Effects of the Catechol-O-Methyltransferase Val108/158Met and Methylenetetrahydrofolate Reductase C677T Gene Polymorphisms on Prostate Cancer Susceptibility

Katekol-O-Metiltransferaz Val108/158Met ve Metilentetrahidrofolat Redüktaz C677T Gen Polimorfizmlerinin Prostat Kanseri Yatkınlığı Üzerindeki Etkileri

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ABSTRACT Objective: Prostate cancer is a multifactorial inherited disease affecting one in six men in industrial countries. A number of mutations in key proteins of the cell cycle machinery or signal transduction pathways have already been associated with prostate cancer; but recently, a number of polymorphisms in proteins that function in less popular cellular pathways have been investigated as possible risk factors. In this study, we evaluated the effects of two gene polymorphisms in enzymes functioning in distinct metabolic pathways on prostate cancer susceptibility. **Material and Methods:** Allele and genotype associations of the *COMT* Val108/158Met and *MTHFR* C677T gene polymorphisms were studied in a total of 112 Turkish prostate cancer patients who constituted our study group while 145 healthy males constituted our control group. Genomic DNA was isolated from the peripheral blood leukocytes of the subjects and analyzed by two different real-time polymerase chain reaction assays. **Results:** The frequency of the heterozygous *COMT* G/A and the homozygous *MTHFR* T/T genotypes were higher in the study group compared to the control group (47.3% versus 34.5%; and 14.3% versus 7.6%, respectively). Prostate cancer subjects with the heterozygous *MTHFR* C/T genotype had higher prostate specific antigen values. Nevertheless, no statistically significant association was found between these polymorphisms alone or in combination and the risk of developing prostate cancer. **Conclusion:** We suggest that functional gene polymorphisms in metabolic proteins predispose subjects to increased risk of developing cancer; but, not as strong as mutations in proto-oncogenes or tumor-suppressor genes.

Key Words: Prostatic neoplasms; prostatic neoplasms; catechol O-methyltransferase; methylenetetrahydrofolate reductase (NADPH2)

ÖZET Amaç: Prostat kanseri endüstrileşmiş ülkelerde her altı erkekten birini etkileyen multifaktöriyel kalıtsal bir hastalıktır. Halihazırda hücre döngüsü çarkının veya sinyal aktarma yolaklarının anahtar proteinlerindeki pek çok mutasyon prostat kanseri ile ilişkilendirilmiştir; fakat, son zamanlarda daha az popüler hücresel yolaklarda işlev yapan proteinlerdeki pek çok polimorfizm de olası risk faktörleri olarak araştırılmaktadır. Bu çalışmada, prostat kanserine yatkınlık yaratabilecek farklı metabolik yolaklarda yer alan enzimlerdeki iki gen polimorfizminin etkilerini değerlendirdik. **Gereç ve Yöntemler:** Çalışma grubumuzu oluşturan toplam 112 Türk prostat kanseri hastasında ve kontrol grubumuzu oluşturan 145 sağlıklı erkekte *COMT* Val108/158Met ve *MTHFR* C677T gen polimorfizmlerinin alel ve genotip ilişkileri çalışıldı. Genomik DNA olguların periferik kan lökositlerinden izole edildi ve iki farklı gerçek-zamanlı polimeraz zincir reaksiyonu testiyle incelendi. **Bulgular:** Heterozigot *COMT* G/A ve homozigot *MTHFR* T/T genotiplerinin sıklığı çalışma grubunda kontrol grubuna göre daha yüksekti (sırasıyla %34.5'a karşılık %47.3 ve %7.6'ya karşılık %14.3). Heterozigot *MTHFR* C/T genotipine sahip prostat kanserli olguların prostat spesifik antijen değerleri de daha yüksekti. Yine de, tek başına veya kombine olarak bu polimorfizmler ile prostat kanseri gelişimi riski arasında istatistiksel olarak anlamlı bir ilişki bulunmadı. **Sonuç:** Metabolik proteinlerdeki fonksiyonel gen polimorfizminin kişileri artmış kanser gelişme riskine yatkın hale getirdiğini, fakat protoonkogenler veya tümör baskılayıcı genlerdeki mutasyonlar kadar güçlü olmadığını düşünüyoruz.

