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Original Article

C677T methylenetetrahydrofolate reductase polymorphism, folate and homocysteine levels and the risk of colorectal cancer

Murat Oksuz[1], Ergun Yilmaz[2]

[1]Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, Cukurova University, Adana, Turkey
[2]Division of Gastroenterology. Ortadogu Hospital, Adana, Turkey

Abstract

Aim: Colorectal cancers are common in the world and many factors play a role. MTHFR (methylenetetrahydrofolate reductase) gene polymorphisms cause a predisposition to cancer formation. C677T polymorphism is the most common among these. To evaluate the role of MTHFR polymorphisms in colorectal carcinogenesis, we examined the associations between MTHFR mutations, homocysteine levels, plasma folate levels and colorectal cancer risk.

Materials and Methods: During two years period, 101 naive colorectal cancer cases and 95 controls were enrolled to the study and groups were matched for age, sex and smoking. The presence of the mutation was determined by real-time PCR amplification of genomic DNA using Light.

Results: Although only 1 of the cancer patients was homozygous mutant Val/Val, this mutation was present in 10.5% of the control group and this difference was statistically significant ($p<0.05$). We observed a significant increased risk of colorectal cancer (OR, 0.04; 95% CI, 0.005-0.304) among those with low plasma folate levels (≤3 ng/ml) compared to subjects with adequate levels. However, in both case and control groups mean folate levels were high (10.3 and 10.7 ng/ml, respectively). Homocysteine levels were significantly higher in patients with colorectal cancer compared to healthy volunteers ($p <0.05$).

Conclusion: The data in our study suggested that the MTHFR val/val mutation did not cause a predisposition to cancer development. The presence of a polymorphism other than val/val mutation in the MTHFR enzyme suggests that it may be associated with elevated levels of homocysteine and low folate in cancer patients.

Keywords: Colorectal cancer; folic acid; homocysteine; 5,10-methylenetetrahydrofolate reductase

INTRODUCTION

Colorectal carcinoma is the third most common type of cancer and is ranked fourth among cancer-associated deaths. It is seen more in developed societies than in others (1). Although its etiology is not clear, genetic factors, diet, lifestyle and environmental factors, altered gut microbiota, gene polymorphisms, point mutations and chromosomal changes have been thought to play an important role (2,3).

The most important enzyme of folate metabolism, methylene tetrahydrofolat reductase (MTHFR), which is composed of 656 amino acids, converts 5,10 methylenetetrahydrofolate (5,10-methylene THF) to 5-methyl tetrahydrofolate (5-methyl THF). 5-Methyl THF provides the methyl group required for DNA methylation and methionine synthesis. 5,10-methylene THF is also oxidized to 10-formyl THF for purine synthesis, the precursor of DNA bases. Any mutation occurring in the gene encoding the MTHFR enzyme causes a decrease in the activity of this enzyme. The most common position changes in the gene encoding the MTHFR enzyme is cytosine (C) to thymin (T) at 677 (C677T) and adenine (A) to cytosine at 1298 (A1298C) polymorphism. The C677T polymorphism occurs in the 4th exon and is the result of the change of the gene encoding the MTHFR enzyme to the 677th nucleotide cytosine to thymine. With this change, valine (p.A226V) replaces alanine in the 226 position of the protein which is the product of the gene and causes a meaningful mutation. A1298C polymorphism occurs in 7th exon. In the 429 codon as a result of the change of 1298th nucleotide adenine to cytosine, the glutamine is replaced by valine (p.V429E). In the serious MTHFR enzyme deficiency, substrates in the MTHFR pathway accumulate, nucleic acid synthesis is affected, and the amount of methyl required for biochemical events decreases. As a

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Corresponding Author: Murat Oksuz, Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, Cukurova University, Adana, Turkey **E-mail:** m_oksuz@hotmail.com

result, hyperhomocysteinemia and homocysteinuria are formed, hemostasis is disrupted, causing various diseases such as stroke, thrombosis, peripheral neuro-nephritis, cardiovascular and cerebrovascular diseases (4,5).

