RESEARCH PAPER

Association analysis of C677T and A1298C polymorphisms in MTHFR gene in patients with colorectal cancer susceptibility

Ramin Lak [1,*]**, Elham Kazemi** [2]**, Pooya Aavani** [3]**, Farzane Alaei** [1]**, Fatemeh Pashizeh** [1]

[1] Pars Advanced and Minimally Invasive Medical Manners Research Center, Pars Hospital, Iran University of Medical Sciences, Tehran, Iran

[2] Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
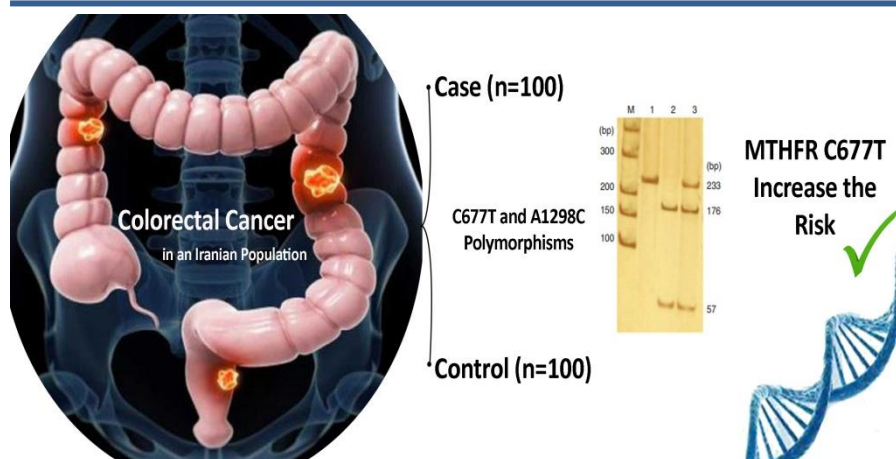
[3] Department of Biology, Emory University, 1510 Clifton Rd. Atlanta, GA 30322 – USA

Highlights

• 5,10-methylenetetrahydrofolate gene may cause impairment of cell function and a variety of cancers like prostate and colorectal cancers.
• MTHFR C677T and A1298C polymorphism can increase the susceptibility risk of colorectal cancer.
• There are significant associations between genotypes 677CT and 677TT with colorectal cancer risk.
• Inflammatory mechanisms are associated with the disorders of female reproductive system.

Graphical Abstract

Abstract

5,10-methylenetetrahydrofolate reductase (MTHFR) plays an important role in folate metabolism. Also, this gene is associated with repair, synthesis and methylation of DNA. Polymorphisms of C677T and A1298C are two common SNP of MTHFR gene. This study was aimed to investigate the association of C677T and A1298C polymorphisms in MTHFR with colorectal cancer in the Iranian population. In this case-control study, 100 patients with colorectal cancer and 100 healthy individuals' samples as a control group were collected. Genomic DNA was isolated from peripheral blood samples, and MTHFR C677T and A1298C genotyping were performed via RFLP-PCR. Significant associations among genotypes 677CT (OR= 2.0910, 95% CI= 1.1728 to 3.7280, p= 0.0124) and 677TT (OR= 5.2073, 95% CI= 1.0299 to 26.3283, p= 0.0460) with risk of colorectal cancer was found. But no significant relationship was detected between A1298C polymorphism and colorectal cancer. MTHFR C677T polymorphism can increase the risk of colorectal cancer in the Iranian population.

Introduction

Colorectal cancer (CRC) is the third most common cancer in men and the fourth leading cause of cancer death in the world. In addition, it is expected to increase by 60% and 2.2 million new and 1.1 million deaths by 2030 (1). Some risk factors including age, tobacco usage, consumption of red meat, family history and genetic factors are involved in colorectal cancer (2). A recent study determined folate, a water-soluble vitamin, plays an important role in colorectal cancer (3). Deficiency in the synthesis of folic acid results in failure in the double-strand chromosome that it is a methyl transporter in the metabolism of the cell and its deficiency leads to genomic hypomethylation and ultimately disrupts the expression of the genes.

