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Research Article

METHYLENETETRAHYDROFOLATE REDUCTASE C677T GENE POLYMORPHISM AND PROSTATE CANCER RISK

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ABSTRACT

Objective: The single nucleotide polymorphism C677T of the methylenetetrahydrofolate reductase (MTHFR) gene encodes a thermolabile enzyme. This polymorphism was found to be implicated in cancer susceptibility. In this study, we analyzed the distribution of the MTHFR C677T polymorphism in two cohorts; patients and controls native of East of Algeria to explore the possible association between this polymorphism and prostate cancer susceptibility.

Methods: Our examination has been conducted in 98 cases and 98 healthy controls. Genotyping was realized by polymerase chain reaction-restriction fragment length polymorphism method.

Results: Compared with CC homozygous, the CT heterozygous was found to have a significantly increased risk of prostate cancer (p=0.04; odds ratio [OR]=2.01, 95% confidence interval [CI]: 1.02–3.95). However, no statistically significant difference was observed concerning the TT homozygous (p=0.74; OR=1.25, 95% CI: 0.51–3.04).

Conclusion: Our results indicate that the genotype CT is a risk factor for prostate cancer in East of Algeria.

Keywords: Methylenetetrahydrofolate reductase C677T, Polymorphism, Prostate cancer, East of Algeria.

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INTRODUCTION

Prostate cancer is one of the most common malignant cancer in terms of prevalence and the second leading cause of death among men [1-3]. The methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the metabolism of folates, which are crucial for deoxyribonucleic acid (DNA) methylation, nucleotide synthesis, and genomic integrity [4]. The MTHFR catalyzes an irreversible conversion of the substrate 5,10-methylenetetrahydrofolate (CH_2-H_4 folate) to 5-methyltetrahydrofolate (CH_3-H_4 folate) [5,6]. The 5,10-MTHF ensures the synthesis of deoxythymidylate monophosphate (dTMP) from deoxyuridylate monophosphate (dUMP). The increases of dTMP in the nucleotide provisions underrate the misincorporation of the dUMP in DNA sequence, so it decreases double-strand breaks. The 5-MTHF ensures methylation of the amino acid homocysteine to methionine, a reaction by which, the universal donor of the one-carbon group is synthesized, the S-adenosyl methionine (SAM), essential for methylation reactions [6,7]. DNA methylation profile change could cause oncogene activation and genomic instability after hypomethylation or influence on tumor suppressor genes following hypermethylation and silences their expression, which can provide prostate tumor development [6,8,9]. The variant MTHFR C677T leads to the substitution of alanine to valine, which generates a thermolabile enzyme, with approximately 35 % of the reduction in activity in heterozygous, and 70% in homozygous [10,11]. The MTHFR C677T polymorphism has been related to many cancers such as acute lymphoblastic leukemia of adult [12], the digestive system [13,14], differentiated thyroid carcinoma [15], breast cancer [16], and prostate cancer [17]. The aim of this study was to examine the relationship between the MTHFR C677T polymorphism and prostate cancer risk in the East Algerian population.

METHODS

Subjects of study

We conducted a case–control study for this purpose we recruited 98 prostate cancer cases and 98 controls. All patients were histologically diagnosed with prostate cancer; thus, all stages of this tumor development have been included [18] blood samples were collected at the Uro-Nephrology Hospital "the Department of Urology and Renal Transplantation," Constantine, Algeria. Control subjects were volunteers, healthy men, without a family history of prostate cancer and they were free of cancer at the time of blood-sample. Patients and controls are native to different regions of East of Algeria. Prostate cancer case and healthy control were matched by age (±5 years). Our research has been approved by the local Ethics Committee. All participants gave their written informed consent for approximately 7 ml peripheral blood sample, after the interview.

Genotyping

Genomic DNA was extracted from leukocytes, using salting out procedure [19]. MTHFR genotyping was performed for the single nucleotide polymorphism (SNP) C677T by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). The primer sequences used were Forward: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' and Reverse: 5'-AGGACGGTGCGGTGAGAGTG-3'. PCR conditions were: Initial denaturation cycle at 94 °C for 5 min, 30 cycles, each cycle contains three stages: Denaturation at 94°C for 30 s, hybridization at 65°C for 30 s, and elongation at 72°C for 40 s, and finally a terminal elongation at 72°C for 10 min. 30 µl of PCR product was digested with restriction enzyme HinfI (Promega, Madison, WI) at 37°C overnight, and digested products were separated on 3% Agarose gel. After that, fragments were visualized using ultra-violet light.