Studies have found that polymorphisms occurring in the gene encoding the MTHFR enzyme increase the incidence of many cancers. For example, in the study in China in patients with colorectal tumor, the rs3753584 T>C, rs9651118 T>C and rs4845882 guanine (G)>A mutations in the MTHFR gene have been shown to be a risk factor for colorectal carcinoma (6). In the study conducted in Korea, it was stated that MTHFR3' -UTR polymorphism is a risk factor for colorectal carcinoma and it can be used as a tumor marker (7). Also in studies conducted in the eastern regions of Turkey, similar to the above studies, MTHFR in patients with colorectal carcinoma 677 C>T and 1298 A>C was found to be higher than the control group (2). In several studies, it was found that individuals with MTHFR gene polymorphism had a high rate of cancer, such as esophagus, stomach, lung and breast, compared to the normal population (8-12). In addition to studies showing that the polymorphism in the MTHFR gene leads to cancer susceptibility, there are many studies that these polymorphisms do not have any effect on prevention or cancer formation (5,13-15).

This study is conducted in the south of Turkey included colorectal cancer patients and normal control individuals. The presence of MTHFR enzyme polymorphism, demographic characteristics of individuals and their relationship with biochemical parameters were investigated.

MATERIALS and METHODS

Ethical Approval

The study was approved by Cukurova University Medical Faculty of Medicine Clinical Research Ethics Committee (04.09.2000/decision no:06).

Study Population

The study consisted of 101 patients with colorectal cancer, aged 27 to 78 years, admitted to the gastroenterology department of the Cukurova University, Balcali Hospital, Adana, province of Turkey. In this region, although the consumption of barbecue and meat cooking and offal is intense, the consumption of fresh fruits and vegetables is also high due to its agricultural area. 95 healthy volunteers (40 females, 55 males) aged 22-75 years with no family history of colorectal cancer were included in the study. Patients included in the study were newly diagnosed and untreated. The control group was selected from individuals who had colonoscopy for control, and constipation, IBS and GIS symptoms. In addition to the biochemical parameters such as demographic features, serum folic acid, vitamin B12 and homocysteine levels, the patients were also questioned whether they used cigarette, alcohol and vitamin drugs.

Biochemical Analyses

Serum samples for biochemical analysis, detection of serum homocysteine (Hcy), vitamin B12, and folic acid levels were collected at the time of diagnosis, before any treatment was performed. In all patients, complete blood count was done. Biochemical parameters and vitamin B12 levels were assessed and colonoscopy was performed in order to detect the malignancy. In all patients, we visualized up to the cecum ot terminal ileum. Folic acid and Vitamin B12 levels were determined by using automated chemiluminescence's system test kit (using test kit for folic acid, FOL 124838, Bayer Corporation, Tarrytown, NY, USA; and for vitaminB12, ACS: 180, 104518, Bayer Corporation, Tarrytown, NY, USA). The normal ranges for folic acid and vitamin B12 were accepted as 3-17 ng/ml and 180 to 710 pg/ml, respectively, according to the product insert of test kit. Folate levels were grouped as adequate and deficient (<3 ng/ml) as recommended by the commercial kit insert. Hcy levels were determined by using Homocysteine ELISA test kit (AX-51301, AXIS shield, Germany) and were measured as µmol/L.

Detection of MTHFR Mutation

DNA from cases and controls were extracted from baseline blood and MTHFR genotype was analysed in a research laboratory in the Haematology Department. The investigators and laboratory staff were blinded to case-control status. The presence of the mutation was determined by real-time PCR amplification of genomic DNA using LightCycler Instrument (Roche Diagnostics, Mannheim, Germany). LightCycler-DNA master hybridisation probes and QIAamp DNA blood midi kit (Qiagen, Hilden, Germany) were also used. The amplification primer sequences (Metabion, Munich, Germany) that comprise to exon 4 were; 5'-CGAAGCAGGGAGCTTTGAGGCTG-3' and 5'-AGGACGGTGCGGTGAGAGTG-3, and hybridisation probes sequences (TIB MOLBIOL, Berlin, Germany) were TGACCTGAAGCACTTGAAGGAGAAGGTGTC-F and LCRed640-CGGGAGCCGAT- TTCATCAT-P. The primers for the MTHFR PCR produced a 233-bp PCR product from genomic DNA. The 3'-phosphorylated detection primer was located downstream of the anchor primer at a distance of 2 nucleotide.

