No Association Between *MTHFR* Gene C677T/A1298C Polymorphisms, Serum Folate, Vitamin B12, Homocysteine Levels, and Prostate Cancer in an Algerian Population.

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Research Article

Keywords: Prostate cancer, Methylenetetrahydrofolatereductase, Genetic polymorphism, Folate, Vitamin B12, Homocysteine

Posted Date: March 2nd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1373580/v1>

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Abstract

Background Methylenetetrahydrofolatereductase (MTHFR) enzyme plays a crucial role in the metabolism of folate and homocysteine, which are necessary for DNA methylation and nucleotide synthesis. Genetic polymorphisms that decrease MTHFR activity are implicated in several diseases as well as diverse malignancies including prostate cancer. The objective of this study was to evaluate an eventual association between *MTHFR* polymorphisms and prostate cancer within an Algerian population, taking into consideration serum levels of folate, vitamin B12, and homocysteine.

Methods and results A total of 106 men with newly diagnosed prostate cancer and 125 healthy controls were examined for *MTHFR* C677T and A1298C polymorphisms using PCR/RFLP and Real-Time PCR TaqMan® respectively. Serum levels of folate, total homocysteine, and vitamin B12 were measured using an automatic biochemistry analyzer. Our study showed no significant difference in genotype frequency of A1298C and C677T in patients compared to the control groups. As for serum levels of folate, total homocysteine, and vitamin B12, there were not associated with prostate cancer ($P \geq 0.05$). However, age and family history increased susceptibility to the disease (OR=1.178, $P=0.00$ and OR = 10.03, $P = 0.007$, respectively).

Conclusion Our results suggest that *MTHFR* C677T and A1298C as folate, total homocysteine, and vitamin B12 do not contribute to the risk of prostate cancer. On the other hand, age and family history are risk factors in an Algerian population. Therefore, further studies with a larger sample size are needed for the confirmation of our finding.

Introduction

Prostate cancer (PCa) is one of the most important diagnosed neoplasms and is the fifth cause of cancer-related death in men around the world. According to GLOBOCAN 2020 database, 1.4 million new cases and 375,000 deaths from prostate cancer were estimated in 2020, with the highest rates are in developed countries [1]. In Algeria, prostate cancer is the second most frequent cancer affecting men (17.0 per 100,000) and the fourth leading cancer-caused mortality (6.8 per 100,000) [1].

Prostate cancer is a complex illness, in which the etiology revolves around the interaction between genetic and environmental factors. The most well-known risk factors include age, family history of PCa, and ethnicity. Additionally, diet, androgen stimulation and smoking have been considered secondary risk factors [2]. A recent study has supported the role of molecular alterations and DNA polymorphisms in the development and progression of PCa [3].

Methylenetetrahydrofolatereductase (MTHFR) is an important regulatory enzyme in the folate and homocysteine metabolic pathways. It catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate (5,10-methylene THF) to 5-methylenetetrahydrofolate (5-methyl-THF), which is the predominant circulatory form of folate and provides one-carbon group for the remethylation of homocysteine to methionine, a reaction for which vitamin B12 is a co-enzyme [4]. Methionine is

considered as the precursor of S-adenosylmethionine (SAM) and the universal methyl donor in biological processes, including the methylation of proteins and DNA [5]. Therefore, MTHFR plays an essential role in dTMP production and biosynthesis, repair, and maintenance of DNA stability [6].

The human *MTHFR* gene is situated in chromosome 1 (1p36.3), it is composed of 11 exons and encodes a protein of 656 amino acids. There are two commonly allelic variant of *MTHFR* gene, they have been described as the following: C677T and A1298C. These *MTHFR* polymorphisms have been associated with decreased MTHFR activity, which lead to an increased plasma homocysteine level and a reduced plasma folate concentration. The *MTHFR* C677T is located at exon 4 leading to amino acid substitution (Ala 222Val). This substitution causes a thermolabile transformation of the enzyme from 35 to 65% activity reduction [4]. The second variant A1298C is located at exon 7, results in a glutamate-to-valine substitution at codon 429 and affects enzyme function to a lesser degree. Nevertheless, the association with C677T polymorphism has a more effect on the decrease of MTHFR enzyme activity [7]. It might be estimated that *MTHFR* polymorphisms and the consequent decrease in enzyme function can produce genomic instability and activation of oncogenes by DNA hypomethylation or hypermethylation, causing cancerization and affecting the progression of malignant tumors [6].