Statistical analysis

Using Epi Info Version 6, differences in allelic and genotypic frequencies have been tested. *p<0.05 was considered statistically significant.

RESULTS

In our study, patients and controls were aged between 49 and 89 years, with an average age of 70.33±7.65 years. General characteristics of the study population represented by; the age of the two cohorts distributed every 10 years, tumor extension, Prostate Specific Antigen (PSA) levels at the time of diagnosis and the existence of a family history of prostate cancer have been shown in Table 1.

Almost all patients have been diagnosed at age 60 and more. Also, 62.24 % of them at late stage and distant metastasis. Furthermore, 58.16 % of patients with PSA levels above 50 ng/ml, underlining delayed diagnosis.

DNA fragments obtained after digestion by the restriction enzyme HinfI and electrophoresis were TT, mutant homozygote (one band of 175 base pairs [bp]); CT, heterozygote (two bands of 198 bp and 175 bp); CC, wild-type homozygote (one band of 198 bp).

Distribution of allelic and genotypic frequencies for the polymorphism MTHFR C677T between prostate cancer patients and controls and their relation with risk of prostatic carcinogenesis is presented in Table 2.

Our results showed that the 677 CT genotype was frequent between cases (p=0.04), (odds ratio [OR]: 2.01, confidence interval [CI]: 1.02–3.95), suggesting that they may be a risk factor for prostate cancer. On the other hand, for the 677 TT genotype, any statistically significant difference between cases and controls was observed (p=0.74), (OR: 1.25, CI: 0.51–3.04). Concerning the T allele frequency of the C677T SNP was 0.41 for patients and 0.36 for controls.

DISCUSSION

This paper reports for the first time in Algeria the relation between the MTHFR C677T polymorphism and susceptibility to developing prostatic cancer. Our results showed that there was an association between the MTHFR 677CT heterozygous and prostate cancer. In agreement with our results, Van Guelpen *et al*. [7], Marchal *et al*. [20], López-Cortés *et al*. [17], and Abedinzadeh *et al*. [21] reported a positive association between the polymorphism C677T and risk of prostatic cancer in the Swedish population, Spanish population, Ecuadorian, and Asians, respectively.

The significant association between the CT genotype and risk of prostate cancer may be explained by the possibility to keep a sufficient stock of methionine to support neoplastic clone progression compared with the TT homozygous [20].

However, various investigations have not found any relation between this polymorphism and prostate cancer in Caucasians population [4], the Swedish population [6], and Americans [22]. As well, the meta-analysis managed by Collin *et al*. [23], Zhang *et al*. [24], and recently, Abedinzadeh *et al*. [21] has concluded that the C677T polymorphism, in general, has no effect on the occurrence of prostate cancer.

Besides, other authors observed that the T allele exerts a protective impact on prostate cancer risk. Like they indicated Marchal *et al*. [20], and Guo *et al.* [25] that the genotype 677 TT is a protective factor in Spanish and Asians, respectively. As well, Cai *et al.* [26] showed that the TT genotype has been associated with a reduced risk of prostate cancer in the Chinese population, and the T allele exerts probably a protective effect on the risk of this cancer type. Furthermore, Li and Xu [27], who had carried out a meta-analysis, have reported the same result for the T allele. Safarinejad *et al*. [28] have studied the association between tumoral aggressiveness and TT, CT, and CC genotypes: The comparison showed that TT homozygous reduced by more than 50% the risk of high-grade prostate cancer (Gleason score >7).

In addition, Küçükhüseyin *et al*. [29] proposed that the CT genotype and the T allele might be associated with decreased risk of prostate cancer among the Turks. Singal *et al*. [30] suggested that the CT genotype was associated with a reduced risk of prostate cancer.

To explain the protective effect of the T allele on prostate cancer, it has been proposed that the mutant 677 TT genotype was associated with significantly decreased DNA methylation status. The reduced activity of MTHFR enzyme, coded by the T allele, decreases the SAM synthesis, the headmaster donor of a methyl group for DNA methylation reactions, favor thereby tumor suppressor genes expression [20]. Second, the thermolabile enzyme increases the pool of the 5,10-methylenetetrahydrofolate, requisite for purine and deoxythymidylate triphosphate synthesis, which is the leader nucleotide, required for DNA reparation, consequently, a decrease of uracil incorporation inside DNA, reduce thereby chromosome breaks [20,31]. Furthermore, *in vitro* experiences approve that inhibition of MTHFR enzyme conduct to decreasing tumor development due to the limited methionine provision, which leads to stop the cell cycle on steps S and G2, damage thus their proliferative potential [20].