Amplification was performed using initial denaturation at 94° C for 2 mins. followed by 40 cycles of 94° C for 0 s, 55° C for 10 s, and 72° C for 15 s, and for melting curve analysis, one cycle of 94° C for 0 s, 40° C for 5 s, 80° C for 0 s. The final cooling was for 30 s at 40°C. The melting point (T_m) of the "wild-type" sample was at 63.1° C, whereas the T_m of the homozygous "mutant" sample was at 55.2 °C. Individuals with two copies of the wild-type sequence (C/C) showed a single melting peak at 63.1° C, homozygous mutant individuals (T/T) showed a single peak at 55.2° C and heterozygous individuals (C/T) resulted in two peaks in this analysis.

Statistical Analysis

The study was a case control study. For quantitative variables, the normality of distribution was checked since the distributions were normal, one-way ANOVA, student t-test were used to compare groups. If normal distribution

was rejected, Mann-Whitney U test was used. Pearson rank correlation test was used to evaluate correlation. Chi-square test or Fisher's exact test were used to compare qualitative variables and Odds ratios with 95% confidence intervals were computed. Adjusted odds ratios (OR) were calculated by using multiple logistic regression analysis. Age, gender, serum Hcy concentration, smoking, alcohol consumption and MTHFR genotypes considered as independent variables for multiple regression analysis. Values are presented as mean±SD and range. A p value of <0.05 is considered significant. Analyses were performed with the SPSS for windows version 10.0 statistical package, programme.

RESULTS

Demographic characteristics and biochemical parameters of patients are shown in Table 1. Of the colorectal cancer patients included in the study, 81 were distal colon and 20 were proximal colon cancer. There was no statistically significant difference in age and sex distribution, body mass index, smoking, alcohol consumption, mean folate and vitamin B12 levels between the patients and the control group (p> 0.05) (Table 1).

MTHFR gene genotypes are shown in Table 2. Although only 1 of the cancer patients (51 years old male) was homozygous mutant Val / Val, this mutation was present in 10 (10.5%) of the control group and this difference was statistically significant (p <0.05). There was no statistically significant difference between homozygous Val/Val and homozygous normal Ala/Ala in terms of age and sex of colorectal cancer patients (OR ratio 0.07, 95% CI, 0.008 to 0.57). Smoking, alcohol consumption, and body mass index were similar to the OR value, age and gender OR value (data not shown). The OR ratio between the homozygous mutantVal / Val and the control group Ala / Ala and Ala / Val was 0.08 (95% CI, 0.01 to 0.64).

Table 1. Means (±SD) and proportions of risk factors for colorectal cancer in groups

Risk factor	Cases(n:101)	Controls(n:95)	P value
Age (years)	52±13	49±11.3	Matching factor
Sex (male/female)(n)	56/45	55/40	Matching factor
Body mass index (kg/m^2)	24.7±2.9	25.1±3.3	0.198
Mean folate (ng/ml)	10.3±5.9	10.7±4.1	0.632
Folate deficiency (n/%) (<3ng/ml)	20/19.8	1/1.1	<0.001
Mean homocysteine (mol/L)*	12.6±4	10.1±2.3	<0.001
Frequency of alcohol intake (occasions/week)	3.24±2.2	4±2.3	0.089
Amount of alcohol intake (grams/week)	45.5±35	52±29.3	0.273
Cigarette smoking (n)	22	24	Matching factor
Mean vitamin B12 (pg/ml)*	495.6±197	459±178	0.189
Rectal cancer(n)	42		
Distal colon cancer (n)†	39		
Proximal colon cancer (n)†	20		

* Measured plasma vitamin B12 and homocysteine levels in ninety-five colorectal cancer patients.† Area from sigmoid colon to splenic flexure was termed as distal colon and more proximal part was termed as proximal colon

Table 2. Frequency of the MTFR genotypes and risk of colorectal cancer among subjects