Previous studies have been reported that *MTHFR* gene polymorphism is known to be an increased risk of prostate cancer. Such results remained inconsistent and controversial [8–11]. Therefore, the aims of this study are to investigate for the first time in the North - Center of Algeria, the genetic polymorphisms of *MTHFR*, as well as their relationship with serum total homocysteine (tHcy), folate, vitamin B12 levels and environmental factors on the risk of PCa.

Materials And Methods

Subjects

This study consisted of 257 histologically confirmed PCa patients and 125 controls. All participants were diagnosed by physicians in the Department of Urology in Mustapha Bacha University Hospital, Algiers, Algeria, from December 2013 to August 2016. Among all patients, 151 (58.75%) were eliminated due to using hormonal therapy, radiotherapy, or chemotherapy. The cohort of the investigation consisted of 106 patients with a median age at diagnosis was 67.98 ± 6.31 years. The risk categories are defined on the basis of cancer stage, Gleason score, and serum prostate-specific antigen (PSA) at diagnosis.

The control group includes volunteer healthy men with an average age of 61.06 ± 6.60 years, living in the same geographical area and without a prior history of cancer or pre-cancerous lesions. Such groups have undergone a digital rectal examination (DRE), serological PSA ($< 4\text{ ng/ml}$) and radiological exams to exclude the possibility of prostate hyperplasia or prostate carcinoma.

All men who participated in the data give their informed consent and the local ethical committee approved this study.

Blood sampling and biochemical analysis

Blood samples were collected from fasting participants using tubes without an anticoagulant and centrifuged at 2000 rpm for 20 minutes at 4°C. The serum was separated, aliquoted and stored at -80°C until analysis. Biochemical assays on the serum were measured at the Department of Biochemistry in the Central Hospital of Army, Algiers. Serum folate and vitamin B12 concentrations were analyzed by competitive binding assays using electro-chemiluminescent detection (Elecsys 2010, Hitachi, Roche or COBASe401). The serum total homocysteine (tHcy) was measured by automated (ARCHITECT /system immunoassay, Abbott Laboratories) fluorescence polarization immunoassay.

DNA extraction and genotyping

Blood samples were collected in EDTA tubes, and genomic DNA was isolated from peripheral blood via salting out method (Miller et al., 1988). The quantity and purity of DNA were checked by Nano spectrophotometer and agarose gel electrophoresis respectively.

Genotyping for *MTHFR* C677T (Rs1801133) was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) procedure [4]. Genomic DNA (50–100 ng) was amplified in a 30 µl PCR reaction mixture containing 1X-taq DNA polymerase buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.4 µM of forward primer: 5-TTTGAGGCTGACCTGAAGCACTTGAAGGAG-3, 0.4 µM of reverse primer: 5-GAGTGGTAGCCCTGGATGGGAAAGATCCCG-3 and 1U of Taq polymerase (Promega Madison, WI USA). The PCR protocol was as follows: initial denaturation (94°C for 5 min); followed by 35 cycles (94°C for 30s, 68°C for 30s, 72°C for 30s) and final extension at 72°C for 7 min. The PCR products were then digested at 37°C overnight with 5U of HinfI restriction enzyme (Promega Madison, WI USA). The digestion products were obtained by electrophoresis on 2% agarose gel contained ethidium bromide and visualized under ultra - violet transilluminator.

The genotype for *MTHFR* A1298C (Rs1801131) was generated using the TaqMan probe real-time PCR method (7500 Fast Real-Time PCR System, Applied Biosystems, USA) with commercially available assays (IDs: C_850486_20; Life Technologies, USA). The 10 µl reaction mixture included 5 µl of TaqMan GTXpress Master Mix (Life Technologies), 10 ng of DNA and 0.5 µl of each primer and probe. The temperature profile consisted of initial denaturation at 95°C for 10 min, followed by 40 cycles of two-step PCR (denaturation 95°C for 15 s, annealing and elongation 60°C for 1 min). The respective FAM and VIC fluorescence dyes on the probes were used for the wild type (A allele) and the mutant type (C allele) to determine the sample genotype.

