RESEARCH COMMUNICATION

MTHFR Gene Polymorphisms and Bladder Cancer Susceptibility: a Meta-Analysis Including Race, Smoking Status and Tumour Stage

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Abstract

Epidemiological studies have investigated that functional polymorphisms in the methylene- tetrahydrofolate reductase (MTHFR) gene may play an essential role in bladder carcinogenesis, but the numerous published studies have reported inconclusive results. The objective of the current study was to conduct an updated analysis in order to investigate the association between polymorphisms in the MTHFR gene and risk of bladder cancer. We searched the Pubmed database for all articles published up to March 31, 2011 that addressed bladder cancer and polymorphisms and variants or mutations of MTHFR for analysis using statistical software. Results for two polymorphisms (C677T and A1298C) in 27 case-control were studies from 15 articles indicated individuals carrying the 677T allele (TC or TT+TC) to have a reduction to a 29% or 21% compared to the wild genotype (CC) in mixed populations (OR: 0.71, 95%CI: 0.55-0.93 or OR: 0.79, 95%CI: 0.64-0.97, respectively) and it is shown that there is significant positive associations between A1298C polymorphism and bladder cancer in Africans (OR: 1.24, 95%CI: 1.02-1.52 for C vs.A; OR: 1.35, 95%CI: 1.10-1.66 for CA vs. AA; OR: 1.29, 95%CI: 1.08-1.55 for CC+CA vs. AA). However, no significant relationship was found in two polymorphisms in the stratified analysis by smoking status. Interestingly, individuals carrying the 677T allele (TT+TC) demonstrated a higher percentage of invasive than superficial cases (OR: 1.38, 95%CI: 1.13-1.69). The results from the current update analysis suggest that C677T and A1298C polymorphisms in the MTHFR gene are associated with bladder cancer risk and prognosis. Further evaluation based on more studies with larger groups of patients are now required.

Keywords: Bladder cancer - methylenetetrahydrofolate reductase - polymorphism - prognosis - susceptibility

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Introduction

An estimated 386 300 new cases and 150 200 deaths from bladder cancer occurred in 2008 worldwide (Jemal et al., 2011). There were 70 530 newly diagnosed (52 760 for male and 17 770 for female) bladder cancer cases and 14 680 related deaths (10 410 for male and 4 270 for female) in the USA in 2010 (Jemal et al., 2010). The highest incidence rates are found in countries in Europe, North America and North Africa, meanwhile the lowest rates are found in Melanesia and Middle Africa (Jemal et al., 2011). Bladder cancer has multifactorial etiology including interactions between genetic background and environmental factors.

Cigarette smoking is the most important risk factor for bladder cancer (Cohen et al., 2000), accounting for 50% of cases in men and 35% in women (Zeegers et al., 2000). Vitamin B12 is a strong antioxidant. High intakes of folate and B vitamins (B12, B6 and B2) are associated with significant decreases in bladder cancer risk (Garcia-Closas et al., 2007). Cigarette smoke contains a range of xenobiotics including oxidants and free radical and accordingly cigarette smoke exposure was associated with decreased levels of serum and red blood cell folate and vitamin B12 antioxidants (Mannino et al., 2003; Tungtrongchitr et al., 2003). In addition, reports indicate that plasma total homocysteine concentration is higher in smokers than in non-smokers (Lwin et al., 2002; Saw et al., 2001). These findings suggest that functional polymorphisms in genes involved in folate metabolism and serum levels of vitamin B12 may play an important role in bladder carcinogenesis.

Methylenetetrahydrofolate reductase (MTHFR) is one of central enzymes in folate metabolism which plays crucial and interrelated roles in folate pathway, DNA synthesis, repair and methylation. MTHFR gene is

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located on short arm of chromosome 1 (1p36.3) and the total length of this gene cDNA is 2.2 kb (Goyette et al., 1998).

MTHFR can catalyze the irreversible conversion of 5, 10-methylenetrahydrofolate to 5- methylenetrahydrofolate. The two common functional polymorphisms in the MTHFR gene have been discovered are C677T and A1298C (Weisber et al., 1998). The C677T polymorphism is located in the amino-terminal catalytic domain and can lead to a thermolabile enzyme with 35-50% reduced activity (Frosst et al., 1995). Meanwhile, the A1298C variant is located in the carboxy-terminal regulatory region and lymphocytes from individuals containing 1298CC genotype have been found to have approximately 60% of wild-type *in vitro* MTHFR activity (Van der Put et al., 1998).