MTHFR genotype	Cases (n / %)	Controls (n / %)	Age and sex-adjusted OR(95% CI)	Multivariate-adjusted OR*(95% CI)
Homozygous normal (Ala/Ala)	50 / 49.5	49 / 51.6	1.00 (reference)	1.00 (reference)
Heterozygous (Ala/Val)	50 / 49.5	36 /37.9	0.90 (0.11-1.08)	0.88 (0.10-1.03)
Homozygous mutant (Val/Val)	1 / 1	10 / 10.5	0.07† (0.008-0.57)	0.07† (0.008-0.65)
Both genotype Ala/Ala and Ala/Val are considered as reference group			0.085† (0.01-0.65)	0.080† (0.01-0.64)

*Adjusted for age, gender, smoking, alcohol consumption, and body mass index.† p<0.005

In our study, the number of individuals with folate <3 ng/dl was also evaluated separately. This level is considered to be severe folate deficiency and is thought to be a risk factor for colorectal cancer. The number of persons with <3 ng/dl folate levels in cancer patients was statistically higher than the control group (20 in cases vs. 1 in control, p value of <0.05) (Table 1). The risk of developing colon cancer was found to be statistically higher in patient with low folate levels compared to individuals with normal folate levels (OR, 0.04; 95% CI, 0.005-0.304). In our study, the relationship between mean serum folate level and MTHFR enzyme polymorphism was evaluated. The mean serum folate level in patients with homozygotic mutant (Val/Val) polymorphism was found to be lower than cases with Ala/Ala and Ala/Val genotypes (Table 3). In addition, homozygous mutant controls had higher folate levels than in our cancer patients Table 3 and Figure 1.

Table 3. Mean folate levels (ng/ml) with MTHFR genotype and case-control status of subjects

MTHFR genotype	Cases mean±SD (n)	Controls mean±SD (n)	Total mean±SD (n)
Homozygous mutant (Val/Val)	2.3 (1)	7.2±2.8 (10) ‡	6.8±3 (11)*
Heterozygous (Ala/Val)	10±6 (50) §	10.1±4.6 (36)	10.1±5.4 (86) †
Homozygous normal (Ala/Ala)	10.8±7 (50)	11.7±3.7(49)	11.3±4.8 (99)
Total patients	101	95	196

* p<0.05 for genotype (Val/Val) versus (Ala/Val), or for genotype (Ala/Ala). † p: 0.123 for genotype (Ala/Ala) versus (Ala/Val). ‡ p< 0.05 for genotype (Val/Val) versus (Ala/Ala), or for genotype (Ala/Val). § p> 0.05 for genotype (Ala/Val) versus for genotype (ala/Ala). p:0.013 within groups by one-way ANOVA

The mean homocysteine level of patients with colorectal cancer was significantly higher than the control group (p <0.05) (Table 1). However, there was no difference in homocysteine levels between the MTHFR groups. There was a significant inverse correlation between serum folate levels and homocysteine levels (Pearsony's correlation coefficient (r) was -508 (p <0.001). When the groups were evaluated separately, this inverse correlation was the same in patients with cancer, but the ratio in healthy subjects was low (r: -698, p <0.001, r: -175, p: 0.09). In addition, the homocysteine level was 16.3 ± 3.6 in patients with a mean folate level less than 3 mg / dl, and 10.7 ± 1.9 mg / dl in patients with a folate level higher than 3 mg / dl, and this difference was statistically significant (p<0.001).

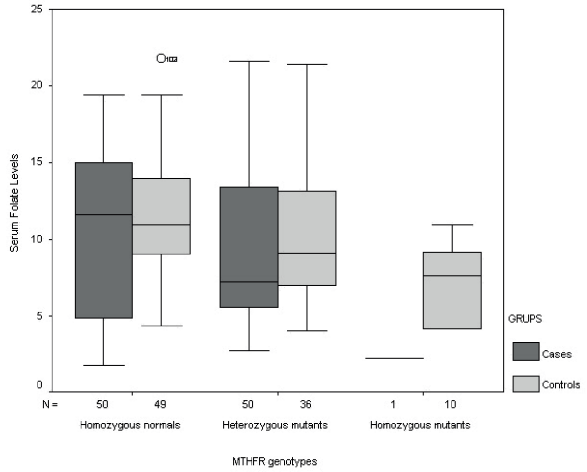

Figure 1. Mean folate levels (ng/ml) by MTHFR genotype in control and cancer groups. P value is calculated by one-way ANOVA test within the groups and p:0.004 for control group and p:0.335 for cancer group.