The human 5, 10-methylenetetrahydrofolate reductase (MTHFR) gene is located on chromosome 1 (1p36.3) and composed of 12 exons, encompasses 19.3 kb of DNA and the gene codes a 74.6-kDa protein of 656 amino acids (4). 5, 10-methylenetrahydrofolate reductase gene plays an important role in the metabolism of folate. MTHFR catalyzes the conversion of 5, 10-methylenetetrahydrofolate (5,10-MTHF) to 5- methyltetrahydrofolate (5-MTHF) which is the main form in the circulation of folate in the blood and lead to the conversion of dUMP (deoxyuridine monophosphate) to dTMP (deoxythymidine monophosphate). Also, MTHFR protein causes biosynthesis of DNA, RNA and homocysteine re-methylation. Considering the important regulatory role of MTHFR on DNA replication, DNA repair, DNA methylation and cell division, this gene can have the potential role for cancer-predisposing and consequently low dietary folate and MTHFR deficiency causing to intestinal tumors in the BALB/c mouse model (5). Low levels of 5, 10-MTHF can lead to an increased dUMP/dTMP ratio, increased risk of DNA mutation and the fracture of DNA strand (6). Also, low levels of 5, 10-MTHF can lead to decreased s-adenosylmethionine (SAM) levels and DNA hypomethylation can cause to genomic instability, activation of cellular oncogenes and DNA damage. However, Low levels of folate can be associated with susceptibility to cancer (7). There are three common polymorphisms of the MTHFR gene, MTHFR C677T (rs1801133), G1793A (rs2274976) and MTHFR A1298C (rs1801131). In the case of the MTHFR C677T polymorphism,the base of cytosineat position 677 changes to a thymidine base while this change leads to conversion in the amino acid at position 222 so that alanineis replaced by valineon the other hand in the 1298A>C transitionresults from a glutamate to-to-alanine replacement at codon 429 (Glu429Ala) and eventually the 1793G>A polymorphism leads to in an arginine-to-glutamine replacement at position 594 (Arg594Gln). MTHFR C677T can be as a risk factor for heart disease, congenital anomalies, diabetes, stroke, psychiatric disorders and some types of cancer (8). Considering the role of MTHFR in impaired DNA methylation, DNA strand breaks and repairand relationship between MTHFR with folate deficiency and colorectal cancerin the present study, the association of C677T and A1298C polymorphisms in MTHFR with colorectal cancer in an Iranian population, has been analyzed.

Materials and Methods

Collection and analysis of blood samples

This study performed on 100 patients with colorectal cancer and 100 healthy individuals' samples as a control group. Peripheral blood samples were collected from Isfahan University of Medical Sciences, Isfahan, Iran into tubes containing anticoagulant sodium citrate and preserved at -20 °C for further usage.

Genomic DNA extraction and primers

Isolation of genomic DNA was done using blood samples and Whole DNA of blood samples was isolated using DNA extraction kit (Add bio Co) and kept at −20°C until usage. The polymerase chain reaction was performed to amplify the MTHFR fragments, in 25 μL PCR reaction containing 0.35 μM forward and reverse primers, 0.5 μL dNTPs mix, 0.2 μL *Taq* polymerase, 35 ng template DNA and 1.5 μM MgCl2 (All PCR reagents were purchased from Fermentas) (Figure 1).
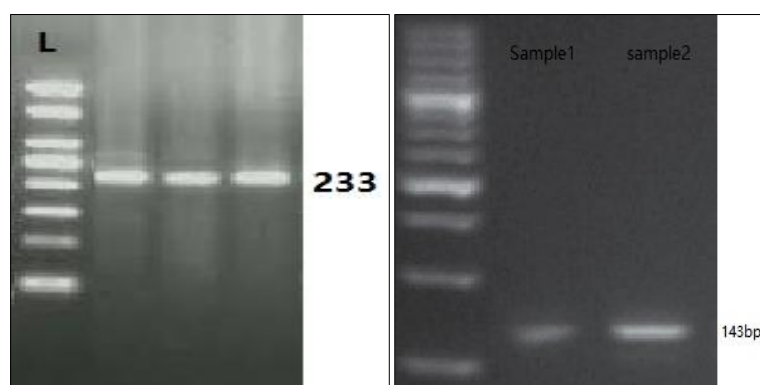
Figure 1. PCR product fragments on 1% agarose gel stained with 1 mg/mL ethidium bromide solution. L, DNA marker, 233-bp fragment of the 677C.T transition,143-bbp fragment of the 1298A.C transition.

