Research Article

Combined Effect of *MTHFR* Genotypes, Tobacco and Occupational Exposure on Bladder Cancer Susceptibility in Algerian Population

Naouel Kherouatou-Chaoui[1*], Djalila Chellat-Rezgoune[1], Asma Ribouh[1], Ahmed Hireche[1], Samah Tellouche[1], Ouarda Semmame[1], Taoufik Djeghri[2], Abderrezak Dahdouh[2], Noureddine Abadi[3], Dalila Satta[1].

[1]Laboratory of cellular and molecular Biology, University Constantine 1, Constantine, Algeria.
[2]Department of Urology and renal transplantation, Daksi renal Clinic. Cité Daksi, Bp 234, Constantine, Algeria.
[3]Laboratory of Biology and molecular Genetic, University Constantine 3, Constantine, Algeria.
***Corresponding author's E-mail:** naouel.kerou@gmail.com

Accepted on: 27-10-2015; **Finalized on:** 30-11-2015.

ABSTRACT

The Methylenetetrahydrofolate reductase (MTHFR) enzyme plays a pivotal role in the folic acid metabolism, which in turn influences DNA methylation, synthesis and repair. Single nucleotide polymorphism (SNPs) in the coding sequences of the gene is associated with several diseases as well as diverse malignancies including bladder cancer. The aim of this study was to evaluate the association and interaction of the *MTHFR C677T* variant with smoking habits, occupational exposure and bladder cancer in Algeria. In this work, we have conducted a case-control study. In total, 95 bladder cancer patients and 109 non-cancer controls in North East Algeria were recruited and genotyped using the PCR-RFLP method. No significant difference for the *C677T* polymorphism was found between the bladder patients and control subjects ($p = 0.06$ for genotype and 0.33 for allele). The strongest evidence was for an interaction between *MTHFR* genotype and smoking. For smokers vs non smokers wild type genotype and all genotypes were associated with bladder cancer ($p < 0.01$) with an increased risk associated with the *MTHFR 677CC* variant. A similar result was found between *MTHFR* genotype and occupational exposure ($p < 0.01$). The analyze of combined effect of polymorphism *C677T*, smoking status and occupational exposure showed that individuals exposed and smokers especially those harboring CC alleles were associated with a significantly increased risk of bladder cancer compared with non smokers. The strongest result obtained by this study was for an additive effect between smoking status, *C677T* alleles and occupational exposures in influencing bladder cancer risk.

Keywords: bladder cancer, *MTHFR C677T* polymorphism, Tobacco, occupational exposure.

INTRODUCTION

Bladder cancer is the ninth most common cancer worldwide, and it is estimated that there were 386,300 newly diagnosed bladder cancer cases and 150,200 related deaths annually,[1] ranking it the seventh most common cancer in men and seventeenth in women worldwide.[2]

In Algeria, bladder cancer represents 16.8 % of all incident cancers accounting 80 new cases per 100.000 persons in 2008.[3] Tobacco smoking and an occupational exposure to aromatic amines are the most important risk factors for this disease[4-6] but other lifestyle, environmental as well as hereditary factors[6,7] have also attracted interest, suggesting individual susceptibility to bladder carcinogenesis.

Folate and methionine metabolism play essential roles in DNA synthesis and DNA methylation, and their metabolism pathways may affect disease susceptibility.[8-10] Methylenetetrahydrofolate reductase (MTHFR) is the essential enzymes in the folate metabolism.[11]

MTHFR acts enzymatically to convert 5, 10 methylenetetrahydrofolate (which acts as the methyl donor in deoxythymidine monophosphate (dTMP) synthesis) to 5-methyltetrahydrofolate, the primary methyl donor for converting homocystéine to methionine.[12] The *MTHFR* gene is located on chromosome 1p36.3. It has been demonstrated that the *C677T* and *A1298C* are two common polymorphisms in the *MTHFR* gene that affect enzyme activity.[13,14] *C677T* is located in exon 4[15] leading to an alanine to valine conversion.[13] The other polymorphism A1298C is located in exon 7 and glutamic acid change to alanine.

A number of molecular epidemiologic studies have been conducted to investigate the associations between the *MTHFR C677T* polymorphism and bladder cancer risk.[16-29] However the results remain conflicting rather than conclusive. In this report, we evaluated the hypothesis that *C677T* polymorphism may affect the risk of bladder cancer in a population based study of bladder cancer from North East Algeria and we assess the combined effect of tobacco, occupational exposures, and *MTHFR C677T* alleles on bladder cancer development. No other studies have been reported in Algeria.