DISCUSSION

In this study, we investigated the presence of val/val, ala/val mutations in MTHFR gene between 677C-T polymorphism between colon cancer patients and healthy individuals. We also investigated the relationship between these mutations and demographic characteristics of individuals. The number of people with val/val mutation in control subjects was statistically significantly higher than patients with colorectal cancer. There was no association between this mutation and the demographic characteristics of individuals, alcohol, smoking and body mass index.

In many studies, individuals with 677C-T polymorphism have been shown to have more colorectal cancer than individuals without this polymorphism (2,6,16). Similar results were found in the meta-analyses of colorectal cancers with MTHFR gene polymorphisms. For example, in a meta-analysis of studies conducted in Caucasian, Asian and African population, 677C-T polymorphism has been shown to be a risk factor for colorectal cancer, especially in Asians (3). In another study, it was found that the relationship between 677C-T polymorphism and cancer showed geographical differences. Although this polymorphism is not a risk factor for colorectal cancer in countries such as Spain and Turkey, it has been suggested to be a risk factor in other countries such as China or Italy (17). Similar to colorectal cancers, this relationship has been demonstrated in various types of cancer, such as lung, prostate and stomach (18,20). However, unlike the above studies, it has been shown that MTHFR gene polymorphisms have the opposite effect in many types of cancer, including colorectal cancer. For example, MTHFR C667 polymorphism was found to be protective for colorectal cancer, especially in non-Asian societies,

whereas in another meta-analysis it was found that this polymorphism did not pose any risk for Asian societies (1,21). In another study, the risk of colorectal cancer is increased in individuals with MTHFR C677T and A1298C polymorphisms, while those with 1298CC genotype have been shown to decrease this risk (22). In our study, the number of individuals with val/val mutant genotype was higher in healthy volunteers ($p<0.05$). This result shows that val/val mutation is not a risk factor for colorectal carcinoma. Although there was 1 case in the patient group, the val / val mutant patient ages in both groups were over 50 years old. Therefore, we cannot say that the val / val mutation is protective, because the incidence of colorectal cancer is already increasing after the age of 50.

The reason why these findings are different from the results obtained in the other studies conducted in our country may be due to environmental factors, low number of cases, regional differences or dietary habits as mentioned in some studies. At the same time, as stated in the above studies, some polymorphisms in the gene encoding MTHFR cause cancer susceptibility and some polymorphisms have preventive effects. In addition, the patients in our study may have another polymorphism, which may have contributed to the development of colorectal carcinoma.

As a result of MTHFR gene polymorphism, homocysteine level increases and folic acid level decreases due to decrease in enzyme activity. Folate is a vital vitamin that can be taken with food, especially in DNA methylation and single carbon reaction. In the deficiency of folate enzyme, methylation of the DNA decreases and creates a risk for cancer formation. The supportive results have been obtained in many studies. For example, folate deficiency caused by MTHFR polymorphism has been shown to be a risk factor for various types of cancer such as ovarian, prostate, breast and brain. However, there are also reports that there is no relationship between folic acid deficiency and cancer formation, and even reduces the risk (23). In our study, there was no statistically significant difference in mean folate levels between cancer patients and healthy volunteers. Our study was similar to the studies suggesting that folic acid had no role in cancer formation, but when the individuals were examined one by one, the number of individuals below the 3 ng/ml folate level was significantly higher in cancer patients than healthy individuals ($p<0.05$). These data suggest that folate deficiency is also a risk factor for colorectal cancer and in this sense, it supports the publications that folate deficiency is associated with cancer. However, when the relationship between mean folate and MTHFR polymorphisms was investigated, the mean folic acid level in patients with val/val genotype in the cancer and control cases was statistically lower than the other genotypes. These findings suggest that val/val genotype leads to folate deficiency, but because this genotype is more common in healthy volunteers, folate deficiency is not a risk factor for direct cancer, or that serious deficiency in folate level causes a higher risk. However, as noted above, the presence of other polymorphisms, nutritional status, or the low number of cases may have affected folic acid levels in individuals. In this sense, new studies with large number of cases will be useful to understand this situation. Another remarkable point is that the serum folate level (10.7 ng/ml) of the people of our region was significantly higher compared to the folate level obtained in western publications (in many publications 3.5 ng/ml).