Primer sequences for two single nucleotide polymorphisms (SNPs), C677T (rs1801133) and A1298C (rs1801131) are presented in Table 1. The PCR was performed in a thermal cycler (Eppendorf, Hamburg, Germany) using the conditions listed in Table 2. The PCR products were evaluated by 1% agarose gel electrophoresis. Then, 5 mL aliquot of the PCR product was digested with 5 units *Hinf*I and *Mbo*II (Fermentas) and being incubated at 37 °C for 16 h and finally digested products were evaluated by polyacrylamide gels and silver nitrate (Figure 2). Results of PCR-RFLP data were checked by DNA direct sequencing.

Table 1. Sequences of the RFLP-PCR Primer.

SNP	Primer	Primer sequence
C677T (rs1801133)	MTHFR1-forwared	5′-CGAAGCAGGGAGCTTTGAGGCTG
	MTHFR1- reverse	5′-AGGACGGTGCGGTGAGAGTG
A1298C (rs1801131)	MTHFR2-forwared	5′-GCAAGTCCCCCAAGGAGG
	MTHFR2- reverse	5′-GGTCCCCACTCCAGCATC

Table 2. Polymerase chain reaction conditions.

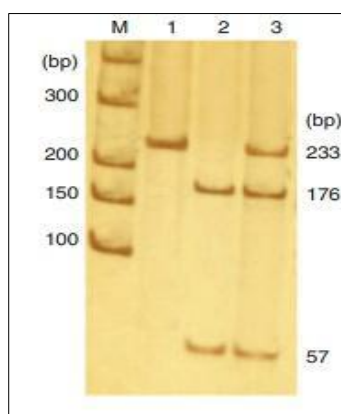| SNP	PCR conditions	PCR product (bp)
C677T (rs1801133)	42 cycles of 94°C for 5 min, 94°C for 45 s, 63°C for 50 s and 72°C for 1 min; final extension at 72°C for 10 min	233
A1298C (rs1801131)	45 cycles of 94°C for 5 min, 94°C for 45 s, 55°C for 1 min and 72°C for 1 min; final extension at 72°C for 10 min	143

Figure 2. Polyacrylamide gel electrophoresis results of the restriction enzyme map. Restriction enzyme map of the 233-bp polymerase chain reaction (PCR) fragment of the methylenetetrahydrofolatereductase (MTHFR) gene following digestion with *Hinf*I. M, DNA marker; Lane 1, CC genotype; Lane 2, TT genotype; Lane 3, CT genotype.

Statistical analysis

Hardy-Weinberg equilibrium in case and control groups for both C677T and A1298C polymorphisms were evaluated by chi-squared test. The differences of genotype and allele frequencies between case and control groups were also evaluated by the same test. The strength of association between C677T and A1298C polymorphisms and colorectal cancer risk was estimated by odds ratio (OR) and 95% confidence interval (CI) which were computed by binary logistic regression analysis. A P-value less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS ver.19 software.