MATERIALS AND METHODS

Subjects

A total of 95 patients with bladder cancer and 109 healthy controls were included in the present study. Cases were diagnosed from 2009 to 2014 from the Department of Urology at Daksi renal Clinic in Constantine. All were from North-East of Algeria, aged between 29 and 92 years and were histologically confirmed. 91, 58 % of them were men.

The control group consisted of unrelated healthy subjects without history of malignant disease who were approximately matched for gender proportion, geographic origin, age range (± 5 years), to those in the case group. Under informed consent peripheral blood samples were collected into tubes with EDTA (pH 8).

Genotyping

Genomic DNA was extracted from leukocytes using NaCl procedure. The quality of genomic DNA was controlled by electrophoresis on 1% agarose gel.

MTHFR genotyping was performed by PCR- RFLP. Briefly, primer sequences were: forward: 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ and reverse: 5′-AGGACGGTGCGGTGAGAGTG-3′. These primers amplified 198-bp fragment with restriction site for HinfI if *C677T* polymorphism is present.

Polymerase chain reaction (PCR) amplification was carried out in a total volume of 50 mL containing ~100 ng of genomic DNA, 2mM dNTPs, 8 pmol of each primer, 1.5mM $MgSO_4$ and 5U Taq polymerase (Biomatik taq), and 2.5 mL of 10 x PCR buffer.

Cycling conditions were as follows: initial preheat 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 65 °C for 30 s and 72 °C for 40 s, and final amplification of 72 °C for 10 min. 30 µL of PCR product was digested with HinfI, and fragments separated on 3% agarose gel: 175-bp and 23-bp fragments for polymorphic T and 198-bp fragment for wild-type C allele. Both negative and positive controls were included for each reaction.

Statistical analysis

All samples were genotyped, and the allele and genotype frequencies of the patients and controls were calculated. Relative risks were estimated by calculating the odds ratios (OR) with 95% confidence intervals (CI) at the 0.05 significance level. OR were calculated using the homozygous wild-type genotype as reference using the software Epi Info (version 6.0). p-values less than 0.05 were considered statistically significant.

RESULTS

Characteristics of the study subjects

The frequency distributions of selected characteristics of the cases and the controls are presented in Table 1.

Except for sex ($p = 0.682$), there were significant differences in the frequency distributions of all selected variables between the cases and controls ($p < 0.001$).

Genotype distributions and association between the MTHFR polymorphism and risk of bladder cancer

Genotype and allele frequency distributions for the *MTHFR C677T* among the cases and controls and their associations with risk of bladder cancer are summarized in Table 2.

No significant difference for the *MTHFR C677T* polymorphism was found between the bladder patients and control subjects ($p = 0.06$ for genotype and 0.33 for allele).

As shown in Table 2 the *MTHFR C677T* polymorphism was not associated with an increased risk of bladder cancer.

Association and stratification analyses between the genotypes of the MTHFR, smoking status and bladder cancer risk

Table 3 examines the interaction between genotype and smoking status. When the effects of smoking are examined separately within the genotypes and within the two group's smokers and non smokers, statistically, there was no evidence of interaction.

But the stratification of patients and controls according to *MTHFR* genotype and tobacco status (Table 4) has suggested that for patients who smoke all genotypes were associated with bladder cancer ($p < 0.01$) with an increased risk for *MTHFR 677CC* variant ($p < 0.001$) when compared to reference group of non–smokers with *MTHFR 677CC* genotype.

Association and stratification analyses between the genotypes of the MTHFR, occupational exposures and bladder cancer risk

We also examined the joint effects of genotype and occupational exposure, a well-established risk factor for bladder cancer. The reference group consisted of non occupationally exposed subjects with the wild type genotype (Table 5). We observed an increased risk for all patients exposed reached the highest value for those carrying the *MTHFR 677CC* genotype.

Finally when we analyzed the combined effect of polymorphism *C677T*, smoking status and occupational exposure we found that individuals exposed and smokers especially harboring CC alleles were associated with a significantly increased risk of bladder cancer compared with non-smokers and non-exposed individuals.

DISCUSSION

In this work, we have undertaken a case-control study to investigate the role of *MTHFR C677T* polymorphism in susceptibility to bladder cancer in Algerian population and to assess combined effect of tobacco, occupational exposures, and *C677T* alleles on bladder cancer development. Both patients and healthy controls belonged to the same ethnic background and all shared a common geographic origin in North East Algeria.