Homocysteine elevation has been shown to be a risk factor for the occurence of various types of cancer, such as colorectal and breast (23). In our study, homocysteine levels were found to be significantly higher in patients with colorectal cancer than in healthy volunteers ($p<0.05$). However, there was no significant difference in homocysteine levels between individuals with val/val genotype and other genotype ($p>0.05$). This may be due to the presence of another possible polymorphism in patients with cancer. Similarly, the presence of an inverse correlation between the folate values and homocysteine values, and the presence of difference between homocysteine levels in patients had the folate levels below and above 3 mg/dl, further enhances the likelihood of possible mutation in cancer patients. Again, there are studies suggesting that alcohol use contributes to the development of colorectal cancer. Alcohol acts as a folate antagonist and causes folate deficiency. Low alcohol use in individuals with MTHFR, rs1801133 and CT/TT reduced cancer formation, but high alcohol use in individuals with MTHFR rs1801133 CC genotype has been shown to increase colorectal cancer development (24). In our study, there was no difference between individuals with MTHFR val/val and other genotypes in terms of alcohol use among healthy individuals and cancer patients.

CONCLUSION

The data in our study indicate that MTHFR val / val mutation cannot cause a predisposition to cancer development. There is no clear relationship between this mutation and demographics (age, gender, smoking and alcohol use). In addition, according to these data, there was no difference in homocysteine levels between patients with cancer and healthy subjects with MTHFR val / val mutation. Although this situation causes a decrease in the level of folic acid level, which may be due to diet, this deficiency has not been found to be very serious. Therefore, high levels of homocysteine and folic acid deficiency in cancer patients suggest that there may be other polymorphisms in MTHFR enzyme than val / val mutation. Finally, we recomment a future study including more val/val genotype in the population.

Competing Interests: The authors declare that they have no competing interest.

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Ethical Approval: The study was approved by Cukurova University Medical Faculty of Medicine Clinical Research Ethics Committee (04.09.2000/ decision no:06).