Results

The results of genetic association study are presented in Tables 3 and 4. With regard to polymorphism C677T, we found that there are significant associations between genotypes CT (OR= 2.0910, 95%CI= 1.1728 to 3.7280, p= 0.0124) and TT (OR= 5.2073, 95%CI= 1.0299 to 26.3283, p= 0.0460) and colorectal cancer risk. Also, there was a significant association between the mentioned polymorphism and colorectal cancer in a dominant model (OR= 2.2508, 95%CI= 1.2781 to 3.9639, p= 0.0050). In addition, there was a significant association between allele T and risk of colorectal cancer (OR= 1.9101, 95%CI= 1.2149 to 3.0030, p= 0.0051). However, we did not find any significant association between A1298C polymorphism and colorectal cancer. Obtained data revealed that there are no significant associations between genotypes AC (OR= 1.2401, 95%CI= 0.6806 to 2.2596, p= 0.4821) and CC (OR= 0.9790, 95%CI= 0.3840 to 2.4960, p= 0.9646) and colorectal cancer. Moreover, there was no significant association between A1298C and colorectal cancer in a dominant model (OR= 1.1748, 95%CI= 0.6733 to 2.0498, p= 0.5705). Allele analysis revealed that there is no significant association between allele C and colorectal cancer (OR= 1.0768, 95%CI= 0.6967 to 1.6644, p= 0.7390).

Table 3. Genotype frequencies of C677T and A1298Cpolymorphisms in cases and controls.

Polymorphism	Genotype	No. and Percentage		OR (95% CI)	*P*-value
		Control (n=100)	Case (n=100)		
C677T	CC (%)	61 (61.00)	(41 41.00)	-	-
	CT (%)	37 (39.39)	52 (52.00)	2.0910(1.1728 to 3.7280)	0.0124*
	TT (%)	2 (2.00)	7 (7.00)	5.2073(1.0299 to 26.3283)	0.0460*
	CT+TT (%)	39 (39.00)	59 (59.00)	2.2508(1.2781 to 3.9639)	0.0050*
A1298C	AA (%)	56 (56.00)	52 (52.00)	-	-
	AC (%)	33 (33.00)	38 (38.00)	1.2401(0.6806 to 2.2596)	0.4821
	CC (%)	11 (11.11)	10 (10.00)	0.9790(0.3840 to 2.4960)	0.9646
	CC+CA (%)	44 (44.00)	48 (48.00)	1.1748(0.6733 to 2.0498)	0.5705

*Significant difference between patient and control group. OR: odds ratio, CI: confidence interval

Table 4. Allele frequencies of C677T and A1298Cpolymorphisms in cases and controls.

Polymorphism	Allele	No. and Percentage		OR (95% CI)	*P*-value
		Control (n= 200)	Case (n= 200)		
C677T	C (%)	159 (79.50)	134 (67.00)		
	T (%)	41 (20.50)	66 (33.00)	1.9101(1.2149 to 3.0030)	0.0051*
A1298C	A (%)	145 (72.50)	142 (71.00)		
	C (%)	55 (27.50)	58 (29.00)	1.0768(0.6967 to 1.6644)	0.7390

*Significant difference between patient and control group.

Discussion

Folate is a form of vitamin B9 that it has an important role in the different function. Mammalian cells cannot produce folate and leafy vegetables, liver, potatoes, bread and fruit are important sources of folat. Recent studies provided crucial evidence that folate deficiency is associated with an increased risk of numerous cancer especially in colorectal cancer because of lower intakes of vegetables and fruit (9) Folate plays an important role in modification reactions including DNA methylation, DNA synthesis, nucleotide synthesis and repair (10). The MTHFR gene encodes a cytoplasmic flavor enzyme and regulatory enzyme involved in folate metabolism through the production 5-methyltetrahydro folate that this form dominant circulating form of folate (11, 12). Thus failure and mutations in the MTHFR gene may cause impairment of cell function and a variety of cancers so myeloid leukaemia, prostate cancer and colorectal cancer. The present study aimed to investigate the association between C677T and A1298C polymorphisms in MTHFR with colorectal cancer in an Iranian population. The results of this study showed a significant association between colorectal cancer and C677T genotypes but in the A1298C polymorphism was not associated with the risk of colorectal in this study. Several studies have been conducted to investigate the association between C677T, A1298C polymorphisms and colorectal cancer in different populations which confirmed the results of this study. In several studies, the 677TT genotype was associated with an increased risk of CRC. The results of the Sameer et al., study showed that the MTHFRC677T polymorphism slightly increases the risk for colorectal cancer development in the ethnic Kashmir population that is consistent with the results of this study (13).