We observed that the *C677T* polymorphism had no effect on risk of bladder cancer, a finding consistent with a number of recent studies. For this polymorphism, no overall association was observed in the meta-analysis of Shi (2014)[29] including a total of 3463 cases and 3927 controls, nor a meta-analysis of Li (2013)[28] involving in 3570 bladder cancer cases and 3926 controls and meta-analysis of Xu (2013)[30] conducted on 3570 cases 3926 controls. Safarinejad (2011)[27] have also reported that

there was no significant association between *C677T* polymorphism and bladder cancer. Similar results were found in some previous studies.[19-24,31,32] Conversely, an inverse relation between *C677T* polymorphism and bladder cancer risk was observed in others published data.[8,18,23,25,33]

These conflicting results may be explained by the metabolic role of the MTHFR enzyme, which is involved in both DNA methylation and DNA synthesis. Because individuals carrying the variant T allele would be less efficient in converting 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the resultant lower level of 5-methyltetrahydrofolate would lead to DNA hypomethylation[34] which can promote carcinogenesis by the derepression of proto-oncogenes or by increasing genomic instability. In this condition, the variant T allele is a risk factor.

On the other hand, lowered risks of cancer may be caused by the increased fidelity of DNA synthesis afforded by the greater availability of the MTHFR substrate 5,10-methylenetetrahydrofolate for DNA synthesis, particularly the increased availability of methyl groups for conversion of uracil to thymidine.[35-37] An inadequate thymidine supply can result in increased incorporation of uracil into DNA, resulting in strand break.[35] In this condition the variant T allele is a protective factor.[35-43]

There may be a balance between the donation of methyl for DNA methylation and the supply of bases for DNA synthesis, i.e. a balance between beneficial and deleterious effects of the *MTHFR 677 T* allele.[44,45]

The comparison of patients and controls group according to their tobacco status (Table 1) suggests that smoking was a high risk factor for bladder cancer development ($p < 0.001$). This result suggests the important role of tobacco in bladder cancer development in the Algerian population. In a meta-analysis of 43 published case-control and cohort studies[46] concluded that current cigarette smokers have an approximately threefold higher risk of bladder cancer than non-smokers. In a combined analysis of 11 case-control studies from six European countries, risk for bladder cancer increased with duration of smoking (number of years smoked) and intensity of smoking (number of cigarettes smoked per day).[47-50]

More than 60 carcinogens are present in tobacco smoke. Of these, 4-amino-biphenyl, acrolein, and oxygen free radicals are known to induce urothelial tumors. Arylamines, including the amino-biphenyls, are postulated to be the primary carcinogen in smoking induced bladder cancer. [51]

The stratification of patients and controls according to *MTHFR* genotype and tobacco status has showed that among smokers, all *C677T* genotypes were associated with an increased risk of bladder cancer especially the *MTHFR CC* polymorphism. This conclusion is in agreement with others studies' reporting that for this locus, the risk is essentially attributed to the tobacco effect.[17,20,31]

We propose that the C allele of *C677T* may affect MTHFR activity, slightly influencing its normal function.[13,52,53] As individuals with the C allele(s) get older, alterations towards carcinogenesis may accumulate via the decreasing functions of MTHFR. Cigarette smoking, a well known cause of DNA damage, will release many DNA damage inducers into the urinary system and cause DNA damage to cells. Therefore, in people who have a risky genetic variant, such as the C allele of *C677T*, and who also have a smoking habit, the joint effect of these factors may synergistically increase their urothelial cancer susceptibility. A similar result was reported by Tsai (2011) when they evaluated the association of polymorphism of *MTHFR* with smoking habits and oral cancer in Taiwan.[54]

The comparison of patients and controls groups according to their occupational exposures (Table 1) shows an association between bladder cancer risk and occupational exposure particularly to aromatic amines. This is in agreement with several previous studies. A review of occupational cohort studies demonstrates an increased risk of bladder cancer—independent of smoking—for individuals exposed to aromatic amines working in various industries including farming, chemical plants, rubber industry, painting and textiles[54-59]. The amount and duration of exposure to environmental carcinogens greatly affects risk of developing bladder cancer.[50]

