesis are generally inadequately identified, irrespective of around 1281 gene mutations that have already been discovered up to now [2].

The human 5,10-methylenetetrahydrofolate reductase (MTHFR) gene is located at chromosomal region 1p36.3 and consists of 11 exons spanning 2.2 kb [7, 8]. MTHFRis an important regulatory enzyme in the metabolism of homocysteine and folate [9] which is essential for DNA synthesis. It works as a key enzyme in the methylation process catalyzing reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Reduction in MTHFR activity increases the accumulation of 5,10-methylenetetrahydrofolate; this change may lead to the overall level of DNA damage in the cell [10]. Thus, folate deficiency induces chromosomal damage,

formation of fragile sites, and found to be associated with tumorigenesis [11]. Deficiency of folate has been reported in general Indian population which may be an additional dietary factor for high risk of GBC in this population [12]. Changes in its activity resulting from polymorphism in the MTHFR could modify interindividual susceptibility to cancer. MTHFR is highly polymorphic in the general population. The two most common single nucleotide polymorphisms (SNPs), C677T and A1298C, have been identified [7, 13]. It has been reported that two single nucleotide polymorphisms (SNPs) of MTHFR gene, C677T (rs1801133 and Ala222Val), which results in the amino acid product changing from alanine to valine [7, 13], and A1298C (rs1801131 and Glu429Ala), which results in the amino acid product changing from glutamic acid to alanine [14] have been linked to the reduced enzyme activity [15]. The second common MTHFR polymorphism, a glutamate to alanine (A3 C) change at position 1298, also influences the specific activity of the enzyme, homocysteine levels, and plasma folate concentration [14].

It has been suggested that MTHFR C677T polymorphism and A1298C modulated the risk of developing various cancers like colorectal, gastric, and endometrial as well as leukemia [16–21]. However, the association involving these two frequent occurring MTHFR polymorphisms and the risk of developing GBC has not been looked at to our best knowledge. Thus, the aim of this work is to study the C677T and A1298C polymorphisms of the 5,10-MTHFR gene in patients with gallbladder cancer and to co-relate with various clinicopathological parameters.

Material and Methods

Subjects

Thirty patients of histopathologically proven GBC as cases, 30 cases of gallstones, and 30 cases of normal healthy control were recruited for study; 5 ml blood was collected in heparinized syringes from cases and controls. Samples were collected from the department of general surgery, and work was performed in the department of surgical oncology laboratory. Patients having diabetes, cardiovascular, renal and venous thrombotic diseases were excluded in the study. This study was ethically approved by the institute's ethics committee. Informed consent was obtained from all individual participants included in the study.

Polymerase Chain Reaction

DNA isolation from blood had been performed by using phenol-chloroform method [22]. Polymerase chain reaction had been performed by using primer for MTHFR A1298C [13] and C677T [23]. PCR program for C677T was 95 °C for 2 min, 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s followed by 35 cycles and final extension at 72 °C for 10 min. While PCR program for A1298C was initial denaturation at 92 °C for 5 min, denaturation at 92 °C for 60 s, annealing at 52 °C for 60 s, and extension at 72 °C for 30 s followed by 35 cycles and final extension at 72 °C for 7 min.

Restriction Fragment Length Polymorphism

Genotyping was performed by restriction fragment length polymorphism (RFLP) method. MTHFR C677T was detected by digestion with HinfI (NEB) restriction enzyme in a total reaction volume of 20 μl and incubated at 37 °C for 1 h. It produced 175 and 23 bp fragments for homozygous condition (TT) and 198, 175, and 23 bp fragments for heterozygous condition (CT). While there was no restriction site for wild type, a 198-bp band was retained.

MboII restriction enzyme was used for A1298C MTHFR polymorphism detection, and digestion was performed in a total reaction volume of 20 μl and incubated at 37 °C for 1 h. It produced six fragments of 84, 56, 31, 30, 28, and 18 bp for 1298AC heterozygous type, five fragments for 56, 31, 30, 28, and 18 bp for 1298CC homozygous type, and four fragments of 84, 31, 30, and 18 bp for 1298 AA genotype.