Also, the results of a study by Ozen and et al., suggested a strong association between both SNPs of MTHFR 677 C>T and 1298 A>C and CRC susceptibility in the Turkish population (14). In addition, the data of the study Gallegos-Arreola et al., determined that the 677C>T polymorphism in MTHFR increased significantly the risk of CRC in the Mexican population (15). But, the multiethnic study conducted by Le Marchand et al., showed the negative correlation of the TT genotype with colorectal cancer, especially those diagnosed at an advanced stage (16). Furthermore, the research results of Cao et al., showed that polymorphisms of the MTHFR 677 C>T could impress susceptibility to colon cancer and interestingly when women and men were evaluated together there was no significant relationship between MTHFR C677T and A1298C polymorphisms with colon cancer but when the two groups of women and men get separated from each other, different results were obtained, men with MTHFR 677 C>T T/T genotype have a significantly higher risk for colon cancer also a significant association between 677 C>T T/T and 1298 A>C A/A genotypes with colon cancer (17). Single nucleotide polymorphisms may influence the expression and structure of a gene depending on their locations. Polymorphisms in the promoter region could affect the gene expression while the exonic polymorphism could alter the function and structure of a protein (18, 19, 20, 21, 22).

Also, mutations in the intron regions might change the gene expression by interfering in the splicing process. Evaluation of the molecular effects of single nucleotide polymorphisms on the interested genes by in vitro and in vivo methods could be a time and cost consuming procedures. But, the employing of computational methods can be a beneficial method to evaluate the molecular aspects of an SNP. A previous study revealed that the C677T transition has a potential effect on both RNA and protein structure. They present that this mutation may be deleterious for the protein structure due to its location near the substrate-binding site. There are many reports about the effects of genotype on cancer, and more research is needed to show more angles (23, 24, 25, 26).

Conclusion

According to results, the 677 C>T polymorphism MTHFR gene can increase the risk factor of developing colorectal cancer, in addition, individuals with T allele in the 677 C>T MTHFR increased risk of developing colorectal cancer also can 677 C>T polymorphism be identified as a risk factor for colorectal cancer but for 1298 A>C, it is needed to supplementary studies with larger sample size. However, the evaluation of gene-gene and gene-environment interaction has not performed and this could be considered as a limitation of this study.

References

1. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut 2017; 66(4): 683-691. https://doi.org/10.1136/gutjnl-2015-310912

2. Anon B, Perray C, Regnault D, Caulet M, Orain I, Godart B, Pages JC, Tallet A, Ouaissi M, Guyetant S, Barin-le Guellec C. A study of the association between UGT1A1* 28 variant allele of UGT1A1 gene and colonic phenotype of sporadic colorectal cancer. Dig Liver Dis 2019; 51(4): 579-583. https://doi.org/10.1016/j.dld.2018.11.032

3. Araghi SO, Kiefte-de Jong JC, Van Dijk SC, Swart KM, van Laarhoven HW, van Schoor NM, de Groot LC, Lemmens V, Stricker BH, Uitterlinden AG, Van Der Velde N. Folic acid and vitamin B12 supplementation and the risk of cancer: long-term follow-up of the B Vitamins for the Prevention of Osteoporotic Fractures (B-PROOF) Trial. Cancer Epidemiol Prev Biomark 2019; 28(2): 275-282. https://doi.org/10.1158/1055-9965.EPI-17-1198

