Analysis of Relation Between C677T Genotype in *MTHFR Gene* and Prostatic Cancer in Iranian Males

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Abstract- Methylenetetrahydrofolate reductase (MTHFR) enzyme is one of the most important enzymes with a pivotal role in the folate metabolism and DNA synthesis pathways. Single nucleotide polymorphism (SNPs) in the coding gene has been related to many medical diseases as well as diverse malignancies including the prostate cancer which is the leading cause of the cancer deaths in men and one of the major public health problems. The goal of this study is to determine the relationship between the MTHFR C677T SNP and the prostate adenocarcinoma in Iranian males attending to the Labbafi-nezhad hospital in Tehran. In this Case-control unmatched study, 67 and 75 paraffinized tissue samples were taken out of the specimens diagnosed previously as the prostatic adenocarcinoma and nodular prostatic hyperplasia for the case and control groups respectively. MTHFR C677T genotyping was done by the use of multiplex ARMS-PCR and frequencies of the alleles were compared between the case and control groups as well as calculating the deviation from Hardy-Weinberg equilibrium and Odds Ratio for the "T" allele regarding the prostatic carcinoma. The observed rates in the control group were not too different from that of expected from Hardy-Weinberg equilibrium (P=0.407). Frequencies of the possible genotypes were as follows: CC, 43.28% *vs.* 42.67%; CT, 49.25% *vs.* 52% and CT, 7.46% vs. 5.33% in the case and control groups respectively (P=0.85). 1.37 times increased risk was found for the homozygote carriers of C677T variant (OR: 1.37, 95% CI: 0.33-5.6; P=0.653) which is however statistically not significant. No association has been evident between the MTHFR 677C>T polymorphism and the risk of prostatic carcinoma in this study confirming the findings of some of the previous attempts; however, (OR: 1.37, 95% CI: 0.33-5.6) implies a slight effect of the homozygote on the carcinogenesis. Thus larger studies especially with a greater number of the smaples are recommended.

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Keywords: MTHFR; Polymorphism; Prostatic carcinoma

Introduction

Methylentetrahydorfolate reductase (MTHFR) enzyme is one of the most important enzymes with a pivotal role in the folate metabolism and DNA synthesis pathways. The enzyme activity is achieved by catalysing the formation of 5-MTHF which is the main circulating form of the folate in the human body serving as the chief donor of the "methyl" group to the homocysteine, the critical amino acid in folate-related DNA handling processes. Distinct single nucleotide polymorphisms of the *MTHFR* gene have been well recognized out of which the C677T is the most common (1), located at 1p36.6 (2) and known by the reference code of "rs-181133" (3). Change of C to T nucleotide causes subsequent substitution of valine instead of alanine in the enzyme protein structure that leads to thermo-liability and thus less functioning state of the enzyme resulting in DNA hypomethylation and inappropriate uracil incorporation both in turn affecting the cellular growth and proliferation pathways as well as playing role in the carcinogenesis (4). The relationship of *MTHFR* C677T polymorphism with many medical diseases has been investigated for years as well as with

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diverse neoplastic conditions among which the prostatic adenocarcinoma is one of the most common public health problems involving one out of every six men being the leading cause of cancer deaths worldwide (5). It has been studied for years in relation to the above mentioned polymorphism with conflicting and sometimes paradoxical results, thus sounding to deserve more related researches. In this study MTHFR C677T polymorphism was investigated in relation to the prostatic adenocarcinoma.

Materials and Methods

Unmatched Case-Control study was designated with regard to the previous literature. Considering the average frequency of the disease (involvement of one out of every six men by prostatic carcinoma) and the rate of *MTHFR* C677T polymorphism in the population which was reported between 20 to 32% (mean: 26%) (1,6), 69 specimens were needed according to Quanto software. Case and Control groups were then collected referring to the Labbafi-Nezhad hospital pathology department archive and selecting 67 and 75 specimens diagnosed since 2006 to 2010 as prostatic adenocarcinoma and nodular prostatic hyperplasia respectively. All prepared slides were reviewed by two pathologists to select appropriate ones followed by sectioning the corresponding paraffin-embedded blocks, into 3-5 micrometer slices. DNA-extraction of the tissue on both groups was done by Rima Kit (reference No.: M-1214) and genotyping of the *MTHFR* C677T polymorphism was carried out via previously defined multiplex ARMS-PCR method using PCR conditions described elsewhere (7) and primer sets modified by inosine nucleotides in order to minimize non-specific reactions as shown in table-1. Reaction patterns have been summarized schematically in figure 1. Finally statistical analysis of the data was done by Chi-square test comparing the frequency of each allele in the groups between which the odds ratio of the calculated T-allele frequency with regard to the prostatic cancer was also compared. In order to confirm appropriate non-carcinomatous population sampling, the deviation of observed genotype frequencies was calculated using student *t*-test from the expected values found by Hardy-Weinberg equilibrium.