Anahtar Kelimeler: Prostat tümörleri; çok biçimlilik, tek nükleotid; katekol O-metiltransferaz; metilentetrahidrofolat redüktaz (NADPH2)

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Prostate cancer is the most commonly diagnosed cancer in men and after lung and colon cancers, it is the third leading cause of cancer-related mortality.[1] Several environmental and genetic factors are involved in its etiology. Genetic factors usually include mutations in certain proto-oncogenes or tumor-suppressor genes that encode for key proteins of the cell cycle machinery or signaling pathways; but recently, also screening of functional polymorphisms in less popular genes, like the ones encoding components of different metabolic pathways, have gained the attention of prostate cancer researchers.

Various carcinogenic metabolites, including catechol estrogens, play a role in malignant transformations. Genotoxic effects of these compounds are associated with risk of developing ovarian, breast, endometrial and prostate cancer.[2-5] An enzyme capable of neutralizing the genotoxic effects of these metabolites is catechol-*O*-methyltransferase (COMT). A common and functional single nucleotide polymorphism in its gene (G→A; Val108/158Met) causes a 3- to 4-fold decrease in enzyme activity. The polymorphic low-activity allele is therefore called COMT-L (*L*ow; Met for Methionine; or A for Adenine) and the more common high-activity allele COMT-H (*H*igh; Val for Valine; or G for Guanine).[6]

Another enzyme important in carcinogenesis is 5,10- methylenetetrahydrofolate reductase (MTHFR) that catalyses the reduction of 5,10-methylenetetrahydrofolate (5,10- methyleneTHF) to 5- methyltetrahydrofolate (5- methylTHF), thus generating the active form of folate required for remethylation of homocysteine to methionine. The functional C677T single nucleotide polymorphism of the *MTHFR* gene falls into the folate binding site of the enzyme and causes a substitution of an alanine amino acid residue to a valine amino acid residue at codon 222 (Ala222Val).[7,8] Individuals with the homozygous polymorphic 677TT genotype have only about 30% of the in vitro MTHFR enzymatic activity as compared to those with the 677CC homozygous wild-type genotype. Deficiency in MTHFR enzyme activity is associated with an increase in plasma homocysteine, which in turn is associated with an increased risk of developing neural tube defects, vascular diseases and cancer.[9]

In this study, we wanted to evaluate the combined effect of the *COMT* Val108/158Met and *MTHFR* C677T gene polymorphisms as possible risk factors for developing prostate cancer in Turkish men.

MATERIAL AND METHODS

SUBJECTS

One-hundred-and-twelve Turkish patients with sporadic prostate cancers who admitted Department of Urology, Ege University Medical Faculty between September 2005 and February 2009 constituted the study group. The tumors of the patients were evaluated with digital rectal examination, transrectal ultrasonography and pelvic computed tomography. All the patients were histologically confirmed cases of prostate adenocarcinoma. Gleason score, pathological stage, and prostate-specific antigen (PSA) values of all subjects are listed in Table 1. The control group consisted of 145 voluntary men, which were examined during the same time period in the Urology Department of Ege University Medical Faculty and clinically found to be healthy. They had no history of malignant or non-malignant prostate disease. No relationship existed between any of the subjects in the study and con-

TABLE 1: Clinical characteristics of the study group subjects.

	n	%
Total number	112	
Age (yr)		
Mean± Standart deviation	65.28 ± 3.53	
PSA (ng/ml)		
< 4	10	8.92
4-12	61	54.46
≥ 12	41	36.62
T Stage		
I/II	72	64.28
III/IV	40	35.72
Gleason Score		
< 7	42	37.50
≥ 7	70	62.50

trol groups. This study complied with the Helsinki Declaration 2008 and was approved by the Ege University Medical Faculty Ethical Committee. All of the participants accepted to participate the study with signed informed consents.

SAMPLES AND DNA EXTRACTION

Peripheral blood samples from all subjects were collected in EDTA-containing tubes and genomic DNA was isolated with the High Pure PCR Template Preparation Kit according to the manufacturer's protocol (Roche Applied Science, Germany). DNA was also isolated from LNCaP, DU145 and PC3 prostate cancer cell lines and the cervical cancer cell line HeLa; and stored together with the other DNA samples at -20°C until use.