Statistical analysis

Statistical analyses were performed using SPSS version 20 (SPSS Science, Chicago, IL). An unconditional logistic regression model was carried out to indicate the effect of *MTHFR* gene polymorphism and other variables on PCa risk. The results expressed as odds ratio (OR) and corresponding 95% confidence intervals (CIs). The analysis of the multivariate logistic regression was performed to calculate the best independent predictors of PCa for variables that have $p \leq 0.25$ in the

univariate model. The Hardy–Weinberg equilibrium was used by chi-square (χ^2) test for genotype distribution. Differences were considered statistically significant when the p-value was less than 0.05.

Results

Characteristics of the study population.

The distributions of the selected demographic, lifestyle habits and clinical characteristics of 106 PCa patients and 125 controls are summarized in Table 1. There was a significant difference between PCa patients and controls regarding age (OR = 1.11, P = 0.00), and family history (OR = 2.53, P = 0.009). However, there was no statistically significant difference in the following indexes: Marital status (P = 0.99), residency (OR = 1.08, P = 0.821), occupational activity (OR = 1.28, P = 0.36) and tobacco (OR = 1.001, P = 0.996).

Table 1

Demographic, clinical characteristics, lifestyle habit and biochemical analyses of the study subjects.

Characteristics	PCa patients (<i>n</i> = 106)	Controls (<i>n</i> = 125)	<i>P</i> value	OR (95% CI)
Age at recruitment (years $\pm$ SD)	67.98 $\pm$ 6.31	61.06 $\pm$ 6.60	0.00	1.11 (1.072–1.156)
Marital status (<i>n</i> , %)				
Married	102 (96.22)	124 (99.2)	0.99	-
Single	04 (3.77)	01 (0.8)		
Residency (<i>n</i> , %)				
Urban	90 (84.1)	105 (84)	0.821	1.08 (0.533–2.208)
Rural	17 (15.9)	21 (16)		
Occupational activity (<i>n</i> , %)				
Manual labor	69 (64.4)	78 (62.4)	0.36	1.28 (0.742–2.23)
Office job	36 (35.6)	47 (37.6)		
Tobacco (<i>n</i> , %)				
Use tobacco	75 (70.45)	78 (74.8)	0.996	1.001 (0.673–1.490)
Family history of PCa (%)	20.2	13.4	0.009	2.532 (1.262–5.079)
Biochemistry (Mean $\pm$ SD)				
Folic acid (ng mL ⁻¹)	12.57 $\pm$ 22.90	9.26 $\pm$ 5.23	0.472	1.014 (0.977–1.051)
Vitamin B12 (pg mL ⁻¹)	295.44 $\pm$ 104.71	362.74 $\pm$ 195.71	0.178	0.997 (0.993–1.001)
Homocysteine (μ mol L ⁻¹)	10.10 $\pm$ 3.19	12.34 $\pm$ 8.27	0.466	0.975 (0.909–1.044)
Total PSA (ng mL ⁻¹)	19.53 $\pm$ 17.69	3.72 $\pm$ 2.77	0.00	-

Univariate odds ratio of prostate cancer risk using unconditional logistic regression analysis. Adjusted OR .SD: Standard deviation. PCa: Prostate cancer.PSA: Prostate-specific antigen. OR: Odds ratio refers to PCa. CI:Confidence intervals. *P* $\leq$ 0.05 statistically significant.

Characteristics	PCa patients (<i>n</i> = 106)	Controls (<i>n</i> = 125)	<i>P</i> value	OR (95% CI)
Clinical characteristics (<i>n</i> , %)				
Gleason score 5–7	88 (92.4)			
Gleason score 8–10	12 (7.6)			
Risk category (<i>n</i> , %)				
Low risk	37 (34.57)			
Intermediate to high risk	38 (35.51)			
Distant metastases	32 (29.90)			
Univariate odds ratio of prostate cancer risk using unconditional logistic regression analysis. Adjusted OR .SD: Standard deviation. PCa: Prostate cancer.PSA: Prostate-specific antigen. OR: Odds ratio refers to PCa. CI:Confidence intervals. <i>P</i> ≤0.05 statistically significant.				

The analysis of biochemical parameters showed that a total PSA value was significantly higher in PCa group ($P = 0.000$). Moreover, we noted no association between serum tHcy (OR = 0.975, $P = 0.466$), folate (OR = 1.014, $P = 0.472$) and vitamin B12 (OR = 0.997, $P = 0.178$,) levels and prostate cancer.