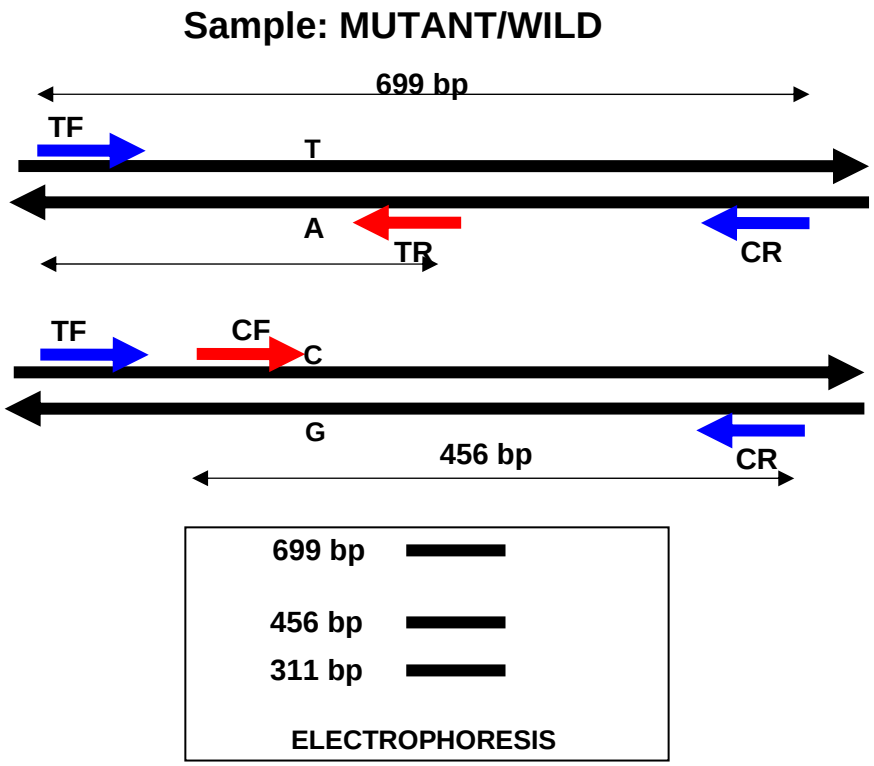

Figure 1. Schematic presentation of PCR reaction patterns.

Table 1. Primers used for *MTHFR* 677C>T genotyping.

Assignation	Nucleotide sequence
CF	CTGAAGCACTTGAAGGAGAAGGIIIIIGCGGGAGC
CR	GACAGCCACCTTTGGGAAACIIIIITTTAAGCAGG
TF	CATCTCTGGGGTCAGAAGCAIIIIIGTCATGAGCC
TR	AGCCTCAAAGAAAAGCTGCGIIIIIATGAAATCGA

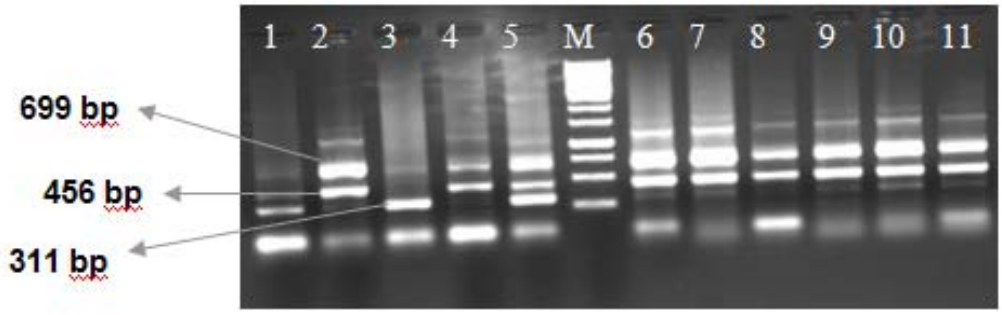

Figure 2. Electrophoreses gel representative of some specimen genotypes. Lanes 2, 4, 6-11: Homozygote CC; lanes 1 and 5: Heterozygote CT; and lane 3: Homozygote TT.