COMT AND *MTHFR* GENOTYPE ANALYSIS

Genotyping of the subjects and cell lines for the *COMT* Val108/158Met and *MTHFR* C677T polymorphisms were performed by real-time polymerase chain reaction (PCR) using the LightCycler® instrument (Roche Applied Science, Germany). For this purpose, specific primers and hybridization probes (TIB MOLBIOL, Germany) for each analyzed polymorphism were used in combination with the LightCycler DNA Master Hybridization Probes Kit (Roche Applied Science, Germany). Real-time PCR was performed according to the protocols of Rakvag et al. and Aslanidis et al. for the *COMT* Val108/158Met and *MTHFR* C677T polymorphisms, respectively.[10,11] Tm values obtained from the amplicons resulting after PCR were 65.45°C for the wild-type *COMT* G allele and 58.75°C for its polymorphic A allele whereas they were 63.1°C for the wild-type *MTHFR* C allele and 55.2°C for its polymorphic T allele.

STATISTICAL ANALYSIS

Allele and genotype frequencies of the *COMT* Val108/158Met and *MTHFR* C677T polymorphisms in the study and control group subjects were compared using the Kruskal Wallis test and Ehwin program. The relationship between the polymorphic status and clinicopathological parameters was examined by using the c^2 test. Significance of the test was set at $p< 0.05$ and the results were evaluated with SPSS for Windows *version.*11.0.

RESULTS

For the *COMT* Val108/158Met polymorphism 31 subjects in the study group carried the wild-type genotype (27.7%), 53 subjects the heterozygote genotype (47.3%) and 28 subjects the polymorphic genotype (25.0%); whereas, in the control group 59 subjects carried the wild-type genotype (40.7%), 50 subjects had the heterozygote genotype (34.5%) and 36 subjects had the polymorphic genotype (24.8%). Although the heterozygous genotype in the study group more, no statistically significant difference was observed between the two groups ($c^2= 5.654$; $p= 0.059$). The same was true when the allele frequencies were investigated. Again, although there was an increase of the Met haplotype in the study group, no statistically significant difference was observed between the two groups ($c^2= 0.233$; $p= 0.629$). All *COMT* Val108/ 158Met polymorphism genotype and haplotype results are summarized in Table 2.

The genotype distribution for the *MTHFR* C677T polymorphism in both groups were as follows: 54 subjects in the study group carried the wild-type genotype (48.2%), 42 subjects had the heterozygote genotype (37.5%), and 16 subjects had the polymorphic genotype (14.3%); whereas 70 subjects in the control group carried the wild-type genotype (48.3%), 64 subjects had the heterozygote genotype (44.1%), and 11 subjects had the polymorphic genotype (7.6%). Although there was an increase of the homozygous genotype in the study group, no statistically significant difference in genotype distribution or allele frequencies of the *MTHFR* C677T polymorphism was observed between two groups ($p> 0.05$). Again, all *MTHFR* C677T polymorphism genotype and haplotype results are summarized in Table 2.

The median ages of the study and control group subjects were 65.28 ± 3.53 and 62.50 ± 13.43 years, respectively. There were no associations between the age, tumor stages and Gleason scores of the study group subjects with the *COMT* or *MTHFR* genotypes (Table 3, 4). Even though statis-

TABLE 2: Genotype distributions and allele frequencies of the *COMT* Val108/158Met and *MTHFR* C677T gene polymorphisms in the study and control groups

Gene Polymorphism	Genotype/Haplotype	Study Group (N = 112) n (%)	Control Group (N = 145) n (%)	P
COMT Val108/158Met	G/G (Wild Type)	31 (27.7)	59 (40.7)	> 0.05
	G/A (Heterozygote)	53 (47.3)	50 (34.5)	
	A/A (Homozygote)	28 (25.0)	36 (24.8)	
	G	125 (55.8)	168 (57.9)	> 0.05
	A	99 (44.2)	122 (42.1)	
MTHFR C677T	C/C (Wild Type)	54 (48.2)	70 (48.3)	> 0.05
	C/T (Heterozygote)	42 (37.5)	64 (44.1)	
	T/T (Homozygote)	16 (14.3)	11 (7.6)	
	C	150 (67.0)	204 (70.3)	> 0.05
	T	74 (33.0)	86 (29.7)	

tically not significant, subjects with a heterozygote *MTHFR* C/T genotype had higher prostate specific antigen (PSA) values (Table 5).

When the combined genotype distribution and haplotype frequency of both polymorphisms was studied, no statistically significant difference was found between the study and control groups (p> 0.05; data not shown).

Genotype distribution of the analyzed gene polymorphisms for the cancer cell lines were as follows; for the COMT Val108/158Met polymorphism the PC3 and HeLa cell lines were found to carry

TABLE 3: Genotype distribution of the COMT Val108/158Met and *MTHFR* C677T polymorphisms with the tumor stage of prostate cancer patients.