Genotype frequencies of MTHFR and haplotypes analysis.

The distributions of the *MTHFR* gene polymorphisms were in Hardy-Weinberg equilibrium in both groups $P \geq 0.05$. The investigation of the relationship between C677T and A1298C *MTHFR* polymorphism and PCa was performed Univariate analyses in Table 2.

Table 2

Frequency distribution of the *MTHFR* C677T, A1298C alleles, genotype and combined genotypes and their relation with PCa.

Polymorphism	PCan (%)	Controlsn (%)	Pvalue	OR (95%CI)
<i>MTHFR</i> C677T	<i>n</i> = 106	<i>n</i> = 125		
CC	50 (47.16)	53 (44)	0.42	1.23 (0.733–2.081)
CT	41 (38.67)	56 (42.4)	0.30	0.75 (0.447–1.28)
TT	15 (14.15)	16 (13.6)	0.90	1.04 (0.486–2.268)
C allele	141 (66.51)	162 (64.8)	0.91	1.06
T allele	71 (33.49)	88 (35.2)	0.87	0.97
<i>MTHFR</i> A1298C	<i>n</i> = 105	<i>n</i> = 106		
AA	62 (59.0)	67 (63.2)	1	0.83 (0.475–1.445)
AC	38 (36.2)	34 (32.1)	0.56	1.18 (0.669–2.095)
CC	05 (4.8)	05 (4.7)	0.51	1 (0.281–3.56)
A allele	48 (22.85)	168 (79.24)	0.67	1.1
C allele	162 (77.14)	44 (20.75)	0.85	0.97
C677T/A1298C	<i>n</i> = 105	<i>n</i> = 105		
CC/AA	21 (20)	28 (27.67)	0.38	0.75
CC/AC	23 (21.9)	18 (17.14)	0.46	1.29
CC/CC	05 (4.76)	03 (2.85)	0.48	1.69
CT/AA	28 (27.67)	29 (27.61)	0.93	0.97
CT/AC	13 (8.66)	15 (14.28)	0.74	0.87
CT/CC	0	01 (0.95)	-	-
TT/AA	13 (13.38)	10 (9.52)	0.54	1.31
TT/AC	02 (1.90)	01 (0.95)	0.56	2.02
TT/CC	0	01 (0.95)	-	-
Univariate odds ratio of prostate cancer risk using unconditional logistic regression analysis.				
MTHFR: Methylene tetrahydrofolate reductase. PCa: prostate cancer. OR: odds ratio refers to PCa. CI: confidence intervals. <i>P</i> < 0.05 Statistically significant				

According to these results, genotype and allele frequencies did not show any significant differences between patients and controls ($P>0.05$). The combined effect of the two polymorphisms, 677CT/1298AC genotype also showed no association with the risk of PCa.

Multivariate Logistic Regression

The results of multiple logistic regression analyses were chosen to find the best independent predictors; in this analysis, only age (OR = 1.178, $P=0.00$) and family history (OR = 10.03, $P=0.007$) appear as independent risk factors for prostate cancer development (Table 3).

Table 3

Results of multivariate adjusted analysis between prostate cancer cases and controls according to *MTHFR* genotypes, biochemistry analysis and demographic parameters.

	<i>P</i> value	OR	95% CI
Age	0.00	1.178	1.083–1.282
Family history	0.007	10.03	1.88–53.62
Adjusted OR by age, residency, occupational activity, tobacco status, family history, serum folate, vitamin B12, homocysteine and <i>MTHFR</i> genotypes refer to risk of prostate cancer.			
OR, odds ratio refers to PCa; CI, confidence intervals.			
$P\leq0.05$ Statistically significant			

Discussion

MTHFR enzyme plays a pivotal role in intracellular folate and homocysteine metabolism, which are fundamental for DNA methylation, synthesis, and genomic integrity [13]. Imbalance in the circulating concentrations of folate, homocysteine and vitamin B12 may cause DNA damage leading to genetic instability which is related to the occurrence of several cancers including prostate cancer [14–16]. In fact, it was reported that folate deficiency will induce in CpG island hypermethylation and uracil misincorporation into DNA strands, resulting in genetic and epigenetic instability observed in murine PCa models. However, a high level of serum folate and homocysteine increases prostate cancer progression. This conflicting results may reflect the potential dualistic role of folate in prostate carcinogenesis [15]. Moreover, high serum level of vitamin B12 and homocysteine has been associated with prostate cancer risk [15, 17]. Unlike previous findings, in our study, we found no relationship between these vitamins, homocysteine and the risk of prostate cancer development. Our results were consistent with the findings of Weinstein et al [18] and Beilby et al [19].