4. Eloualid A, Abidi O, Charif M, Benrahma H, Louanjli N, Chadli E, Ajjemami M, Barakat A, Bashamboo A, McElreavey K, Rhaissi H. Association of the MTHFR A1298C variant with unexplained severe male infertility. PloS one 2012; 7(3): e34111. https://doi.org/10.1371/journal.pone.0034111

5. Lin KM, Yang MD, Tsai CW, Chang WS, Hsiao CL, Jeng LB, Yueh TC, Lee MC, Bau DT. The role of MTHFR genotype in colorectal cancer susceptibility in Taiwan. Anticancer Res 2018; 38(4): 2001-2006. https://doi.org/10.21873/anticanres.12438

6. Kim YI, Pogribny IP, Basnakian AG, Miller JW, Selhub J, James SJ, Mason JB. Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. Am J Clin Nutr 1997; 65(1): 46-52. https://doi.org/10.1093/ajcn/65.1.46

7. Chittiboyina S, Chen Z, Chiorean EG, Kamendulis LM, Hocevar BA. The role of the folate pathway in pancreatic cancer risk. PloS one 2018; 13(2): e0193298. https://doi.org/10.1371/journal.pone.0193298

8. Rai V. Evaluation of the MTHFR C677T polymorphism as a risk factor for colorectal cancer in Asian populations. Asian Pac J Cancer Prev 2016; 16(18): 8093-8100. https://doi.org/10.7314/APJCP.2015.16.18.8093

9. Kok DE, Steegenga WT, Smid EJ, Zoetendal EG, Ulrich CM, Kampman E. Bacterial folate biosynthesis and colorectal cancer risk: more than just a gut feeling. Crit Rev Food Sci Nutr 2020; 60(2): 244-256. https://doi.org/10.1080/10408398.2018.1522499

10. James SJ, Pogribny IP, Pogribna M, Miller BJ, Jernigan S, Melnyk S. Mechanisms of DNA damage, DNA hypomethylation, and tumor progression in the folate/methyl-deficient rat model of hepatocarcinogenesis. J Nutr 2003; 133(11): 3740S-3747S. https://doi.org/10.1093/jn/133.11.3740S

11. Amirfallah A, CalibasiKocal G, Unal OU, Ellidokuz H, Oztop I, Basbinar Y. DPYD, TYMS and MTHFR genes polymorphism frequencies in a series of Turkish colorectal cancer patients. J Pers Med 2018; 8(4): 45. https://doi.org/10.3390/jpm8040045

12. Taioli E, Garza MA, Ahn YO, Bishop DT, Bost J, Budai B, Chen K, Gemignani F, Keku T, Lima CS, Le Marchand L. Meta-and pooled analyses of the methylenetetrahydrofolatereductase (MTHFR) C677T polymorphism and colorectal cancer: a HuGE-GSEC review. Am J Epidemiol 2009; 170(10): 1207-1221. https://doi.org/10.1093/aje/kwp275

13. Sameer AS, Shah ZA, Nissar S, Mudassar S, Siddiqi MA. Risk of colorectal cancer associated with the methylenetetrahydrofolatereductase (MTHFR) C677T polymorphism in the Kashmiri population. Genet Mol Res 2011; 10(2): 1200-1210. https://doi.org/10.4238/vol10-2gmr1067

14. Ozen F, Sen M, Ozdemir O. Methylenetetrahydrofolatereductase gene germ-line C677T and A1298C SNPs are associated with colorectal cancer risk in the Turkish population. Asian Pac J Cancer Prev 2014; 15(18): 7731-7735. https://doi.org/10.7314/APJCP.2014.15.18.7731