Statistical Analysis

Statistical analysis had been performed by using SPSS (version 16.0, Chicago, IL, USA) software. Allele frequency has been calculated for each genotype under the assumption of Hardy-Weinberg equilibrium. The association between GBC and MTHFR polymorphism were analyzed by determining the odds ratio (OR) and confidence interval (CI) by doing univariate logistic regression. Correlation of both polymorphisms with different clinicoparameters was calculated by Pearson's correlation test.

Results

The clinical characteristics of all the groups are listed in Table 1. The mean age of GBC, cholelithiasis, and healthy control were 56 ± 14, 44 ± 13, and 44 ± 13 years, respectively. Most of the patients were females in our study group. Female: male ratio was 6.5:1 in GBC group; 93.7 % GBC patients were of adenocarcinoma histopathology and 6.7 % cases of squamous cell carcinoma. Gallstones were present in the 63.3 % cases of gallbladder cancer. Of those, 16.7% were single and 46.7% multiple stones (Table 1).

The distribution of C677T and A1298C genotypes in all the three groups is shown in Table 2. No statistically significant differences were noted among MTHFR 677CC, CT, and TT genotypes ($p = 0.85$). Genotype frequency of A1298C

Table 1 Clinical characteristic of GBC patients, cholelithiasis and healthy control

Variable	Gallbladder cancer	Cholelithiasis	Healthy control
Age			
Mean ± SD	56 ± 14 years	44 ± 13 years	44 ± 13 years
Gender			
Female	26 (86.7 %)	20 (66.7 %)	21 (70 %)
Male	4 (13.4 %)	10 (33.3 %)	9 (30 %)
HPE			
Adenocarcinoma	28 (93.3 %)	–	–
Squamous cell carcinoma	2 (6.7 %)		
Stage			
I	0 (0)	-	–
II	9 (30)		
III	9 (30)		
IV	12 (40)		
Gallstone present	19 (63.3 %)	30 (100 %)	
Single stone	5 (16.7 %)	8 (26.7 %)	
Multiple stone	14 (46.7 %)	22 (73.3 %)	

Table 3 Distribution of A1298C MTHFR polymorphism with presence of gallstone in gallbladder cancer patients

A1298C MTHFR polymorphism	Gallstone present	Total
A1298C		
CC	2 (100.0 %)	2
AC	15 (60.0 %)	25
AA	2 (66.7 %)	3
Total	19	30

polymorphism with AC, CC, and AA has showed a statistically significant difference among the groups ($p < 0.001$). AC genotype was present in 60% of gallbladder cancer cases in which gallstones were present. While both CC and AA genotypes were present in only two cases of gallbladder cancer (Table 3).

Univariate logistic regression analysis has been performed to check the association of C677T and A1298C polymorphism among the three groups (gallbladder cancer, cholelithiasis, and normal control). When GBC has been assumed as case and normal healthy volunteer as control, A1298C was found to be highly significant ($p < 0.001$) while C677T was not significant ($p = 1.0$). In another case, when cholelithiasis group has been considered as case and healthy volunteer as control, then no statistically significant difference was found ($p = 1.0$), whereas A198C have exhibited a statistically significant difference (AC—CI 6.5, $p < 0.001$). Similarly, when GBC was taken as case and cholelithiasis patients as control group, then again, C677T did not show any significant difference in all the genotypes ($p = 1.0$) and a statistically significant difference was observed in A1298C polymorphism (AC—CI 4.902, $p < 0.05$) (Table 4).

Correlation between the MTHFR polymorphism of cases and controls with different factors has been evaluated. Overall, there was no correlation between the C677T with any of the parameters. A1298C MTHFR genotype was significantly correlated with grade ($r = 0.337$; $p < 0.001$) and histopathology ($r = 0.466$; $p < 0.001$) of the patients (Table 5).