REFERENCES

1. Xin Y, Wang DX, Qian H, et al. 5,10-Methylenetetrahydrofolate Reductase Polymorphisms and ColonCancer Risk: a Meta-analysis. Asian Pac J Cancer Prev 2014;15:8245-50.
2. Filiz O, Metin S, Ozturk O. Methylenetetrahydrofolate Reductase Gene Germ-Line C677T and A1298C SNPs are Associated with Colorectal Cancer Risk in the Turkish Population. Asian Pac J Cancer Prev 2014;15:7731-5.
3. Lingyan X, Zhiqiang Q, Feng W, et al. Methylenetetrahydrofolate reductase C677T Polymorphism and colorectal cancer susceptibility: a meta-analysis. Biosci Rep 2017; 37:1-15.
4. Miris D. Molecular Biology of Methylenetetrahydrofolate Reductase (MTHFR) Enzyme and Its Association with Diseases. The Medical Journal of Kocatepe 2004;5: 9-16.
5. Luciana MR, Fernando A, Lima M, et al. Can MTHFR C677T and A1298C Polymorphisms Alter the Risk and Severity of Sporadic Breast Cancer in Brazilian Women? Clinical Breast Cancer 2017;1:1-8.
6. Sheng Z, Shuchen C, Yu C, et al. Investigation of methylenetetrahydrofolate reductase tagging polymorphisms with colorectal cancer in Chinese Han population. Oncotarget 2017;38: 63518-27.
7. Young JJ, Jong WK, Hye MP, et al. Genetic variants in 3′-UTRs of methylenetetrahydrofolate reductase (MTHFR) predict colorectal cancer susceptibility in Koreans. Scientific Reports 2015;5:1-13.
8. Shen GR, Li WZ, Liu YC, et al. Association between a microRNA-214 binding site polymorphism in the methylenetetrahydrofolate reductase gene and esophageal squamous cell carcinoma. Br Genetics and Molecular Research 2016;15:1-8.
9. Long C, Ning L, Bai HZ, et al. Association between the MTHFR C677T polymorphism and gastric cancer susceptibility: A meta-analysis of 5,757 cases and 8,501 controls. Oncology Letters 2015;10:1159-65.
10. Yousef AM, Maha S, Ismail S, et al. Allele and Genotype Frequencies of the Polymorphic Methylenetetrahydrofolate Reductase and Lung Cancer in the Jordanian Population: a Case Control Study. Asian Pacific Journal of Cancer Prevention 2015;16:3101-9.
11. lilin HY, xiangs H. MTHFR C677T polymorphism and breast, ovarian cancer risk: a meta-analysis of 19,260 patients and 26,364 controls. OncoTargets and Therapy 2017;10 :227-38.
12. Ramos S, Figuera LE Soto Q, et al. Association of the C677T polymorphism in the methylenetetrahydrofolate reductase gene with breast cancer in a Mexican population. Br Genetics and Molecular Research 2015;14: 4015-4026.
13. Beibei Z, Weiming Z, Liang Y, et al. The association between MTHFR gene C677T polymorphism and ALL risk based on a meta-analysis involving 17,469 subjects. Clinica Chimica Acta 2017; 466:85-92.
14. Sui J, Jin DX, Zhen JZ, et al. Association of MTHFR C677T and A1298C polymorphisms with oral cancer susceptibility: evidencefrom a meta-analysis. OncoTargets and Therap 2017;10:303-10.
15. Michael L, Alexander S, Susanna M, et al. The methylenetetrahydrofolate reductase (MTHFR) variant C677T (A222V) influences overall survival of patientswith glioblastoma multiforme. Neuro Oncology 2008;3:548-52.
16. Federica M, Marina B, Andrea B, et al. Effect of MTHFR Polymorphisms on Gastrointestinal Cancer Risk in Italy. World J Oncol 2015;6:394-7.
17. Shiao SPK and Yu CH. Meta-Prediction of MTHFR Gene Polymorphism Mutations and Associated Risk for Colorectal Cancer. Biological Research for Nursing 2016;18:357-69.
18. Xin W, Kai Y, Liran H. Meta-analysis of methylenetetrahydrofolate reductase polymorphism and lung cancer risk in Chinese. Int J Clin Exp Med 2015;8:1521-5.
19. Mehdi A, Masoud ZS, Hossein N, et al.Association between MTHFR C677T Polymorphism and Risk of Prostate Cancer: Evidence from 22 Studies with 10,832 Cases and 11,993 Controls. Asian Pac J Cancer Prev 2015;16:4525-30.
20. Lei ZXia, Yi L, Xiao ZX, et al. Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and gastric cancers susceptibility. World J Gastroenterol 2014;20:11429-38.
21. Vandana R. Evaluation of the MTHFR C677T Polymorphism as a Risk Factor for Colorectal Cancer in AsianPopulations. Asian Pac J Cancer Prev 2015;16:8093-100.
22. Meng Z, Xuelian l, Cheng Z, et al Association of methylenetetrahydrofolate reductase C677T and A1298C polymorphisms with colorectal cancer risk: A meta analysis. Biomedical Reports 2013;1:781-91.
23. Donghong Z, Xuemei W, Wei W, et al Elevated Homocysteine Level and Folate Deficiency Associated with Increased Overall Risk of Carcinogenesis: Meta-Analysis of 83 Case-Control Studies Involving 35,758 Individuals. Plos One 2015;10:1-16.
24. Thomas S, Taiki Y, Sanjeev B, et al. Alcohol consumption, genetic variants in thealcohol- and folate metabolic pathways and colorectal cancer risk: the JPHC Study. Scientific Reports 2016;6:1-8.