As for the relationship between *MTHFR* polymorphisms and prostate cancer, the results showed no significant differences in our population. The results were consistent with several meta-analyses which have reported that C677T and A1298C polymorphisms were not associated with prostate cancer in Caucasian, mixed, and Asian populations [11, 20–22]. However, some published data showed a positive

association between the *MTHFR* C667T polymorphism and PCa development [10, 23]. Moreover, it was reported that the genotype 677 TT exerts a protective effect on prostate cancer risk in the Spanish and Asian populations [24, 25]. As well as, Cicek et al.[26] suggested that 677T and 1298A may be associated with the reduction of prostate cancer progression. These conflicting results may be due to the methods of control selection, their dietary differences, geographical regions, ethnic origins, as well as their exposure to diverse environmental risk factors.

Tobacco is identified as an environmental risk factor for cancer causing 30% of all human cancers including PCa [27]. Two several biological mechanisms were proposed to promote carcinogenesis in the prostate: First of all, tobacco contains a multiple carcinogenic compounds, which can indirectly induce PCa through their interaction with androgen receptors [28]. Secondly, it was reported that men smokers have elevated levels of circulating testosterone, androstenedione, and DHT [29]. Nevertheless; other studies have not supported the link between tobacco smoking and prostate cancer [30]. Similarly, our results showed that tobacco consumption was not associated with prostate cancer (OR = 1.001, $P = 0.996$). The same results have been found in the Western and Eastern populations of Algeria [31–32].

However, age is among the strongest prostate cancer risk factors, more than 80% of prostate cancer is detected in men who are 65 years or older [2]. Actually, it was shown that 42% of 50 years old men have already developed microscopic evidence of this disease [33]. Our results reveal that age has influence on the development of prostate cancer (OR = 1.178, $P = 0.00$).

Furthermore, in our study, the family history is an independent risk factor of PCa (OR = 2.53, $P = 0.009$). Several studies reported an increased PCa risk with a positive family history in different populations [34, 35]. Diverse meta-analyses have found that there was a 2-3-fold increased risk of prostate cancer in men who have first-degree family members affected and the risk was slightly lower among those with a second-degree family member. In addition, men can be vulnerable to prostate cancer at an early age in case of having many family members affected [36, 37]. Moreover, our results are similar to those of Western and Eastern populations of Algeria [31, 38]. Due to the significance of these findings, it goes without saying that an earlier diagnosis is essential, especially for men with a higher degree of prostate cancer risk.

Conclusion

the present study reveals no association between the two genetic polymorphisms of *MTHFR* enzyme and prostate cancer risk in the North-Center of Algeria population. In addition, folate, homocysteine, vitamin B12 and tobacco are not risk factors for prostate cancer in our population. On the other hand, age and family history play a significant role in the development of PCa. However, it is vital to state that further additional studies with a larger sample size are required for the confirmation of our finding.

Abbreviations

MTHFR Methylenetetrahydrofolatereductase

PCa Prostate cancer

5,10-methylene THF 5,10-methylenetetrahydrofolate

dTMP desoxythymidinemonophosphate

tHcy total homocysteine

Declarations

Funding

The authors declare no funds, grants, or other support were received during the preparation of this manuscript.

Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

The institutional ethical committee approved this study.

Acknowledgments

We wish to thank all participants for their contribution in this work. We also would like to extend our gratitude and appreciation to Professor RedaDjidjik, Professor GhoutiKacimi, Doctor AsmahSaida *Merad*, Doctor Mohamed El-HadiCherifi, Doctor OuniKhawla and all personnel at the Department of Urology, for their help in carrying out this work.

Author Contributions

Authors' contributions Design of experiments by RM and MK. Analysis of data by RM and MK. First draft of manuscript by RM, and subsequent drafting by RM, MK, and AC. All authors read and approved the final manuscript.

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