Table 2. Age distribution of the case and control groups.

Variable	Prostatic Carcinoma (n=67)		BPH (n=75)		*P*-value*
	N	%	n	%	
Age					0.057
<=60	28	41.79%	20	26.66%	
>60	39	58.21%	55	73.34%	

* *P*-value<0.05 is significant

Observed genotype frequencies in the control groups were not significantly different from the

expected rates based on Hardy-Weinberg equilibrium (χ^2 =1.86, *P*=0.407, significance level: less than 3.84) .

Age distribution of the patients in the case and control groups have been shown in table 2, revealing no noticeable age biases (*P*=0.057).

Genotype frequencies were calculated in the case and control groups as below (Table 3), followed by measuring the mutant T-allele frequency which is 0.32 in prostatic cancer patients and 0.31 in the control group (Table 4), neither revealing any statistically significant differences (*P*=0.85 and 0.89 respectively).

Table 3. Frequency of genotypes in the case and control groups.

Genotype of *MTHFR* C677T	Prostatic Carcinoma		BPH		*P*-value*
	n	%	n	%	
					0.85
CC	29	43.28	32	42.67	
CT	33	49.25	39	52	
TT	5	7.46	4	5.33	

* *P*-value < 0.05 is significant

Table 4. T-allele frequency in the case and control groups.

	Prostatic carcinoma	BPH	*P*-value*
T allele frequency	0.32	0.31	0.89

* *P*-value < 0.05 is significant

Table 5. Odds ratio of genotypes in the case and control groups.

Genotype	Crude OR (95% CI)	*P-value***
TT *vs*.CC	1.37 (0.33-5.6),	0.6533
(TT or CT) *vs.* CC*	1.43 (0.37-5.6),	0.603
TT *vs.* (CT or CC)**	0.97 (0.50-1.9),	0.941

* *P*-value < 0.05 is significant ** Dominant T allele ** Recessive T allele

Also odds ratio (OR) for the T-allele was defined with regard to the occurrence of the prostatic carcinoma both in dominant and recessive forms which have been summarized in table 5;1.37 fold increased risk of the homozygote TT genotype is seen which is however statistically insignificant(OR:1.37, 95% CI :0.33-5.6, *P*=0.653).

Discussion

The relationship of *MTHFR* C677T polymorphism with many medical diseases has been investigated for years as well as with diverse neoplastic conditions; Increased risk of the cardiovascular disease, essential hypertension and cerebrovascular accident (8-11) even in the children (12-16) are mentionable among the former as are documented hypercoagulability state and recurrent fetal losses (17-19), congenital anomalies (20) increased risk of Down syndrome (21-23) cognitive and behavioral diseases (24-27) and many metabolic alterations including diabetic nephropathy, Wilson's encephalopathy, etc (28-30).

Many types of malignancies have been found associated with *MTHFR* C677>T polymorphism among which CML, AML, ALL and multiple myeloma (31,32), esophagogastric and colorectal cancers (33-35), pancreatobiliary tumors (36,37), squamous cell carcinoma of head and neck and the lungs (38,39) and differentiated thyroid carcinomas (7) can be mentioned. However, squamous or basal cell carcinoma of the skin, urinary bladder cancers and ovaries tumor seems to be unrelated (40-42). In this study *MTHFR* C677T polymorphism was investigated in relation to the prostatic adenocarcinoma.

This study was designated as a case-control model which has been commonly used in the previous literature (43-50), choosing adequate sample number with the help of the Quanto software regarding the mean 26% frequency rate of the mentioned polymorphism in the population.

