RESEARCH COMMUNICATION

Effects of the MTHFR C677T Polymorphism on Prostate Specific Antigen and Prostate Cancer

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Abstract

Prostate cancer is the most common malignancy and the second leading cause of cancer related deaths among men in many countries. Serum levels of prostate-spesific antigen (PSA) have attracted attention for prediction purposes. The methylenetetrahydrofolate reductase (MTHFR) gene play a critical role in cancer development, but its potential impact on prostate cancer has not been well studied. The C677T variant lies in exon 4 at the folate binding site of the MTHFR gene and results in substitution of an alanine by a valine residue. The present study was carried out 55 cases with prostate cancer and 50 healthy men. Polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), and agarose gel electrophoresis techniques were employed to determine MTHFR C677T mutation. The frequencies of the CT genotype (p= 0.025) and T allele (p= 0.023) was found to be higher in control subjects when compared with patients group. No statistical difference was found between the alleles of MTHFR and PSA levels after (PSA-BT)/ before (PSA-AT) antiandrogen treatment or tumor stages. We suggest that the heterozygote CT genotype and the 677T allele of the MTHFR polymorphism might be associated with an decreased prostate cancer risk.

Keywords: Prostate cancer - PSA - MTHFR - polymorphism - RFLP

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Introduction

Prostate cancer is the most common malignancy and the second leading cause of cancer-related deaths among men in many countries. The incidence and prevalence account for 15.3% in developed countries and 4.3% in developing countries. Diet, hormone levels, age, and both chemical and carcinogen exposure are the potential risk factors for prostate cancer. Although the etiology of prostate cancer is largely unknown, both genetic and environmental factors might contribute to the development of prostate cancer (Gambert et al., 2001; Parkin et al., 2001; Ilic et al., 2011).

Prostate cancer has a long latency period however serum levels of prostate-specific antigen (PSA) showed an early rise by the specific activity of tumour cells, therefore PSA is the major serum marker of prostate cancer. Recently total serum levels of homocysteine has been identified as a marker of prostate cancer (Lasalvia-Prisco et al., 2003).

Genetic polymorphisms that alter the activity of the enzymes of biotransformation have been reported to be associated with cancer development and progression. Previous reports revealed that polymorphisms in the MTHFR gene play a critical role in cancer development, but their potential impact on prostate cancer has not been well studied (Rosenblat et al., 2001; De Mattia et al., 2009).

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme (EC 1.5.1.20) in folate metabolism which results in both DNA synthesis, repair and intracellular methylation reactions. MTHFR regulates the entrance of folates into the methylation cycle (Goyette et al., 1998; Krajinovic et al., 2004). Together with other enzymes MTHFR irreversibly catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulating form of folate and the methyl donor for re-methylation processes, which is involved in the homocysteine/ methionine conversion. Methionine is then activated to S-adenosylmethionine, a universal methyl donor in numerous transmethylation reactions including methylation of DNA, proteins, lipids, and synthesis of polyamines. This conversion is critical in controlling intracellular homocysteine levels and maintaining adequate levels of S-adenosylmethionine. MTHFR enzyme function may effect cancer risk in two ways. The impaired MTHFR activity might result in accumulation of 5,10-methylenetetrahydrofolate and therefore a reduction in 5-methyltetrahydrofolate levels which lowers

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the source of methyl groups for DNA methylation, an important epigenetic process in gene regulation, leading to modifications in DNA conformation and gene expression. In the case of a reduction in the substrate of the MTHFR, 5,10-methylenetetrahydrofolate, the levels of thymidylate biosynthesis are decreased leading to deoxynucleotide pool imbalances and increased uracil misincorporation into DNA which potentially causes to chromosomal damage, DNA double-strand breaks, impaired DNA repair, and DNA hypomethylation (Frosst and Blom et al., 1995; Frosst and Rosenblatt et al., 1995; Blount et al., 1997; Bailey et al., 1999; Stern et al. 2000; Friso et al., 2002; Cicek et al., 2004).

MTHFR gene is located on 1p36.3 and has 11 exons spanning 2.2 kb (cDNA Gen Bank accession number U09806) (Goyette et al., 1998; Pereira et al., 2006). Several polymorphic sites have been identified on MTHFR gene. A common 677 C-T transition (rs1801133) in the MTHFR gene is a well identified genetic determinant of hyperhomocysteinemia, and results in a termolabile protein with a decreased enzymatic activity. The C677T variant lies in exon 4 at the folate binding site of the MTHFR gene and results in the substitution of an alanine by a valine (Ala222Val) residue. Some reports have shown an association between MTHFR gene polymorphism with cancer development. However, not all researchers found this association (Frosst and Blom et al., 1995; Bailey et al., 1999; Kimura et al., 2000; Stern et al., 2000; Heijman et al., 2003; Cicek et al., 2004; Krajinovic et al.,2004; Signal et al., 2004; De Mattia et al., 2009).