Discussion

In this study, genotype frequencies for MTHFR C677T polymorphism did not differ between cancer cases and controls ($\chi^2 = 2.44$; $p = 0.85$). We were not able to find any evidence for the existence of this gene effect. The overall genotype difference was not statistically significant. Thus, we did not find any association of C677T polymorphism in GBC with different parameters. We observed no major significant association of the MTHFR C677T polymorphism with GBC risk. A single study done by Srivastava et al. also concluded that genetic risk for GBC is not modulated by MTHFR C677T polymorphism [24]. They inferred from their results that MTHFR C677T polymorphism is neither a risk nor a

Table 2 Comparison of MTHFR C677T and A1298C genotypes between GBC, cholelithiasis, and control

Polymorphism	Gallbladder cancer	Cholelithiasis	Healthy control	χ^2 value	*p* value
C677T					
CT	6 (20 %)	5 (16.7 %)	7 (23.3 %)	2.44	0.85
CC	23 (76.7 %)	25 (83.3 %)	23 (76.3 %)		
TT	1 (3.3 %)	0 (0)	0 (0)		
A1298C					
AC	25 (83.3 %)	18 (60 %)	6 (20 %)	28.87	<0.001*
CC	2 (6.7 %)	2 (6.7 %)	1 (3.3 %)		
AA	3 (10 %)	10 (33.3 %)	23 (76.7 %)		

*Significance level)

Table 4 Univariate logistic regression analysis to look at the association between C677T and A1298C polymorphism and gallbladder cancer

Polymorphism	OR (CI)	*p* value
(I) When gallbladder cancer (GBC) have been taken as cases and healthy volunteers as control group		
a. C677T		
CC	0 (0–0)	1.0
CT	0 (0–0)	1.0
Reference category—TT; the difference is not statistically significant		
b. A1298C		
CC	15.333 (1.046–224.779)	<0.05*
AC	31.944 (7.148–142.755)	<0.001*
Reference category—AA; the difference is statistically significant		
(II) When cholelithiasis have been taken as cases and healthy volunteers as control group		
a. C677T		
CT	0.685 (0.190–2.468)	0.562
Reference category—TT; the difference is not statistically significant		
b. A1298C		
CC	4.600 (0.373–56.752)	0.234
AC	6.517 (1.981–21.432)	<0.001*
Reference category—AA; thus, the value is significant		
(III) When gallbladder cancer (GBC) have taken as cases and cholelithiasis as control group		
a. C677T		
CC	0 (0–0)	1.0
CT	0 (0–0)	1.0
Reference category—TT; the difference is not statistically significant		
b. A1298C		
CC	3.333 (0.319–34.830)	0.315
AC	4.902 (1.173–20.479)	<0.05*
Reference category—AA; thus, the value is significant		

*Significance level)

Table 5 Correlation of C677T and A1298C polymorphism with different clinicopathological factors

Clinicopathological factors	C677T	A1298C
Age		
Pearson correlation	0.195	−0.127
p value	0.065	0.233
N	90	90
Group		
Pearson correlation	−0.031	0.492*
p value	0.773	<0.001
N	90	90
Grade		
Pearson correlation	−0.030	0.337*
p value	0.777	<0.001
N	90	90
Histopathology		
Pearson correlation	−0.030	0.466*
p value	0.77	<0.001
N	90	90
Stage		
Pearson correlation	0.156	−0.217
p value	0.410	0.249
N	30	30

*Significance level

protective factor for GBC, suggesting that MTHFR genetic variation does not affect the susceptibility for gallbladder tumorigenesis [23]. Other study also reported that the C677T MTHFR polymorphism did not significantly contribute to the inherited genetic susceptibility to breast and prostate cancer, while they got some evidence for possible genetic contribution of MTHFR polymorphism to the development of head and neck carcinoma [25]. It has already been reported that frequency of C677T polymorphism is low in Indian population [26]. A preliminary study has been done which suggests that individuals with the TT genotype have a decreased risk of adult acute leukemia and increased risk of endometrial cancer, cervical neoplasia, and breast cancer [27]. A recent meta-analysis also suggested a significant association between thyroid cancer and MTHFR C677T polymorphism [28]. Another study performed by Yilmaz et al. suggested no association of MTHFR C677T with lung cancer among the Turkish population [29]. Some other case control studies found to have increased risk for other cancers including breast [30, 31], colorectal [32], lung [33], and head and neck [25].