Paraffin-embedded tissue blocks were used for genotyping by the multiplex ARMS-PCR method. Review of the literature reveals similar approaches (43-50) with one study working on peripheral blood leukocytes instead of the prostatic tissue blocks (51) looking good as an alternative, as well as restriction fragment length polymorphism (RFLP) application in two researches (38,46) which gives rise to quite consistent results according to Ye and Dhillon (44).

Method of data analysis also seems to be comparable with all previous articles (43-50) using the odds ratio (OR) for the mutant allele in both homo- and heterozygote states with regard to the prostatic carcinoma. Also calculating the deviation of the genotypes from the Hardy-Weinberg equilibrium serves as a reliable and logistic method in studying the association of diseases and genetic polymorphisms (45-47).

Considering the results, review of the literature reveals comparable findings most of the time; Collin found no relationship between the *MTHFR* C677T polymorphism and the prostate cancer, neither in homo- nor in heterozygote states after reviewing eight known polymorphism including the *MTHFR* C677T in a large meta-analysis of twelve studies on more than 10,000 carcinoma and 40,000 control specimens (51). So have concluded Kimura Cicek and Relijic (47,48,39) as well as some else during 2000 to 2007 after which some conflicting data has been on public Marchal insists on the *MTHFR* C677T polymorphism as the only one associated with the prostatic carcinoma (OR: 2.9, 95% CI: 1.46-3.3); In 2010 this relationship has been shown by Safavinezhad in Iranian male population with referring to the possible role of folate supplementation preventive therapy (53) which has been confirmed also by Wu, et al in Taiwan (54) who have found *MTHFR* C677T SNP detection useful for anticancer interventions. In conclusion, in our study the relative age distribution of the case & control groups is rather comparable and no significant deviation from the Hardy-Weinberg equilibrium is noted. Frequency of the mutant T-allele is not statistically too different between the case and control groups; however, the Odds Ratio may be

indicative of partial increased risk of the homozygote individuals for developing *adenocarcinoma* of the prostate; thus further studies are recommended with a greater number of the specimens which might confirm the findings.