It is important to note that only certain aromatic amines have been demonstrated to cause bladder cancer in laboratory models. The risk of developing bladder cancer following long term exposure to 1-naphthylamine, 2-naphthylamine, benzidine, and 4-aminobiphenyl has been demonstrated in case-control studies for over 60 years.[60,61] More recently, the International Agency for Research on Cancer (IARC) has also classified *ortho*-toluidine and chloroaniline (MOCA) as carcinogenic.[62] MOCA and *ortho*-toluidine act as mutagens causing bladder cancer in a similar fashion to 4-aminobiphenyl by creating metabolites, which form adducts with DNA.[59,63] IARC classified the occupational exposure to hair dyes for barbers/hairdressers as probably carcinogenic (IARC Group 2A); however, there is no clear evidence to support an association between bladder cancer and the personal use of hair dye and therefore the risk remains unclassifiable (IARC Group 3).[62]

Our results showed that occupationally exposed patients had higher risk for bladder cancer. This result confirms the occupational exposure as a bladder cancer risk factor. Furthermore, the analysis of gene-occupational exposure interaction indicated a significant effect between occupationally exposed patients and *C677T* polymorphism.

There are few reports about relationship between MTHFR polymorphism and occupational exposure. Previous case-control studies reported no association with the *MTHFR 677TT* genotype and urinary arsenic metabolite.[64]

However, Steinmaus (2007) reported association between *MTHFR* and arsenic metabolism.[65]

The study of the combined effect of tobacco, occupational exposures and *MTHFR* genotypes in bladder cancer development (Table 6), has suggested that the inheritance of MTHFR C677T allele(s) was associated with an increased risk of bladder cancer in smokers exposed patients, compared to non-smokers non exposed subjects with wild-type genotypes.

The study indicates that the *MTHFR C677T* mutation is not a risk factor of bladder cancer.

However, sample size was not large enough to completely evaluate the interaction between gene_environment factors in our study. Thus, our findings support the need for future studies of the potential interaction between genetic variation in MTHFR and environmental exposures among larger populations in which folate status has been characterized.

We conclude that tobacco smoking and occupational exposure interact additively and increase the risk of developing bladder cancer in north East Algeria.

Acknowledgement: Authors appreciate the kind participation of the patients and controls. We are grateful to all members of Biology and Genetics Laboratory, the Renal Clinic of Daksi for their contributions to this work.

Table 1: Frequency distributions of selected variables between the bladder cancer cases and control subjects.

Variables	Cases (N= 95)		Controls (N= 109)		Pa
	N	%	N	%	
Age (years)					
< 50	13	13.68	37	33.94	
50-69	51	53.68	56	51.38	< 0.001
≥ 70	31	32.63	16	14.68	
Sex					
Male	87	91.58	98	89.91	
Female	8	8.42	11	10.09	0.682
Smoking status					
smokers	22	23.16	71	65.14	
non smokers	73	86.84	38	34. 86	< 0.001
Occupational exposures					
HAP	18	18.94	3	2.75	
aromatic amines	32	33.68	13	11.93	
Nitrosamines	8	8.42	7	6.42	< 0.001
Any substance	37	38.95	86	78.90	

Table 2. Allelic and genotype differences for *MTHFR C677T* polymorphism for cases vs. controls comparison.

MTHFR C677T Genotype	Cases (N= 95) N (%)	Controls (N=101) N (%)	OR	95% CI	p value
CC	46 (48.42 %)	49 (43.12 %)			
CT	42 (44.21 %)	40 (38 .53 %)	1.12	0.59 -2.11	0.825
TT	7 (7.37 %)	20 (18.35 %)	0.37	0.13-1.05	0.06
CT or TT	49 (51.58%)	60 (56.88 %)	0.87	0.48-1.57	0.722
C allele	134 (70.52 %)	138 (62.38 %)			
T allele	56 (29.48 %)	80 (37.62%)	0.72	0.47-1.12	0.15

Table 3: Odds ratios and 95% confidence intervals for *C677T* polymorphism and bladder cancer overall, by cigarette smoking.

Smoking status	Genotype	Cases	Controls	OR	P value
Smokers	CC	36 (37.89%)	16 (14.68%)	-	
	CT	30 (31.58%)	15 (13.76%)	0.89 [0.35-2.28]	0.958
	TT	07(7.37%)	07 (6.42%)	0.44 [0.11-1.72]	0.30
	CT+TT	37 (38.95%)	22 (20.18%)	0.75 [0.31-1.77]	0.601
Non smokers	CC	10 (10.53%)	33 (30.27%)		
	CT	12 (12.62%)	25 (22.94%)	1.58[0.53-4.77]	0.50
	TT	00(0%)	13 (11.93%)	0 [0-1.57]	0.132
	CT+TT	12(12.62%)	38 (34.87%)	1.04[0.36-3.02]	0.872

Table 4: Association between *MTHFR* genotypes and bladder cancer risk among smokers and non-smokers patients.