The contradictory results from studies of different populations may be explained by the method by which controls have been selected, the ethnic origin of cases and controls, geographical region, lifestyle and exposition to some factors [32,33].

CONCLUSION

The results suggest for the first time, but in a small sample of subjects, that the 677 CT heterozygous is a risk factor for prostatic carcinogenesis. Further researches should be undertaken that includes other possible genes that participate in the homocysteine metabolism, such as

Table 1: General characteristics of the study population

Characteristics	Patients n=98 (%)	Controls n=98 (%)
Age (years)		
50–59	3 (3.06)	13 (13.26)
60–69	33 (33.67)	39 (39.79)
≥70	62 (63.26)	46 (46.93)
Early stage (T1, T2)	37 (37.75)	-
Late stage and distant metastasis	61 (62.24)	-
PSA level at diagnosis (ng/ml)		
<4	3 (3.06)	-
4–10	9 (9.18)	-
10–50	29 (29.59)	-
≥50	57 (58.16)	-
Family history		
Yes	21 (21.42)	-
No	77 (78.57)	-

n: Number. PSA: Prostate specific antigen

Table 2: Distribution of allelic and genotypic frequencies for the polymorphism MTHFR C677T between prostate cancer patients and controls and their relation with risk of prostatic carcinogenesis

Genotype and Allele	Patients n=98 (%)	Controls n=98%	OR (95% CI)	p value
MTHFR C677T				
CC	32 (32.65)	45 (45.92)	-	-
CT	50 (51.02)	35 (35.71)	2.01 (1.02–3.95)	0.04
TT	16 (16.32)	18 (18.37)	1.25 (0.51–3.04)	0.74
CT or TT	66 (67.35)	53 (54.08)	1.75 (0.94–3.26)	0.07
C allele	114 (58.16)	125 (63.77)	-	-
T allele	82 (41.84)	71 (36.22)	1.62 (0.90–2.94)	0.11

n: Number, OR: Odds ratio, CI: Confidence interval.
MTHFR: Methylenetetrahydrofolate reductase

methionine synthase. The influence of the environment, the gene-gene as well as gene-environment interactions should also be performed to clarify their possible roles in prostatic carcinogenesis.

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AUTHORS CONTRIBUTION

Tellouche-Bouhouhou Samah performed the genotyping, drafted the manuscript, Mrs. Chellat-Rezgoune Djalila "supervisor" and Satta Dalila corrected and revised the manuscript, Abadi Noureddine director of Biology and Molecular Genetics laboratory, Dahdouh Abderrezak thesis advisor. The final manuscript has been approved by all authors.

CONFLICT OF INTERESTS

All authors declare that they have any conflict of interest. This article has not been published previously, nor is it under consideration for publication elsewhere.