References

1. Kang SS, Wong PW, Susmano A, Sora J, Norusis M, Ruggie N. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. Am J Hum Genet1991;48(3):536-45.
2. Taioli E, Garza MA, Ahn YO, Bishop DT, Bost J, Budai B, Chen K, Gemignani F, Keku T, Lima CS, Le Marchand L, Matsuo K, Moreno V, Plaschke J, Pufulete M, et al. Meta- and pooled analyses of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and colorectal cancer: a HuGE-GSEC review. Am J Epidemiol 2009;170(10):1207-21.
3. Goyette P, Pai A, Milos R, Frosst P, Tran P, Chen Z, Chan M, Rozen R.Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). Mamm Genome 1998;9(8):652-6. Erratum in: Mamm Genome 1999 Feb;10(2):204.
4. Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am J Epidemiol 2004;159(5):423-43.
5. Muslumanoglu MH, Tepeli E, Demir S, Uludag A, Uzun D, Atli E, Canturk KM, Ozdemir M, Turgut M.The analysis of the relationship between A1298C and C677T polymorphisms of the MTHFR gene with prostate cancer in Eskisehir population. Genet Test Mol Biomarkers 2009;13(5):641-5.
6. Wilcken B, Bamforth F, Li Z, Zhu H, Ritvanen A, Renlund M, Stoll C, Alembik Y, Dott B, Czeizel AE, Gelman-Kohan Z, Scarano G, Bianca S, Ettore G, Tenconi R, et al. Geographical and ethnic variation of the 677C>T allele of 5,10 methylenetetrahydrofolate reductase (MTHFR): findings from over 7000 newborns from 16 areas world wide. J Med Genet 2003;40(8):619-25.
7. Fard-Esfahani P, Fard-Esfahani A, Saidi P, Fayaz S, Mohabati R, Majdi M.An increased risk of differentiated thyroid carcinoma in Iran with the 677C→T homozygous polymorphism in the MTHFR Gene. Cancer Epidemiol 2011;35(1):56-8.
8. Trabetti E.Homocysteine, MTHFR gene polymorphisms, and cardio-cerebrovascular risk. J Appl Genet 2008;49(3):267-82.
9. Lin PT, Cheng CH, Wei JC, Huang YC.Low plasma pyridoxal 5'-phosphate concentration and MTHFR 677C-->T genotypes are associated with increased risk of hypertension. Int J Vitam Nutr Res 2008;78(1):33-40.
10. Niu WQ, You YG, Qi Y. Strong association of methylenetetrahydrofolate reductase gene C677T polymorphism with hypertension and hypertension-in-pregnancy in Chinese: a meta-analysis. J Hum Hypertens 2012;26(4):259-67.
11. Hill LD, York TP, Kusanovic JP, Gomez R, Eaves LJ, Romero R, Strauss JF 3rd.Epistasis between COMT and MTHFR in maternal-fetal dyads increases risk for preeclampsia. PLoS One 2011;6(1):e16681.
12. Sen S, Reddy PL, Grewal RP, Busby M, Chang P, Hinderliter A.Hyperhomocysteinemia is Associated with Aortic Atheroma Progression in Stroke/TIA Patients. Front Neurol 2010;1:131.
13. Dhar S, Chatterjee S, Ray S, Dutta A, Sengupta B, Chakrabarti S. Polymorphisms of methylenetetra-hydrofolate reductase gene as the genetic predispositions of coronary artery diseases in eastern India. J Cardiovasc Dis Res 2010;1(3):152-7.
14. Wernimont SM, Raiszadeh F, Stover PJ, Rimm EB, Hunter DJ, Tang W, Cassano PA. Polymorphisms in serine hydroxymethyltransferase 1 and methylenetetrahydrofolate reductase interact to increase cardiovascular disease risk in humans. J Nutr 2011;141(2):255-60.
15. Chutinet A, Suwanwela NC, Snabboon T, Chaisinanunkul N, Furie KL, Phanthumchinda K. Association between genetic polymorphisms and sites of cervicocerebral artery atherosclerosis. J Stroke Cerebrovasc Dis 2012;21(5):379-85.
16. Alsayouf H, Zamel KM, Heyer GL, Khuhro AL, Kahwash SB, de los Reyes EC.Role of methylenetetrahydrofolate reductase gene (MTHFR) 677C>T polymorphism in pediatric cerebrovascular disorders. J Child Neurol 2011;26(3):318-21.
17. Kim SY, Park SY, Choi JW, Kim do J, Lee SY, Lim JH, Han JY, Ryu HM, Kim MH.Association between MTHFR 1298A>C polymorphism and spontaneous abortion with fetal chromosomal aneuploidy. Am J Reprod Immunol 2011;66(4):252-8.
18. Jeddi-Tehrani M, Torabi R, Zarnani AH, Mohammadzadeh A, Arefi S, Zeraati H, Akhondi MM, Chamani-Tabriz L, Idali F, Emami S, Zarei S.Analysis of plasminogen activator inhibitor-1, integrin beta3, beta fibrinogen, and methylenetetrahydrofolate reductase polymorphisms in Iranian women with recurrent pregnancy loss. Am J Reprod Immunol 2011;66(2):149-56.