Smoking status	Genotype	Cases	Controls	OR	P value
Non smokers	CC CT+TT	10 (10.53%) 12(12.62%)	33 (30.27%) 38 (34.87%)	1.04[0.36-3.02]	0.872
Smokers	CC CT+TT	36 (37.89%) 37 (38.95%)	16 (14.68%) 22 (20.18%)	7.43 [2.71-20.90] 5.55 [2.12-14.86]	0.00002 0.0001

Table 5: Association between, *MTHFR* genotypes and bladder cancer risk among exposed and non-exposed patients to occupational exposures.

Occupational exposure	Genotype	Cases	Controls	OR	P value
Non exposed	CC CT+TT	17 19	40 46	0.97 [0.42-2.28]	0.898
Exposed	CC CT+TT	29 30	9 14	7.58 [2.71-21.82] 5.04 [1.99-13.00]	0.0000231 0.00028

Table 6: Association between *MTHFR* genotypes, tobacco status, occupational exposures and bladder cancer.

Combined effect of tobacco and occupational exposure	Genotype	Cases	Controls	OR	P value
Non smokers **Not exposed**	CC CT+TT	6 5	29 29	0.83 [0.19-3.57]	0.958
Smokers exposed	CC CT+TT	22 17	2 8	53.17 [8.34-447.72] 10.27 [2.64-42.66]	0.0000001 0.000195

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D, Global cancer statistics, CA Cancer J Clin, 61, 2011, 69-90.
2. Ploeg M, Aben KK, Kiemeney LA, The present and future burden of urinary bladder cancer in the world, World J Urol, 27, 2009, 289-293.
3. Hamdi Cherif M, Zaidi Z, Abdellouche D, Hamdi S, Lakhdari N, Djema BA, Registre du cancer de sétif (Algérie): incidence, prévalence et survie, 1986-2005, Africain J cancer, 2, 2010, 245-258.
4. Freedman ND, Silverman DT, Hollenbeck AR, Schatzkin A, Abnet CC, Association between smoking and risk of bladder cancer among men and women, JAMA, 306, 2011, 737-745.
5. Garrett BE, Dube SR, Trosclair A, Caraballo RS, Pechacek TF, Centers for Disease Control and Prevention (CDC), Cigarette smoking-United States, 1965-2008, MMWR Surveill Summ, 60, 2011, 109-113.
6. Chu H, Wang M, Zhang Z, Bladder cancer epidemiology and genetic susceptibility, J Biomed Res, 27, 2013, 170-178.
7. Cohen SM, Shirai T, Steineck G, Epidemiology and etiology of premalignant and malignant urothelial changes. Scand J Urol Nephrol, 2, 2000, 105-115.
8. Heijmans BT, Boer JM, Suchiman HE, Cornelisse CJ, Westendorp RG, Kromhout D, Feskens EJ, Slagboom PE, A common variant of the methylenetetrahydrofolate reductase gene (1p36) is associated with an increased risk of cancer, Cancer Res, 63, 2003, 1249–1253.
9. Crider KS, Yang TP, Berry RJ, Bailey LB, Folate and DNA Methylation: A Review of Molecular Mechanisms and the Evidence for Folate's Role, Adv Nutr, 3, 2012, 21–38.
10. Kenwal R, Gupta S, Epigenetic modifications in cancer, Clin Genet, 81, 2012, 303–311.
11. Sirachainan N, Sasanakul W, Visudtibhan A, Tapanapruksakul P, Charoenkwan P, Kadegasem P, Udomsubpayakul U, Chuansumrit A, The effect of polymorphisms of MTHFR C677T, A1298C, MS A2756G and CBS 844ins68 bp on plasma total homocysteine level and the risk of ischemic stroke in Thai children, Thromb Res, 122, 2008, 33–37.
12. Kim YI, Folate and carcinogenesis: evidence, mechanisms, and implications, J Nutr Biochem, 10, 1999, 66–88.
13. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase, Nat Genet, 10, 1995, 111-113.
14. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R, A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity, Mol Genet Metab, 64, 1998, 169–172.
15. Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG, Rozen R, Human methylenetethrahydrofolate reductase: isolation of cDNA, mapping and mutation identification, Nat Genet, 7, 1994, 195-200.