To the best of our knowledge, this is the first report to examine the association between the MTHFR A1298C gene polymorphisms and risk of gallbladder cancer. In our study, the frequencies of the MTHFR 1298CC, 1298AC, and 1298AT genotypes were 6.7, 83.3, and 10 % in GBC group ($\chi^2 = 28.87$; $p < 0.001$). AC genotype was found unusually high in comparison to control and was highly significant. Only 20 % distribution of AC was observed in control while it was elevated to 60 % in cholelithiasis and even higher in GBC (83.3%). Moreover, the distribution of AC genotype was also prominent in GBC cases in which gallstones were present. Out of 19 GBC samples with gallstones, 15 samples had AC genotype suggesting an overlapping role of AC genotype in GBC and development of gallstones. To further understand the role of this polymorphism in bridging GBC and gallstone development, univariate logistic regression analysis was performed. Firstly, when GBC was taken as cases and normal gallbladder as control, AC genotype in A1298C MTHFR polymorphism showed almost 33 times higher risk of development of GBC than control ($p < 0.001$; OR = 31.944) while CC genotype had 15 times higher risk ($p < 0.05$; OR = 15.333). Secondly, when cholelithiasis have taken as cases and normal gallbladder as control group, AC genotype showed a significantly higher risk of

development of cholelithiasis ($p < 0.001$; OR = 6.516). Thereby, it signifies the role of this genotype in this polymorphism both in the development of GBC and cholelithiasis. Finally, when GBC was taken as case and cholelithiasis as control, then AC genotype of this polymorphism showed significantly related to the risk of development of GBC than cholelithiasis ($p < 0.05$; OR = 4.902). Thus, we can conclude that cholelithiasis sample with AC genotype of A1298C polymorphism is at a significantly higher risk of development of GBC. In addition, A1298C polymorphism was significantly correlated with the grade ($r = 0.337$, $p < 0.001$) and histopathology ($r = 0.446$, $p < 0.001$). Thus, we can also suggest here that A1298C polymorphism is contributing some risk to the development of gallbladder cancer. Nair et al. (2013) conducted a prospective case-control study to see the genetic association between methylenetetrahydrofolate reductase (MTHFR) A1298C polymorphism and recurrent pregnancy loss (RPL). In conclusion, they suggested that a significant risk of pregnancy loss associated with MTHFR A1298C polymorphism such that the presence of rare allele C and genotypes AC and CC significantly increases the risk of pregnancy loss [34, 35]. Zhang et al. also tried to see whether MTHFR C677T and A1298C polymorphisms could be associated with the sex-specific overall survival (OS) in patients with metastatic colon cancer treated with FU-based chemotherapy. They suggested the role of A1298C polymorphism in MTHFR as a prognostic marker in female patients with metastatic colon cancer [36]. Interestingly, others also concluded that A1298C polymorphism may be a predictor of colon cancer risk and have functional relevance [37]. A report also deduced that C677T and A1298C are not associated with the risk of developing lung cancer, with and without dietary folate intake [19]. Our findings support that the MTHFR A1298C polymorphism may be as relevant in predicting GBC but further studies are needed.

In conclusion, our study suggests that increased frequency of the A1298C MTHFR gene polymorphism observed may be associated with risk of developing GBC. Further research is needed to establish the relationship proposed by the present results. However, we did not find any association with C677T and gallbladder cancer.