In the present study, investigation of the effects of MTHFR C677T polymorphism on prostate cancer development and progression in Turkish population was the aim.

Materials and Methods

Study participants and clinical investigation

The MTHFR C677T polymorphism was studied in 55 cases with prostate cancer. All the subjects were selected from Istanbul Uskudar Hospital and the diagnosis of prostate carcinoma was confirmed by the clinical and laboratory examinations and confirmed by pathologic examination. The blood samples were collected from the patients before and after the antiandrogene treatment had been started. 50 healthy men without any symptoms of prostate cancer were included as the control group.

PCR-based detection of MTHFR mutation.

Blood specimens were collected in tubes containing EDTA, and DNA samples were extracted from whole blood with a salting-out procedure (Miller et al., 1998). Polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), and agarose gel electrophoresis techniques were used to determine MTHFR C677T mutation. After amplification of the isolated DNA with PCR, the MTHFR C677T mutation was detected by cutting the PCR product with the restriction endonuclease HinfI (MBI Fermentas, Ontario, Canada) (Frosst and Blom et al., 1995; Frosst and Rosenblatt et al., 1995). The digested DNAs were separated on 3% agarose gel in 1XTris borate EDTA buffer followed by staining with ethidium bromide solution. The genotypes were typed by visualization under ultraviolet light.

Statistical methods

Statistical analysis was performed by using SPSS software package programme (revision 11.5 SPSS Inc., Chicago, IL, USA). Clinical laboratory data are expressed as mean±SD. Mean values were compared between patients and controls by unpaired Student's t-test. Differences in the distribution of genotypes and alleles between cases and controls were tested using the Chi-square statistic. Allele frequencies were estimated by gene counting methods. Values of $p<0.05$ were considered statistically significant.

Results

Demographic characteristics were summarised in Table I. The patients and the controls had similar distribution of age, smoking and body mass index. The patient group had a significantly higher level of alcohol consuming (30.0% vs. 8.0%; $p<0.05$), PSA (56.7± 125.1 vs. 1.26±0.92; $p<0.01$), free prostate specific antigen (10.5± 31.9 vs. 0.30±0.29; $p<0.05$), alkaline phosphatase (192.5±71.8 vs. 90.4±18.7; $p<0.001$), BUN (22.9±9.45 vs. 18.8±4.97; $p<0.05$) and creatinine (1.01±0.25 vs. 0.91±0.19; $p<0.05$) when compared to the control group.

The distributions of genotypes and alleles of MTHFR C677T are shown in Table 2. The frequencies of CC, TT, and CT genotypes among the patients with prostate cancer were 58.2%, 3.6%, and 38.2%, respectively, and among the control subjects 36.0%, 4.0% and 60.0%, respectively. The frequencies of the CT genotype ($p=0.025$) and T allele ($p=0.023$) was found to be higher in control subjects when compared with patients group. The 677 CT heterozygote

Table 1. Demographic Characteristics of the Study Population

	Groups		
	Controls n= 50	Prostate cancer n=55	P value
Age (years) (X±SD)	66.1±5.12	69.1±10.2	NS
Smoking (Yes/No)	30.0/ 70.0	38.1/ 61.9	NS
Alcohol consumers /nonconsumers (%)	8.00/ 92.0	30.0/ 70.0*	<0.05
Positive family history (first degree) (Yes/No,%)	6.00/94.0	46.2/53.8	<0.001
BMI (kg/m^2) (X±SD)	26.3±3.03	26.1±2.86	NS
FPSA-BT(ng/ml)	0.30±0.29	10.5±31.8	<0.05
FPSA-AT(ng/ml)	-	7.1±23.8	
PSA-BT (ng/ml)	1.26±0.92	56.7±125.1	<0.01
PSA-AT (ng/ml)	-	8.04±19.5	
Alkalene phosphatase (X±SD)	90.4±18.7	192.5±71.8	<0.001
Creatinine (X±SD)	0.91±0.19	1.01±0.25	<0.05
BUN (X±SD)	18.8±4.97	22.9±9.45	<0.05
Early stage/Late stage (%)	-	47.6/52.4	
Distant metastasis (Yes/No,%)	-	33.3/66.7	

BMI, body mass index; PSA, prostate specific antigen; FPSA, free PSA; BT, before treatment; AT, after treatment; BUN, blood urea nitrogen; NS, not significant; n, number

Table 2. Genotype and Allele Frequencies of C677T of MTHFR Gene in the Study Groups

	Prostate cancer (n=55)	Control (n=50)
MTHFR C677T Genotypes		
CC	32 (58.2 %)	18 (36.0%)
TT	2 (3.60%)	2 (4.00%)
CT	21 (38.2%)	30 (60.0%)*
MTHFR C677T Alleles		
C	85 (72.3%)	66 (66.0%)
T	25 (22.7%)	34(34.0%)**