16. Kimura F, Florl AR, Steinhoff C, Golka K, Willers R, Seifert HH, Schulz WA, Polymorphic methyl group metabolism genes in patients with transitional cell carcinoma of the urinary bladder, Mutat Res, 458, 2001, 49-54.
17. Lin J, Spitz MR, Wang Y, Schabath MB, Gorlov IP, Hernandez LM, Pillow PC, Grossman HB, Wu X, Polymorphisms of folate metabolic genes and susceptibility to bladder cancer: a case–control study, Carcinogenesis, 25, 2004, 1639–1647.
18. Moore LE, Wiencke JK, Bates MN, Zheng S, Rey OA, Smith AH, Investigation of genetic polymorphisms and smoking in a bladder cancer case–control study in Argentina, Cancer Lett, 211, 2004, 199–207.
19. Sanyal S, Festa F, Sakano S, Zhang Z, Steineck G, Norming U, Wijkström H, Larsson P, Kumar R, Hemminki K, Polymorphisms in DNA repair and metabolic genes in bladder cancer, Carcinogenesis, 25, 2004, 729–734.
20. Karagas MR, Park S, Nelson HH, Andrew AS, Mott L, Schned A, Kelsey KT, Methylene tetrahydrofolate reductase (MTHFR) variants and bladder cancer: a population-based case–control study, Int J Hyg Environ Health, 208, 2005, 321–327.
21. Moore LE, Malats N, Rothman N, Real FX, Kogevinas M, Karami S, García-Closas R, Silverman D, Chanock S, Welch R, Tardón A, Serra C, Carrato A, Dosemeci M, García-Closas M, Polymorphisms in one-carbon metabolism and trans sulfuration pathway genes and susceptibility to bladder cancer, Int J Cancer, 120, 2007, 2452–2458.
22. Ouerhani S, Oliveira E, Marrakchi R, Ben Slama MR, Sfaxi M, Ayed M, Chebil M, Amorim A, El Gaaied AB, Prata MJ, Methylene tetrahydrofolate reductase and methionine synthase polymorphisms and risk of bladder cancer in a Tunisian population, Cancer Genet Cytogenet, 176, 2007, 48–53.
23. Wang M, Zhu H, Fu G, Wang M, Zhang Z, Lu Q, Wang S, Zhang Z, Polymorphisms of methylenetetrahydrofolate reductase and methionine synthase genes and bladder cancer risk: a case-control study with meta-analysis, Clin Exp Med, 9, 2009, 9-19.
24. Rouissi K, Ouerhani S, Oliveira E, Marrakchi R, Cherni L, Ben Othman F, Ben Slama MR, Sfaxi M, Ayed M, Chebil M, Amorim A, Prata MJ, Benammar Elgaaied A, Polymorphisms in one-carbon metabolism pathway genes and risk for bladder cancer in a Tunisian population, Cancer Genet Cytogenet, 195, 2009, 43-45.
25. Izmirli M, Inandiklioglu N, Abat D, Alptekin D, Demirhan O, Tansug Z, Bayazit Y, MTHFR Gene Polymorphisms in Bladder Cancer in the Turkish Population, Asian Pacific J Cancer Prev, 12, 2011, 1833-1835.
26. Kouidhi S, Rouissi K, Khedhiri S, Ouerhani S, Cherif M, Benammar-Elgaaied A, Methylene-tetrahydrofolate reductase (MTHFR) gene polymorphisms and bladder cancer susceptibility: A meta-analysis that includes race, smoking status and tumor stage, J Toxicol Environ Health Sci, 3, 2011, 328-334.
27. Safarinejad MR, Shafiei N, Safarinejad S, Genetic susceptibility of methylene tetrahydrofolate reductase (MTHFR) gene C677T, A1298C and G1793A polymorphisms with risk for bladder transitional cell carcinoma in men, Med Oncol, 28, 2011, 398-412.