		Tumor Stage				
Gene Polymorphism	Genotype	I*	II*	III*	IV*	P
COMT Val108/158Met	GG	4 (30.8)	17 (27.0)	7 (26.9)	3 (30.0)	> 0.05
	GA	4 (30.8)	30 (47.6)	15 (57.7)	4 (40.0)	
	AA	5 (38.5)	16 (25.4)	4 (15.4)	3 (30.0)	
MTHFR C677T	CC	5 (38.5)	33 (52.4)	8 (30.8)	8 (80.0)	> 0.05
	CT	7 (53.8)	22 (34.9)	11 (42.3)	2 (20.0)	
	TT	1 (7.7)	8 (12.7)	7 (26.9)	0 (0.0)	

* n (%).

TABLE 4: Genotype distribution of the *COMT* Val108/158Met and *MTHFR* C677T polymorphisms with the Gleason score of prostate cancer patients.

		Gleason Score				
Gene Polymorphism	Genotype	6*	7*	8*	9*	P
COMT Val108/158Met	GG	12 (28.6)	14 (28.6)	2 (18.2)	3 (33.3)	> 0.05
	GA	18 (42.9)	22 (44.9)	7 (63.6)	5 (55.6)	
	AA	12 (28.6)	13 (26.5)	2 (18.2)	1 (11.1)	
MTHFR C677T	CC	23 (54.8)	22 (44.9)	4 (36.4)	4 (44.4)	> 0.05
	CT	14 (33.3)	20 (40.8)	4 (36.4)	4 (44.4)	
	TT	5 (11.9)	7 (14.3)	3 (27.3)	1 (11.1)	

* n (%).

TABLE 5: Genotype distribution of the *COMT* Val108/158Met and *MTHFR* C677T polymorphisms with prostate specific antigen of prostate cancer patients.

	Genotype	n	PSA	P**
COMT Polymorphism				
Val108/158Met	GG	31	59.97	> 0.05
	GA	53	57.58	
	AA	28	50.61	
MTHFR Polymorphism				
C677T	CC	54	55.05	> 0.05
	CT	42	59.62	
	TT	16	53.22	

PSA: ng/ml, * Mean rank, **Kruskal-Wallis Test.

the wild-type genotype, and the LNCaP and DU145 cell lines the heterozygote genotype. For the MTHFR C677T polymorphism the PC3, HeLa and LNCaP cells lines were found to carry the heterozygote genotype and the DU145 cell line the homozygote genotype.

DISCUSSION

In this study, we evaluated the impact of the *COMT* Val108/158Met and *MTHFR* C677T polymorphisms as risk factors in the evolution of prostate cancer in a total of 257 subjects. Both genes encode enzymes that have important roles in two distinct metabolic pathways.

The COMT enzyme is involved in the metabolism of estrogens in tissues like prostate, breast and uterus. As a phase II metabolic enzyme, its activity increases the protective conjugation of reactive catechol estrogens produced by the cytochrome P450 superfamily member CYP1B1, another enzyme involved in the metabolic pathway of estrogens.[12] Since the Val108/158Met polymorphism decreases the enzymatic activity of COMT by 3- to 4- fold, it can be expected that this may result in the local increase of unconjugated carcinogenic estrogen metabolites; thus, in an increased risk of local carcinogenesis. The involvement of the polymorphic *COMT* allele in carcinogenesis was first shown for breast cancer; but, was than later rejected by another study.[13,14] According to our *COMT* results the incidence of the heterozygous (G/A) genotype was higher in the prostate cancer group compared to the control group, even though we could not find a statistically significant difference between the two groups. Our results are similar to the results of Suzuki et al., where the G/A genotype of the *COMT* gene showed a weak tendency toward increased prostate cancer risk. In their study they could also show that this heterozygous *COMT* genotype, together with estrogen-related enzyme aromatase *(CYP19)* and estrogen receptor alpha (*ESR1*) gene polymorphisms, increased the risk of developing cancer in Japanese men with a familial prostate carcinoma history.[5] Another and similar study was conducted on French prostate cancer subjects, and again the polymorphic *COMT* allele increased the risk of prostate cancer when it was found in combination with another gene polymorphism; this time with the V432L polymorphism of the *CYP1B1* gene, which increases its enzymatic activity.[15] In a study performed by Cunningham et al., 46 polymorphisms in 25 genes involved in the androgen and estrogen metabolic pathways were investigated as risk factors for sporadic and familial prostate cancer. At the end of their study, they showed that polymorphisms in *ESR1* and *CYP1B1*, among other gene polymorphisms, were associated with clinical variables like disease stage, grade and/or node status; but this was not true for polymorphisms in the *COMT* and *CYP19* genes. However, these associations were statistically not significant.[16] Low et al. analyzed a total of five polymorphisms in the *COMT*, *CYP19*, *ESR1* and sex hormone binding globulin (*SHBG*) genes and found only one polymorphism in the *ESR1* gene to be associated with increased risk for prostate cancer in British men.[17] However, it should be taken into consideration that the later two studies were undertaken on a smaller number of prostate cancer and control subjects, compared to the study with French men.