Acknowledgments We would like to thank all the patients and healthy volunteers who agreed to participate in the study.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

Funding This study was funded by Departmental Research Grant.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Lazcano-Ponce EC, Miquel JF, Muñoz N, Herrero R, Ferrecio C, Wistuba II, et al. Epidemiology and molecular pathology of gallbladder cancer. CA Cancer J Clin. 2001;51:349–64.
2. Hundal R, Shaffer EA. Gallbladder cancer: epidemiology and outcome. Clin Epidemiol. 2014;7(6):99–109.
3. Shaffer EA. Gallbladder cancer: the basics. Gastroenterol Hepatol (N Y). 2008;4:737–41.
4. Randi G, Franceschi S, La Vecchia C. Gallbladder cancer worldwide: geographical distribution and risk factors. Int J Cancer. 2006;118:1591–602.
5. Randi G, Malvezzi M, Levi F, Ferlay J, Negri E, Franceschi S, et al. Epidemiology of biliary tract cancers: an update. Ann Oncol. 2009;20:146–59.
6. Unisa S, Jagannath P, Dhir V, Khandelwal C, Sarangi L, Roy TK. Population-based study to estimate prevalence and determine risk factors of gallbladder diseases in the rural Gangetic basin of North India. HPB (Oxford). 2011;13:117–25.
7. Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG, et al. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping, and mutation identification. Nat Genet. 1994;7:551–4.
8. Goyette P, Pai A, Milos R, Frosst P, Tran P, Chen Z, et al. Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). Mamm Genome. 1998;9:652–6.
9. Huh HJ, Chi HS, Shim EH, Jang S, Park CJ. Gene–nutrition interactions in coronary artery disease: correlation between the MTHFR C677T polymorphism and folate and homocysteine status in a Korean population. Thromb Res. 2006;117:501–6.
10. Qin YT, Zhang Y, Wu F, Su Y, Lu GN, Wang RS. Association between MTHFR polymorphisms and acute myeloid leukemia risk: a meta-analysis. PLoS One. 2014;9:e88823. doi:10.1371/journal.pone.0088823.
11. Krajinovic M, Lamothe S, Labuda D, Lemieux-Blanchard E, Theoret Y, Moghrabi A, et al. Role of MTHFR genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia. Blood. 2004;103:252–7.
12. Yajnik CS, Deshpande SS, Lubree HG, Naik SS, Bhat DS, Uradey BS, et al. Vitamin B12 deficiency and hyperhomocysteinemia in rural and urban Indians. J Assoc Physicians India. 2006;54:775–82.
13. van der Put NM, Gabreëls F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet. 1998;62:1044–51.
14. Stern LL, Mason JB, Selhub J, Choi SW. Genomic DNA methylation, a characteristic of most cancers, is present in peripheral leucocytes of individuals who are homozygous for the C677T polymorphism in the methylene tetrahydrofolate reductase gene. Cancer Epidemiol Biomark Prev. 2000;9:849–53.
15. Zhong S, Chen Z, Yu X, Li W, Tang J, Zhao J. A meta-analysis of genotypes and haplotypes of methylenetetrahydrofolate reductase gene polymorphisms in breast cancer. Mol Biol Rep. 2014;41:5775–85.
16. Chen J, Giovannucci E, Kelsey K, Rimm EB, Stampfer MJ, Colditz GA, et al. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. Cancer Res. 1996;56:4862–4.
17. Esteller M, Garcia A, Martinez-Palones JM, Xercavins J, Reventos J. Germ line polymorphisms in cytochrome-P450 1A1 (C4887 CYP1A1) and methylenetetrahydrofolate reductase (MTHFR) genes and endometrial cancer susceptibility. Carcinogenesis. 1997;18:2307–11.
18. Houlston RS, Tomlinson IP. Polymorphisms and colorectal tumour risk. Gastroenterology. 2001;121:282–301.