28. Li K, Hu Yp, Yang Z, Sun T, Association between MTHFR Ala222Val (rs1801133) polymorphism and bladder cancer susceptibility: a systematic review and meta-analysis, Tumor Biology, 34, 2013, 2565-2572.
29. Shi R, Zhao Z, Zhou H, Zhou J, Tan W, Lack of association between MTHFR Ala222Val and Glu429Ala polymorphisms and bladder cancer risk: A meta-analysis of case-control studies, Biomedical Reports, 2, 2014, 396-403.
30. Xu W, Zhang H, Wang F, Wang H, Quantitative assessment of the association between MHTFR C677T (rs1801133, Ala222Val) polymorphism and susceptibility to bladder cancer, Diagn Pathol, 8, 2013, 95.
31. Ouerhani S, Rouissi K, Marrakchi R, Ben Slama MR, Sfaxi M, Chebil M, El Gaaied AB, Combined effect of NAT2, MTR and MTHFR genotypes and tobacco on bladder cancer susceptibility in Tunisian population, Cancer Detect Prev, 32, 2009, 395–402.
32. Chung CJ, Pu YS, Su CT, Huang CY, Hsueh YM, Polymorphisms in one-carbon metabolism pathway genes, urinary arsenic profile, and urothelial carcinoma, Cancer Causes Control, 21, 2010, 1605-1613.
33. Cai DW, Liu XF, Bu RG, Chen XN, Ning L, Cheng Y, Wu B, Genetic polymorphisms of MTHFR and aberrant promoter hypermethylation of the RAS SF1 A gene in bladder cancer risk in a Chinese population, J Int Med Res, 37, 2009, 1882-1889.
34. Jacob RA, Gretz DM, Taylor PC, James SJ, Pogribny IP, Miller BJ, Henning SM, Swendseid ME, Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women, J Nutr, 128, 1998, 1204-1212.
35. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB, Ames BN, Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage, Proc Natl Acad Sci USA, 1997, 94, 3290-3295.
36. Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA, Morgan G, Polymorphisms in the methylene tetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults, Proc Natl Acad Sci USA, 96, 1999, 12810–12815.
37. Wiemels JL, Smith RN, Taylor GM, Eden OB, Alexander FE, Greaves MF; United Kingdom Childhood Cancer Study investigators, Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk of molecularly defined subtypes of childhood acute leukemia, Proc Natl Acad Sci USA, 98, 2001, 4004–4009.
38. Solomon E, Borrow J, Goddard AD, Chromosome aberrations and cancer, Science, 254, 1991, 1153-1160.
39. Jurgens B, Schmitz-Drager BJ, Schulz WA, Hypomethylation of L1 LINE sequences prevailing in human urothelial carcinoma, Cancer Res, 56, 1996, 5698–5703.
40. Laird PW, Jaenisch R, The role of DNA methylation in cancer genetics and epigenetics, Annu Rev Genet, 30, 1996, 441-464.
41. Chen RZ, Pettersson U, Beard C, Jackson-Grusby L, Jaenisch R, DNA hypomethylation leads to elevated mutation rates, Nature, 395, 1998, 89-93.
42. Florl AR, Löwer R, Schmitz-Dräger BJ, Schulz WA, DNA methylation expression of LINE-1 and HERV-K provirus sequences in urothelial and renal cell carcinomas, Br J Cancer, 80, 1999, 1312–1321.