*Chi-square= 4.991 p= 0.025; OR: 1.571 (95% CI: 1.048-2.357); **Chi-square= 5.626 p=0.023; OR: 1.530 (95% CI: 1.052- 2.226)

Table 3. Effects of MTHFR C677T Alleles on PSA Levels and Tumor Stages in Patient Group

MTHFR C677T Alleles	CC Genotype	TT+CT Genotype
PSA-BT (ng/ml)	57.3±101.6	55.7±158.8
PSA-AT (ng/ml)	4.39±7.02	14.3±30.7
FPSA-BT (ng/ml)	16.8±43.0	3.22±4.68
FPSA-AT (ng/dl)	1.90±3.02	13.0±34.8
Early stage (T1 and T2) (%)	56.0	47.1
Late stage (T3 and T4) (%)	44.0	52.9
Distant metastasis (Yes/No,%)	26.7/73.3	44.4/55.6

PSA, prostate specific antigen; FPSA, free PSA; BT, before treatment; AT, after treatment; n, number of individuals.

genotype and 677T allele had significantly decreased the risk of prostate cancer when compared with the CC+TT genotypes and 677CC genotype, respectively (OR=1.57 and OR=1.53, respectively, p<0.05). As for these findings showed that the 677CT genotype and 677T allele might have a protective effect against prostate cancer.

To investigate the effects of the alleles of MTHFR on PSA levels after (PSA-BT)/ before (PSA-AT) antiandrogen treatment and tumor stages, student's t test was elevated and reported no statistical difference (p>0.05). In addition a higher frequency of T677 allele (TT+CT genotypes) was observed in patients who have distant metastase rate, but this relation was also not significantly valued (for CCgenotype: 26.7% vs. for T allele: 44.4%) (Table 3).

Discussion

The association between MTHFR C677T polymorphism and prostate cancer has been investigated in several previous studies and different conclusions have been reported. Heijmans BT et al. (Heijman et al., 2003) reported a positive association of MTHFR C677T polymorphism with prostate cancer, whereas Kimura F et al. (Kimura et al., 2000) found a weak positive association with higher tumor grade. Cicek et al. (Cicek et al., 2004) reported a reduced risk of prostate cancer progression with 677T allele. In addition some studies reported any effect of the MTHFR C677T variants on prostate cancer (Reljic et al., 2007).

In the present study, the 677 CT heterozygote genotype and 677T allele had significantly decreased the risk of prostate cancer when compared with the CC+TT genotypes and 677CC genotype, respectively (p<0.05). The results of the present study showed that the 677CT genotype and 677T allele might have a protective effect against prostate cancer. However, any relationship between PSA levels or metastase rate or tumor grade and the MTHFR C677T polymorphism was observed in this study.

Polymorphic variants of MTHFR C677T were found to influence its functional activities whereas homozygous TT variant results in approximately 65% reduction in enzyme activity and heterozygotes CT have 30% of normal enzyme activity (Frosst and Blom et al., 1995). In the case of reduced activity of the MTHFR enzyme the intracellular levels of 5,10-methylene-THF was increased and so causes DNA synthesis via thymidine synthesis which supports the DNA stability and reduces uracil misincorporation into DNA. In addition availability of 5-methyl-THF for DNA methylation especially in tumour suppressor genes which plays an important role in carcinogenesis was decreased (Goyette et al., 1998; Choi et al., 2002; Paz et al., Krajinovic et al., 2004; 2002; Sohn et al., 2004). Therefore MTHFR can be accepted as both risk and protect factor in carcinogenesis (Choi et al., 2002). Taken together, different tumours evolve by different pathological pathways and the tissue-specific impacts of MTHFR in cancer is determined by its target genes (Mao et al., 2008). Thus, it appears that effect of MTHFR polymorphism on cancer susceptibility is not uniform.

On the other hand etiological factors, such as smoking habit and alcohol consumption is known to increase the risk for several disorders. Puri et al. (Puri et al., 2005) and Wang et al. (Wang et al., 2008) reported that alcohol consumption has been associated with DNA methylation in head, neck, and esophageal cancer. Similarly in the present report higher ratio of alcohol use was observed.

In addition folate and methionine metabolisms might have an important role the development of cancers. In addition some studies reported adequate ingestion of folate may be sufficient to overcome the adverse effects of MTHFR variants (Gemmati et al., 2004; Shrubsole et al.,2004)

The present study is a preliminary study to establish whether MTHFR C677T polymorphism has a causative role in the pathogenesis of prostate cancer in Istanbul population. The main limitation was the sample size to examine the multiple interactions of the data. Also, evaluation of the interaction of folate and MTHFR variant was not applicable due to the lack of folate intake data. Additional studies with larger sample sizes are needed to define the the influence of MTHFR C677T genotyping on clinical outcomes in prostate cancer patients.

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