19. Shen H, Spitz MR, Wang LE, Hong WK, Wei Q. Polymorphism of methylenetetrahydrofolate reductase and risk of lung cancer: a case control study. Cancer Epidemiol Biomarkers Prev. 2001;10: 397–401.
20. Wiemels JL, Smith RN, Taylor GM, Eden OB, Alexander FE, Greaves MF, et al. Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk of molecularly defined subtypes of childhood acute leukemia. Proc Natl Acad Sci U S A. 2001;98:4004–9.
21. Paz MF, Avila S, Fraga MF, Pollan M, Capella G, Peinado MA, et al. Germ-line variants in methyl-group metabolism genes and susceptibility to DNA methylation in normal tissues and human primary tumors. Cancer Res. 2002;62:4519–24.
22. Sambrook J, Russell DW. Preparation and analysis of eukaryotic genomic DNA. In: Sambrook J, Russell DW, editors. Molecular cloning: a laboratory manual. 3rd ed. New York: Cold Spring Harbor Laboratory Press, Cold Spring Harbor; 2001. p. 6.4–6.11.
23. James SJ, Pogribna M, Pogribny IP, Melnyk S, Hine RJ, Gibson JB, et al. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. Am J Clin Nutr. 1999;70:495–501.
24. Srivastava A, Pandey SN, Pandey P, Choudhuri G, Mittal B. No association of methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism in susceptibility to gallbladder cancer. DNA And Cell Biology. 2008;3:127–32.
25. Reljic A, Simundic AM, Topic E, Nikolac N, Justinic D, Stefanovic M. The methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and cancer risk: the Croatian case–control study. Clin Biochem. 2007;40:981–5.
26. Kumar J, Das SK, Sharma P, Karthikeyan G, Ramakrishnan L, Sengupta S. Homocysteine levels are associated with MTHFR A1298C polymorphism in Indian population. J Hum Genet. 2005;50:655–63.
27. Kim DH, Ahn YO, Lee BH, Tsuji E, Kiyohara C, Kono S. Methylenetetrahydrofolate reductase polymorphism, alcohol intake, and risks of colon and rectal cancers in Korea. Cancer Lett. 2004;216:199–205.
28. Chen Y, Wang B, Yan S, Wang YG. Significant association between MTHFR C677T polymorphism and thyroid cancer risk: evidence from a meta-analysis. Genet Test Mol Biomarkers. 2014;18:695–702.
29. Yilmaz M, Kacan T, Sari I, Kilickap S. Lack of association between the MTHFRC677T polymorphism and lung cancer in a Turkish population. Asian Pac J Cancer Prev. 2014;15(15):6333–63337.
30. Weiwei Z, Liping C, Dequan L. Association between dietary intake of folate, vitamin B6, B12 & MTHFR, MTR genotype and breast cancer risk. Pak J Med Sci. 2014;30(1):106.
31. Jiang-Hua Q, De-Chuang J, Zhen-Duo L, Shu-de C, Zhenzhen L. Association of methylenetetrahydrofolate reductase and methionine synthase polymorphisms with breast cancer risk and interaction with folate, vitamin B6, and vitamin B12 intakes. Tumour Biol. 2014;35(12):11895–901.
32. Chang SC, Lin PC, Lin JK, Yang SH, Wang HS, Li AF. Role of MTHFR polymorphisms and folate levels in different phenotypes of sporadic colorectal cancers. Int J Colorectal Dis. 2007;22(5): 483–9.
33. Rai V. Folate pathway gene MTHFR C677T polymorphism and risk of lung cancer in Asian populations. Asian Pac J Cancer Prev. 2014;15(21):9259–64.
34. Chango A, Boisson F, Barbe F, Quilliot D, Droesch S, Pfister M, et al. The effect of 677C–NT and 1298A–NC mutations on plasma homocysteine and 5,10-methylenetetrahydrofolate reductase activity in healthy subjects. Br J Nutr. 2000;83:593–6.
35. Nair RR, Khanna A, Singh R, Singh K. Association of maternal and fetal MTHFR A1298C polymorphism with the risk of pregnancy loss: a study of an Indian population and a meta-analysis. Fertil Steril. 2013;99:1311–8.
36. Zhang W, Press OA, Haiman CA, Yang DY, Gordon MA, Fazzone W, et al. Association of methylenetetrahydrofolate reductase gene polymorphisms and sex-specific survival in patients with metastatic colon cancer. J Clin Oncol. 2007;25:3726–31.
37. Curtin K, Bigler J, Slattery ML, Caan B, Potter JD, Ulrich CM. MTHFR C677T and A1298C polymorphisms: diet, estrogen, and risk of colon cancer. Cancer Epidemiol Biomarkers Prev. 2004;13: 285–92.