19. Seremak-Mrozikiewicz A, Drews K, Kurzawinska G, Bogacz A, Grzeskowiak E, Mrozikiewicz PM.The significance of 1793G>A polymorphism in MTHFR gene in women with first trimester recurrent miscarriages. Neuro Endocrinol Lett 2010;31(5):717-23.
20. Blanton SH, Henry RR, Yuan Q, Mulliken JB, Stal S, Finnell RH, Hecht JT. Folate pathway and nonsyndromic cleft lip and palate. Birth Defects Res A Clin Mol Teratolv 2011;91(1):50-60.
21. Wang SS, Qiao FY, Feng L, Lv JJ. Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome in China. J Zhejiang Univ Sci B 2008;9(2):93-9.
22. Sadiq MF, Al-Refai EA, Al-Nasser A, Khassawneh M, Al-Batayneh Q. Methylenetetrahydrofolate reductase polymorphisms C677T and A1298C as maternal risk factors for Down syndrome in Jordan. Genet Test Mol Biomarkers 2011;15(1-2):51-7.
23. Trabetti E. Homocysteine, MTHFR gene polymorphisms, and cardio-cerebrovascular risk. J Appl Genet 2008;49(3):267-82.
24. Mavros M, Chiriţă V, Popescu O, Ferencz B. The genetic polymorphism of MTHFR gene in schizophrenia. Rev Med Chir Soc Med Nat Iasi 2008;112(1):76-82.
25. Golimbet VE, Panteleeva GP, Bologov PV, Korovaĭtseva GI, Abramova LI. Molecular-genetic approach to the clinical and nosologic differentiation of schizoaffective disorder. Zh Nevrol Psikhiatr Im S S Korsakova 2010;110(10):48-52.
26. Hill M, Shannahan K, Jasinski S, Macklin EA, Raeke L, Roffman JL, Goff DC. Folate supplementation in schizophrenia: a possible role for MTHFR genotype. Schizophr Res 2011;127(1-3):41-5.
27. Saetre P, Vares M, Werge T, Andreassen OA, Arinami T, Ishiguro H, Nanko S, Tan EC, Han DH, Roffman JL, Muntjewerff JW, Jagodzinski PP, Kempisty B, Hauser J, Vilella E, et al. Methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms and age of onset in schizophrenia: a combined analysis of independent samples. Am J Med Genet B Neuropsychiatr Genet 2011;156(2):215-24.
28. Lin L, Guo XZ, Li M. Analysis on relationship of Chinese medicine syndrome pattern with urinary albumin excretion rate and its related factors in early stage diabetic nephropathy. Zhongguo Zhong Xi Yi Jie He Za Zhi 2010;30(9):912-4.
29. Rahimi M, Hasanvand A, Rahimi Z, Vaisi-Raygani A, Mozafari H, Rezaei M, Zargooshi J, Najafi F, Shakiba E. Synergistic effects of the MTHFR C677T and A1298C polymorphisms on the increased risk of micro- and macro-albuminuria and progression of diabetic nephropathy among Iranians with type 2 diabetes mellitus. Clin Biochem 2010;43(16-17):1333-9.
30. Vasilopoulos Y, Sarafidou T, Bagiatis V, Skriapa L, Goutzelas Y, Pervanidou P, Lazopoulou N, Chrousos GP, Mamuris Z. Association between polymorphisms in MTHFR and APOA5 and metabolic syndrome in the Greek population. Genet Test Mol Biomarkers 2011;15(9):613-7.
31. Gromadzka G, Rudnicka M, Chabik G, Przybyłkowski A, Członkowska A. Genetic variability in the methylenetetrahydrofolate reductase gene (MTHFR) affects clinical expression of Wilson's disease. J Hepatol 2011;55(4):913-9.
32. Moon HW, Kim TY, Oh BR, Min HC, Cho HI, Bang SM, Lee JH, Yoon SS, Lee DS. MTHFR 677CC/1298CC genotypes are highly associated with chronic myelogenous leukemia: a case-control study in Korea. Leuk Res 2007;31(9):1213-7.
33. Karas Kuzelicki N, Milek M, Jazbec J, Mlinaric-Rascan I. 5,10-Methylenetetrahydrofolate reductase (MTHFR) low activity genotypes reduce the risk of relapse-related acute lymphoblastic leukemia (ALL). Leuk Res 2009;33(10):1344-8.
34. Larsson SC, Giovannucci E, Wolk A. Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis. Gastroenterology 2006;131(4):1271-83.
35. Etienne-Grimaldi MC, Francoual M, Formento JL, Milano G. Methylenetetrahydrofolate reductase (MTHFR) variants and fluorouracil-based treatments in colorectal cancer. Pharmacogenomics 2007;8(11):1561-6.
36. Vossen CY, Hoffmeister M, Chang-Claude JC, Rosendaal FR, Brenner H. Clotting factor gene polymorphisms and colorectal cancer risk. J Clin Oncol 2011;29(13):1722-7.
37. Kwak SY, Kim UK, Cho HJ, Lee HK, Kim HJ, Kim NK, Hwang SG. Methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) gene polymorphisms as risk factors for hepatocellular carcinoma in a Korean population. Anticancer Res 2008;28(5A):2807-11.
38. Mazaki T, Masuda H, Takayama T. Polymorphisms and pancreatic cancer risk: a meta-analysis. Eur J Cancer Prev 2011;20(3):169-83.
39. 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(13-14):981-5.