REFERENCES

1. Sepehr S, Sepehri H, Khodagholi F, Delphi L, Dashtbozorgi S. Apple pectin (AP) induced apoptosis via nitric oxide (NO) in human prostate cancer cells DU145. Int J Pharm Pharm Sci 2015;7:281-5.
2. Purushothaman A, Kumar G, Gangadharan P, Roshni PR. A comparison of leuprolide acetate versus bilateral orchiectomy for patients with metastatic prostate cancer. Asian J Pharm Clin Res 2016;9:51-4.
3. Kaur P, Khatik GL. Identification of novel 5-styryl-1,2,4-oxadiazole/triazole derivatives as the potential anti-androgens through molecular docking study. Int J Pharm Pharm Sci 2016;8:72-7.
4. Reljic A, Simundic AM, Topic E, Nikolac N, Justinic D, Stefanovic M, *et al.* The methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and cancer risk: The croatian case-control study. Clin Biochem 2007;40:981-5.
5. Guenther BD, Sheppard CA, Tran P, Rozen R, Matthews RG, Ludwig ML, *et al.* The structure and properties of methylenetetrahydrofolate reductase from *Escherichia coli* suggest how folate ameliorates human hyperhomocysteinemia. Nat Struct Biol 1999;6:359-65.
6. Johansson M, Van Guelpen B, Hultdin J, Wiklund F, Adami HO, Bälter K, *et al*. The MTHFR 677C→T polymorphism and risk of prostate cancer: Results from the CAPS study. Cancer Causes Control 2007;18:1169-74.
7. 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;15:46-50.
8. Nelson WG, De Marzo AM, Isaacs WB. Prostate cancer. N Engl J Med 2003;349:366-81.
9. Sherzay N, Chitakar E. Epigenetics: Effect of environmental factors on human genome. Int J Pharm Pharm Sci 2016;8:1-6.
10. Sonbolestan ES, Sazgar H, Zia-Jahromi N, Farsani FM. Investigation the association between MTHFR gene polymorphism and homocysteine in iranian pregnant women. Asian J Pharm Clin Res 2017;10:411-4.
11. 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;10:111-3.
12. Pereira TV, Rudnicki M, Pereira AC, Pombo-de-Oliveira MS, Franco RF 5,10-methylenetetrahydrofolate reductase polymorphisms and acute lymphoblastic leukemia risk: A meta-analysis. Cancer Epidemiol Biomarkers Prev 2006;15:1956-63.
13. Larsson SC, Giovannucci E, Wolk A. Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: A meta-analysis. Gastroenterology 2006;131:1271-83.
14. Ryan BM, Weir DG. Relevance of folate metabolism in the pathogenesis of colorectal cancer. J Lab Clin Med 2001;138:164-76.
15. Fard-Esfahani P, Fard-Esfahani A, Saidi P, Fayaz S, Mohabati R, Majdi M, *et al.* An increased risk of differentiated thyroid carcinoma in iran with the 677C→T homozygous polymorphism in the MTHFR gene. Cancer Epidemiol 2011;35:56-8.
16. Wang ZG, Cui W, Yang LF, Zhu YQ, Wei WH. Association of dietary intake of folate and MTHFR genotype with breast cancer risk. Genet Mol Res 2014;13:5446-51.
17. López-Cortés A, Jaramillo-Koupermann G, Muñoz MJ, Cabrera A, Echeverría C, Rosales F, *et al.* Genetic polymorphisms in MTHFR (C677T, A1298C), MTR (A2756G) and MTRR (A66G) genes associated with pathological characteristics of prostate cancer in the ecuadorian population. Am J Med Sci 2013;346:447-54.
18. Sobin LH, Gospodariwicz M, Wittekind C, editors. TNM classification of malignant tumors. UICC International Union Against Cancer. 7th ed. Hoboken, NJ: Wiley-Blackwell; 2009. p. 243-8.
19. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
20. 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;34:805-10.
21. Abedinzadeh M, Zare-Shehneh M, Neamatzadeh H, Abedinzadeh M, Karami H. Association between MTHFR C677T polymorphism and risk of prostate cancer: Evidence from 22 studies with 10,832 cases and 11,993 controls. Asian Pac J Cancer Prev 2015;16:4525-30.
22. Cicek MS, Nock NL, Li L, Conti DV, Casey G, Witte JS, *et al.* Relationship between methylenetetrahydrofolate reductase C677T and A1298C genotypes and haplotypes and prostate cancer risk and aggressiveness. Cancer Epidemiol Biomarkers Prev 2004;13:1331-6.
23. 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;18:2528-39.
24. Zhang WB, Zhang JH, Pan ZQ, Yang QS, Liu B. The MTHFR C677T polymorphism and prostate cancer risk: New findings from a meta-analysis of 7306 cases and 8062 controls. Asian Pac J Cancer Prev 2012;13:2597-604.
25. Guo S, Jiang X, Chen X, Chen L, Li X, Jia Y, *et al.* The protective effect of methylenetetrahydrofolate reductase C677T polymorphism against prostate cancer risk: Evidence from 23 case-control studies. Gene 2015;565:90-5.
26. Cai D, Ning L, Pan C, Liu X, Bu R, Chen X, *et al*. Association of polymorphisms in folate metabolic genes and prostate cancer risk: A case-control study in a Chinese population. J Genet 2010;89:263-7.
27. Li XL, Xu JH. MTHFR polymorphism and the risk of prostate cancer: A meta-analysis of case-control studies. Prostate Cancer Prostatic Dis 2012;15:244-9.
28. 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;70:1645-57.
29. Küçükhüseyin Ö, Kurnaz Ö, Akadam-Teker AB, Narter F, Yılmaz-Aydoğan H, İsbir T, *et al.* Effects of the MTHFR C677T polymorphism on prostate specific antigen and prostate cancer. Asian Pac J Cancer Prev 2011;12:2275-8.
30. Singal R, Ferdinand L, Das PM, Reis IM, Schlesselman JJ. Polymorphisms in the methylenetetrahydrofolate reductase gene and prostate cancer risk. Int J Oncol 2004;25:1465-71.
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;63:1249-53.
32. Sumathi R, Tamizharasi S, Sivakumar T. Formulation and evaluation of polymeric nanosuspension of naringenin. Int J App Pharm 2017;9:60-70.