The MTHFR enzyme, on the other hand, is involved in the metabolism of folates. It irreversibly converts 5,10- methyleneTHF to 5- methylTHF, the predominant circulating folate and carbon donor for remethylation processes.[18] This conversion is critical in controlling intracellular homocysteine levels and maintaining adequate levels of S-ade-

nosylmethionine (SAM), the universal methyl donor of all methylation reactions.[19,20] A reduction in 5- methylTHF levels would lower the source of methyl groups for DNA methylation and thus promotes cancer in organs like the liver and prostate.[21] Meanwhile, the immediate precursor of SAM is methionine which is necessary to maintain nucleotide pools and synthesize polyamines. Limited supply of methionine can cause a cell cycle arrest at S and G2-M phase in methionine-dependent tumor cells, and by this way sensitizes them to cell cycle-specific chemotherapeutic agents.[22,23] Since the C677T polymorphism decreases the enzymatic activity of MTHFR by 30%, it is expected to lead to carcinogenesis by affecting both DNA methylation and DNA synthesis. According to our results on the *MTHFR* C677T polymorphism, the homozygote (T/T) genotype ratio was higher in the study group; whereas, the heterozygote (C/T) genotype ratio was higher in the control group. However, there was no statistically significant difference between these two groups. Marchal et al. analyzed four polymorphisms in three genes that encode folate-metabolizing enzymes, and only found a significant association between the *MTHFR* C677T gene polymorphism and prostate cancer.[24] Different from our study, they found the heterozygote genotype more frequent in the study group compared to the control group. Similarly, Singal et al. found the *MTHFR* C/T heterozygote genotype to be associated with a reduced risk of prostate cancer as did Van Guelpen et al., although the result of the latter study was statistically not significant.[25,26] Interestingly, other studies showed no association of this particular *MTHFR* gene polymorphism with prostate cancer.[27,28] Moreover, in a meta-analysis where 3511 cases and 2762 controls were studied, the polymorphic T allele was even found having a protective effect for developing prostate cancer.[29] A larger meta-analysis which was accomplished on 10745 cases and 40158 controls, found no association of this polymorphic T allele with prostate cancer.[30] As it is seen from these, the association of this *MTHFR* C677T gene polymorphism with prostate cancer is still contradictory.

We also investigated the relationship of the *COMT* and *MTHFR* genotypes with clinical parameters of the study group subjects, however we did not find any associations. Only subjects with a heterozygote *MTHFR* C/T genotype had higher PSA values, even though statistically not significant. Heijmans et al. also evaluated the effects of the *MTHFR* C677T gene polymorphism as a risk factor for carcinogenesis and showed that the homozygote T/T genotype was significantly associated with prostate, kidney and bladder cancer in men of an older age.[31] Another study, in turn, found that the polymorphic T allele of *MTHFR* tended to be associated with tumor grade.[32] The reason why we could not find any association between these parameters and the investigated gene polymorphisms might be the fact that our study group was too small in size.