40. Cui LH, Shin MH, Kim HN, Song HR, Piao JM, Kweon SS, Choi JS, Yun WJ, Kim YC, Oh IJ, Kim KS. Methylenetetrahydrofolate reductase C677T polymorphism in patients with lung cancer in a Korean population. BMC Med Genet 2011;12:28.
41. Kang SY, Lee SJ, Hong SH, Chung YK, Oh HS, Kim SW, Yim DJ, Kim NK. Polymorphisms of 5,10-methylenetetrahydrofolate reductase and thymidylate synthase in squamous cell carcinoma and basal cell carcinoma of the skin. Mol Med Report 2010;3(5):741-7.
42. Terry KL, Tworoger SS, Goode EL, Gates MA, Titus-Ernstoff L, Kelemen LE, Sellers TA, Hankinson SE, Cramer DW. MTHFR polymorphisms in relation to ovarian cancer risk. Gynecol Oncol 2010;119(2):319-24.
43. Chang SC, Yang YC, Zhang ZF. Single nucleotide polymorphisms in genes of one-carbon metabolism pathway and bladder cancer survival. Cancer Epidemoil Biomarkers Prev 2011;20(4):716-7.
44. Ye S, Dhillon S, Ke X, Collins AR, Day IN. An efficient procedure for genotyping single nucleotide polymorphisms. Nucleic Acids Res 2001;29(17):E88-8.
45. Faure-Delanef L, Quéré I, Chassé JF, Guerassimenko O, Lesaulnier M, Bellet H, Zittoun J, Kamoun P, Cohen D. Methylenetetrahydrofolate reductase thermolabile variant and human longevity. Am J Hum Genet 1997;60(4):999-1001.
46. Wang J, Shete S. A test for genetic association that incorporates information about deviation from Hardy-Weinberg proportions in cases. Am J Hum Genet 2008;83(1):53-63.
47. Kimura F, Franke KH, Steinhoff C, Golka K, Roemer HC, Anastasiadis AG, Schulz WA. Methyl group metabolism gene polymorphisms and susceptibility to prostatic carcinoma. Prostate 2000;45(3):225-31.
48. Cicek MS, Nock NL, Li L, Conti DV, Casey G, Witte JS. Relationship between methylenetetrahydrofolate reductase C677T and A1298C genotypes and haplotypes and prostate cancer risk and aggressiveness. Cancer Epidemiol Biomarkers Prev 2004;13(8):1331-6.
49. Johansson M, Van Guelpen B, Hultdin J, Wiklund F, Adami HO, Bälter K, Grönberg H, Stattin P. The MTHFR 677C --> T polymorphism and risk of prostate cancer: results from the CAPS study. Cancer Causes Control 2007;18(10):1169-74.
50. Bai JL, Zheng MH, Xia X, Ter-Minassian M, Chen YP, Chen F. MTHFR C677T polymorphism contributes to prostate cancer risk among Caucasians: A meta-analysis of 3511 cases and 2762 controls. Eur J Cancer 2009;45(8):1443-9.
51. Collin SM, Metcalfe C, Zuccolo L, Lewis SJ, Chen L, Cox A, Davis M, Lane JA, Donovan J, Smith GD, Neal DE, Hamdy FC, Gudmundsson J, Sulem P, Rafnar T, et al. Association of folate-pathway gene polymorphisms with the risk of prostate cancer: a population-based nested case-control study, systematic review, and meta-analysis. Cancer Epidemiol Biomarkers Prev 2009;18(9):2528-39.
52. Marchal C, Redondo M, Reyes-Engel A, Perea-Milla E, Gaitan MJ, Machuca J, Diaz F, Caballero J, Carnero J. Association between polymorphisms of folate-metabolizing enzymes and risk of prostate cancer. Eur J Surg Oncol 2008;34(7):805-10.
53. Safarinejad MR, Shafiei N, Safarinejad S. Relationship between three polymorphisms of methylenetetrahydrofolate reductase (MTHFR C677T, A1298C, and G1793A) gene and risk of prostate cancer: a case-control study. Prostate 2010;70(15):1645-57.
54. Wu HC, Chang CH, Tsai RY, Lin CH, Wang RF, Tsai CW, Chen KB, Yao CH, Chiu CF, Bau DT, Lin CC. Significant association of methylenetetrahydrofolate reductase single nucleotide polymorphisms with prostate cancer susceptibility in taiwan. Anticancer Res 2010;30(9):3573-7.