15. Gallegos-Arreola MP, Garcia-Ortiz JE, Figuera LE, Puebla-Pérez AM, Morgan-Villela G, Zuniga-Gonzalez GM. Association of the 677C→ T polymorphism in the MTHFR gene with colorectal cancer in Mexican patients. Cancer Genomics-Proteomics 2009; 6(3): 183-188. ISSN: 17906245

16. Le Marchand L, Wilkens LR, Kolonel LN, Henderson BE. The MTHFR C677T polymorphism and colorectal cancer: the multiethnic cohort study. Cancer Epidemiol Biomark Prev 2005; 14(5): 1198-1203. https://doi.org/10.1158/1055-9965.EPI-04-0840

17. Cao HX, Gao CM, Takezaki T, Wu JZ, Ding JH, Liu YT, Li SP, Su P, Cao J, Hamajima N, Tajima K. Genetic polymorphisms of methylenetetrahydrofolatereductase and susceptibility to colorectal cancer. Asian Pac J Cancer Prev 2008; 9(2): 203-208. ISSN: 2476762X

18. Ismaili A, Yari K, Moradi MT, Sohrabi M, Kahrizi D, Kazemi E, Souri Z. IL-1B (C+ 3954T) gene polymorphism and susceptibility to gastric cancer in the Iranian population. Asian Pac J Cancer Prev 2015; 16(2): 841-844. https://doi.org/10.7314/APJCP.2015.16.2.841

19. Yang M, Abdalrahman H, Sonia U, Mohammed AI, Vestine U, Wang M, Ebadi AG, Toughani M. The application of DNA molecular markers in the study of Codonopsis species genetic variation, a review. Cell Mol Biol 2020; 66(2): 23-30. https://doi.org/10.14715/cmb/2020.66.2.3

20. Wen L, Zhang Y, Yang B, Han F, Ebadi AG, Toughani M. Knockdown of Angiopoietin-like protein 4 suppresses the development of colorectal cancer. Cell Mol Biol 2020; 66(5): 117-124. https://doi.org/10.14715/cmb/2020.66.5.21

21. Kazemi E, Kahrizi D. Lack of association between gastric cancer and hopq alleles in Helicobacter pylori. Genetika 2016; 48(3): 893-902. https://doi.org/10.2298/GENSR1603893K

22. Kazemi E, Kahrizi D, Moradi MT, Sohrabi M, Amini S, Mousavi SA, Yari K (a). Association between Helicobacter pylori hopQI genotypes and human gastric cancer risk. Cell Mol Biol 2016; 62(1): 6-9. https://doi.org/10.14715/cmb/2016.62.1.2

23. Yang M, Shi D, Wang Y, Ebadi AG, Toughani M. Study on Interaction of Coomassie Brilliant Blue G-250 with Bovine Serum Albumin by Multispectroscopic. Int J Pept Res Ther 2021; 27(1): 421-431. https://doi.org/10.1007/s10989-020-10096-6

24. Kazemi E, Kahrizi D, Moradi MT, Sohrabi M, Yari K (b). Gastric cancer and helicobacter pylori: impact of hopQII gene. Cell Mol Biol 2016; 62(2): 107-110. https://doi.org/10.14715/cmb/2016.62.2.18

25. Mason JB. Folate status and colorectal cancer risk: a 2016 update. Mol Aspects Med 2017; 53: 73-79. https://doi.org/10.1016/j.mam.2016.11.010

26. Ulrich CM. Nutrigenetics in cancer research—folate metabolism and colorectal cancer. J Nutr 2005; 135(11): 2698-2702. https://doi.org/10.1093/jn/135.11.2698

How to cite this paper:

Lak R, Kazemi E, Aavani P, Alaei F, Pashizeh F. Association analysis of C677T and A1298C polymorphisms in MTHFR gene in patients with colorectal cancer susceptibility. Cent Asian JMed Pharm Sci Innov 2021; 1(2): 74-80.