In the present study, we also investigated the presence of the *COMT* Val108/158Met and *MTHFR* C677T gene polymorphisms in four cell lines that are commonly used in cancer research: the human prostate adenocarcinoma cell lines LNCaP (lymph node metastasis), DU145 (brain metastasis) and PC3 (bone metastasis); and the human cervical cancer cell line HeLa. It is known that LNCaP is androgen-sensitive and does express PSA; whereas, PC3 is not androgen-sensitive but does express PSA; and DU145 is neither hormone sensitive nor does it express PSA. Although, methionine-dependency was shown for the PC3 prostate cancer cell line, its *MTHFR* C677T genotype was not analyzed beforehand, as well as its association with that specific phenotype.[22] Interestingly, we found that the PC3, LNCaP and HeLa cancer cell lines where heterozygous; and the DU145 prostate adenocarcinoma cell line was homozygous for the *MTHFR* C677T gene polymorphisms. We therefore suggest that the polymorphic T allele is associated with reduced risk of developing prostate cancer, despite studies that do not support this view. Interestingly, we found that the androgen-sensitive LNCaP prostate carcinoma cell line carried the heterozygous genotype for the *COMT* Val108/158Met polymorphism. However overall, the polymorphic A allele of the *COMT* polymorphism was found less frequ-

ent in the analyzed cancer cell lines compared to the polymorphic T allele of the *MTHFR* polymorphism.

This study is the first one examining the combined effect of the *COMT* Val108/158Met and *MTHFR* C677T gene polymorphisms as possible risk factors for developing prostate cancer. The enzymes encoded by these two genes have important roles in distinct metabolic pathways: the estrogen and folate pathways. Although many studies suggest that both gene polymorphisms on their own are associated with risk of developing cancer, we could not find a combined effect when the polymorphic genotypes or allelles were present together in prostate cancer subjects.

In summary, in this study we evaluated the effects of the *COMT* Val108/158Met and *MTHFR* C677T gene polymorphisms on prostate cancer susceptibility in Turkish men. We could not find a statistically significant association of the respective variants either alone or together with the risk of developing prostate cancer. Nevertheless, the ratios of the heterozygous *COMT* G/A genotype and the homozygous *MTHFR* T/T genotype were slightly higher in the study group subjects than in the controls. Furthermore, prostate cancer subjects with the heterozygous *MTHFR* C/T genotype had higher PSA values. We therefore suggest that functional polymorphisms in genes that encode metabolic proteins contribute to increased risk of developing cancer; although, not as dramatic as mutations in proto-oncogenes or tumor-suppressor genes that encode key proteins of the cell cycle machinery or signaling pathways.

REFERENCES

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al. Cancer statistics, 2006. CA Cancer J Clin 2006;56(2):106-30.
2. Garner EI, Stokes EE, Berkowitz RS, Mok SC, Cramer DW. Polymorphisms of the estrogen-metabolizing genes CYP17 and catechol-O-methyltransferase and risk of epithelial ovarian cancer. Cancer Res 2002;62(11):3058-62.
3. McGrath M, Hankinson SE, Arbeitman L, Colditz GA, Hunter DJ, De Vivo I. Cytochrome P450 1B1 and catechol-O-methyltransferase polymorphisms and endometrial cancer susceptibility. Carcinogenesis 2004;25(4):559-65.
4. Mitrunen K, Jourenkova N, Kataja V, Eskelinen M, Kosma VM, Benhamou S, et al. Polymorphic catechol-O-methyltransferase gene and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2001;10(6):635-40.
5. Suzuki K, Nakazato H, Matsui H, Koike H, Okugi H, Kashiwagi B, et al. Genetic polymorphisms of estrogen receptor alpha, CYP19, catechol-O-methyltransferase are associated with familial prostate carcinoma risk in a Japanese population. Cancer 2003;98 (7):1411-6.
6. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 1996;6(3):243-50.
7. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10(1):111-3.
8. 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(4):551.
9. Bailey LB, Rampersaud GC, Kauwell GP. Folic acid supplements and fortification affect the risk for neural tube defects, vascular disease and cancer: evolving science. J Nutr 2003;133(6):1961S-1968S.
10. Aslanidis C, Nauck M, Schmitz G. High-speed prothrombin G-->A 20210 and methylenetetrahydrofolate reductase C-->T 677 mutation detection using real-time fluorescence PCR and melting curves. Biotechniques 1999;27 (2):234-6, 238.
11. Rakvåg TT, Klepstad P, Baar C, Kvam TM, Dale O, Kaasa S, et al. The Val158Met polymorphism of the human catechol-O-methyltransferase (COMT) gene may influence morphine requirements in cancer pain patients. Pain 2005;116(1-2):73-8.
12. Sissung TM, Price DK, Sparreboom A, Figg WD. Pharmacogenetics and regulation of human cytochrome P450 1B1: implications in hormone-mediated tumor metabolism and a novel target for therapeutic intervention. Mol Cancer Res 2006;4(3):135-50.
13. Gaudet MM, Chanock S, Lissowska J, Berndt SI, Peplonska B, Brinton LA, et al. Comprehensive assessment of genetic variation of catechol-O-methyltransferase and breast cancer risk. Cancer Res 2006;66(19):9781-5.
14. Lavigne JA, Helzlsouer KJ, Huang HY, Strickland PT, Bell DA, Selmin O, et al. An association between the allele coding for a low activity variant of catechol-O-methyltransferase and the risk for breast cancer. Cancer Res 1997;57(24): 5493-7.
15. Cussenot O, Azzouzi AR, Nicolaiew N, Fromont G, Mangin P, Cormier L, et al. Combination of polymorphisms from genes related to estrogen metabolism and risk of prostate cancers: the hidden face of estrogens. J Clin Oncol 2007;25(24):3596-602.
16. Cunningham JM, Hebbring SJ, McDonnell SK, Cicek MS, Christensen GB, Wang L, et al. Evaluation of genetic variations in the androgen and estrogen metabolic pathways as risk factors for sporadic and familial prostate cancer. Cancer Epidemiol Biomarkers Prev 2007; 16(5):969-78.
17. Low YL, Taylor JI, Grace PB, Mulligan AA, Welch AA, Scollen S, et al. Phytoestrogen exposure, polymorphisms in COMT, CYP19, ESR1, and SHBG genes, and their associations with prostate cancer risk. Nutr Cancer 2006;56(1):31-9.
18. Bailey LB, Gregory JF 3rd. Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: metabolic significance, risks and impact on folate requirement. J Nutr 1999;129(5):919-22.