43. Siegfried Z, Eden S, Mendelsohn M, Feng X, Tsuberi BZ, Cedar H, DNA methylation represses transcription *in vivo*, Nature Genet, 22, 1999, 203-206.
44. James SJ, Cross DR, Miller BJ, Alterations in nucleotide pools in rats fed diets deficient in choline, methionine and/or folic acid, Carcinogenesis, 13, 1992, 2471-2474.
45. Ulrich CM, Kampman E, Bigler J, Schwartz SM, Chen C, Bostick R, Fosdick L, Beresford SA, Yasui Y, Potter JD, Colorectal adenomas and the C677 MTHFR polymorphism: evidence for gene environment interaction, Cancer Epidemiol Biomark Prev, 8, 1999, 659-668.
46. Zeegers MP, Tan FE, Dorant E, van Den Brandt PA, The impact of characteristics of cigarette smoking on urinary tract cancer risk: a meta-analysis of epidemiological studies, Cancer, 89, 2000, 630-639.
47. Brennan P, Bogillot O, Cordier S, Greiser E, Schill W, Vineis P, Lopez-Abente G, Tzonou A, Chang-Claude J, Bolm-Audorff U, Jöckel KH, Donato F, Serra C, Wahrendorf J, Hours M, T'Mannetje A, Kogevinas M, Boffetta P, Cigarette smoking and bladder cancer in men: a pooled analysis of 11 case-control studies, Int J Cancer, 86, 2000, 289-94.
48. Brennan P, Bogillot O, Greiser E, Chang-Claude J, Wahrendorf J, Cordier S, Jöckel KH, Lopez-Abente G, Tzonou A, Vineis P, Donato F, Hours M, Serra C, Bolm-Audorff U, Schill W, Kogevinas M, Boffetta P, The contribution of cigarette smoking to bladder cancer risk in women (pooled European data), Cancer Causes Control, 12, 2001, 411-417.
49. Kuper H, Boffetta P, Adami HO, Tobacco use and cancer causation: association by tumour type, J Internal Medicine, 252, 2002, 206-224.
50. Kiriluk KJ, Prasad SM, Patel AR, Steinberg GD, Smith ND, Bladder cancer risk from occupational and environmental exposures, Urol Oncol, 2012, 30, 199–211.
51. Zeegers MP, Kellen E, Buntinx F, van den Brandt PA, The association between smoking, beverage consumption, diet and bladder cancer: a systematic literature review, World J Urol, 21, 2004, 392-401.
52. van der Put NM, Gabreëls F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, van den Heuvel LP, Blom HJ, A second common mutation in the methylene tetrahydrofolate reductase gene: an additional risk factor for neural-tube defects, Am J Hum Genet, 62, 1998, 1044-1051.
53. James SJ, Melnyk S, Pogribna M, Pogribny IP, Caudill MA, Elevation in S adenosylhomocysteine and DNA hypomethylation: potential epigenetic mechanism for homocysteine-related pathology, J Nutr, 132, 2002, 2361-2366.
54. Tsai CW, Hsu CF, Tsai MH, Tsou YA, Hua CH, Chang WS, Lin CC, Bau DT Methylene tetrahydrofolate Reductase (MTHFR) Genotype, Smoking Habit, Metastasis and Oral Cancer in Taiwan, Anticancer res, 31, 2011, 2395-2400.
55. Dolin PJ, A descriptive study of occupation and bladder cancer in England and Wales, Br J Cancer, 65, 1992, 476–478.
56. Viel JF, Challier B, Bladder cancer among French farmers: Does exposure to pesticides in vineyards play a part, Occup Environ Med, 52, 1995, 587–592.
57. Ward EM, Sabbioni G, DeBord DG, Teass AW, Brown KK, Talaska GG, Roberts DR, Ruder AM, Streicher RP, Monitoring of aromatic amine exposures in workers at a chemical plant with a known bladder cancer excess, J Natl Cancer Inst, 88, 1996, 1046–1052.
58. Carreón T, Hein MJ, Viet SM, Hanley KW, Ruder AM, Ward EM, Increased bladder cancer risk among workers exposed to o-toluidine and aniline, A reanalysis Occup Environ Med, 67, 2010, 348–350.
59. Pira E, Piolatto G, Negri E, Romano C, Boffetta P, Lipworth L, McLaughlin JK, La Vecchia C, Bladder cancer mortality of workers exposed to aromatic amines: A 58-year follow-up, J Natl Cancer Inst, 102, 2010, 1096–1099.
60. Case RA, Hosker ME, Mcdonald DB, Pearson JT, Tumours of the urinary bladder in workmen engaged in the manufacture and use of certain dyestuff intermediates in the British chemical industry: I. The role of aniline, benzidine, α-naphthylamine, and β-naphthylamine, Br J Ind Med, 11, 1954, 75-104.
61. Melick WF, Escue HM, Naryka JJ, Mezera RA, Wheeler EP, The first reported cases of human bladder tumors due to a new carcinogen-xenylamine, J Urol, 74, 1955, 760–766.
62. Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Cogliano V, Carcinogenicity of some aromatic amines, organic dyes, and related exposures, Lancet Oncol, 9, 2008, 322–323.
63. Swaminathan S, Frederickson SM, Hatcher JF, Reznikoff CA, Butler MA, Cheever KL, Savage RE Jr, Neoplastic transformation and DNA-binding of 4,4'-methylenebis (2-chloroaniline) in SV40-immortalized human uro epithelial cell lines, Carcinogenesis, 17, 1996, 857–864.
64. Porter KE, Basu A, Hubbard AE, Bates MN, Kalman D, Rey O, Smith A, Smith MT, Steinmaus C, Skibola CF, Association of genetic variation in cystathionine-beta-synthase and arsenic metabolism, Environ Res, 110, 2010, 580-587.
65. Steinmaus C, Moore LE, Shipp M, Kalman D, Rey OA, Biggs ML, Hopenhayn C, Bates MN, Zheng S, Wiencke JK, Smith AH, Genetic polymorphisms in MTHFR 677 and 1298, GSTM1 and T1, and metabolism of arsenic, J Toxicol Environ Health, 70, 2007, 159–170.

Source of Support: Nil, **Conflict of Interest:** None.