19. Kitada C, Ohtaki T, Masuda Y, Masuo Y, Nomura H, Asami T, et al. Design and synthesis of ETA receptor antagonists and study of ETA receptor distribution. J Cardiovasc Pharmacol 1993;22(Suppl 8):S128-31.
20. Goyette P, Frosst P, Rosenblatt DS, Rozen R. Seven novel mutations in the methylenetetrahydrofolate reductase gene and genotype/phenotype correlations in severe methylenetetrahydrofolate reductase deficiency. Am J Hum Genet 1995;56(5):1052-9.
21. Counts JL, Goodman JI. Hypomethylation of DNA: an epigenetic mechanism involved in tumor promotion. Mol Carcinog 1994;11(4): 185-8.
22. Pavillard V, Drbal AA, Swaine DJ, Phillips RM, Double JA, Nicolaou A. Analysis of cell-cycle kinetics and sulfur amino acid metabolism in methionine-dependent tumor cell lines; the effect of homocysteine supplementation. Biochem Pharmacol 2004;67(8): 1587-99.
23. Stern PH, Hoffman RM. Enhanced in vitro selective toxicity of chemotherapeutic agents for human cancer cells based on a metabolic defect. J Natl Cancer Inst 1986;76(4):629-39.
24. Marchal C, Redondo M, Reyes-Engel A, Perea-Milla E, Gaitan MJ, Machuca J, et al. Association between polymorphisms of folate-metabolizing enzymes and risk of prostate cancer. Eur J Surg Oncol 2008;34(7):805-10.
25. Singal R, Ferdinand L, Das PM, Reis IM, Schlesselman JJ. Polymorphisms in the methylenetetrahydrofolate reductase gene and prostate cancer risk. Int J Oncol 2004;25(5): 1465-71.
26. Van Guelpen BR, Wirén SM, Bergh AR, Hallmans G, Stattin PE, Hultdin J. Polymorphisms of methylenetetrahydrofolate reductase and the risk of prostate cancer: a nested case-control study. Eur J Cancer Prev 2006;15(1):46-50.
27. Muslumanoglu MH, Tepeli E, Demir S, Uludag A, Uzun D, Atli E, et al. 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.
28. 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.
29. 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.
30. Collin SM, Metcalfe C, Zuccolo L, Lewis SJ, Chen L, Cox A, 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.
31. Heijmans BT, Boer JM, Suchiman HE, Cornelisse CJ, Westendorp RG, Kromhout D, et al. A common variant of the methylenetetrahydrofolate reductase gene (1p36) is associated with an increased risk of cancer. Cancer Res 2003;63(6):1249-53.
32. Kimura F, Franke KH, Steinhoff C, Golka K, Roemer HC, Anastasiadis AG, et al. Methyl group metabolism gene polymorphisms and susceptibility to prostatic carcinoma. Prostate 2000;45(3):225-31.