A number of studies have indicated that two polymorphisms were involved in the etiology of bladder cancer. However, the results from those studies remain conflicting rather than conclusive. Previously, a meta-analysis involving seven studies written by Wang et al. (2009) didn't show any significant association between MTHFR two polymorphisms and bladder cancer. In the last two years, some new articles have been published to further investigate this issue. Considering the important role of MTHFR gene in bladder carcinogenesis, we performed an update analysis on all eligible case-control studies or only case studies involving cancer stage to estimate the bladder cancer risk associated with two polymorphisms (contains race, smoking and cancer stage). To our knowledge, this is the most comprehensive meta-analysis conducted to date with respect to the association between MTHFR gene polymorphisms and bladder cancer.

Materials and Methods

Identification of eligible studies

We conducted a literature search of the Pubmed database (http://www.ncbi.nlm.nih.gov/) (updated on March 31, 2011) using combinations of the following keywords 'polymorphism' or 'variant' or 'mutation' and 'bladder cancer' or 'carcinoma' and 'MTHFR' or 'methylenetetrahydrofolate reductase'. There was no language restriction in the search. All studies that evaluated the associations between polymorphisms of MTHFR gene bladder cancer risk were retrieved. Studies that were included in our meta-analysis had to meet all of the following criteria: (1) evaluation of MTHFR C677T and/or A1298C polymorphisms and bladder cancer risk; (2) case-control design; (3) genotype frequency was available; (4) only the full-text manuscripts were included; (6) genotype distributions of control consistent with Hardy–Weinberg equilibrium (HWE) and (7) some date containing cancer stage (Rouissi et al., 2011).

The following exclusion criteria are also applied: (1) no control population; (2) no available genotype frequency; (3) HWE of controls were less than 0.05; (4) for studies with overlapping or repeating data, only the most recent or complete studies with the largest numbers of cases and controls were included; (5) studies haven't been published.

Data extraction

We carefully extract the information from all eligible publications independently according to the inclusion criteria listed above. The following data were collected from each study: first author's last name, year of publication, race of origin, sample size (cases/controls), age range in cases and controls, study design, and HWE of controls.

Statistical analysis

We use crude risk ratios (OR) with 95% confidence intervals (CI) to measure the strength of the association between MTHFR two polymorphisms and bladder cancer based on the genotype frequencies in cases and controls. In our analysis, we recognized 677T or 1298C as 'M' and C677 or A1298 as 'W'. We analyzed this relationship between C677T or A1298C and bladder cancer risk using three different models which are respectively: allelic contrast (M vs. W), heterozygote comparison (MW vs. WW), and dominant model (MM+MW vs. WW). It should also be noted that different ethnic descents are categorized as European, Asian, African and Mixed (if the included population isn't a pure race). Subgroup analysis stratified by smoking status (smokers and non-smokers) was conducted only under the dominant model with all studies for which stratification data on smoking were available. Furthermore, bladder cancer are classified into superficial (pTa and pT1) and muscle invasive (≥ pT2) stages. Thus in our study, cancer stage was also performed only in the dominant model similarly to smoking status. We evaluated the heterogeneity assumption using a chi-square-based Q-test. A Probability (P-value) value of more than 0.05 for the Q-test indicates a lack of heterogeneity among the studies. The statistical significance of the summary OR was determined with the Z-test. In order to better evaluate the extent of heterogeneity between studies, the I2 test was also used (Higgins et al., 2003). As a guide, I2 values which are less than 25% are considered 'low', values near 50% are 'moderate' and values greater than75% are considered 'high'. If $P \leq 0.05$, or $I2 \geq 50\%$ then a random-effects model, also called the DerSimonian–Laird method, is adopted, otherwise we choose a fixed-effects model following the Mantel–Haenszel method as described in DerSimonian et al., 1986 and also in Mantel and Haenszel, 1959. The funnel plot asymmetry and publication were assessed with Egger's test where a $P < 0.05$ was considered statistically significant (Egger et al., 1997). The departure of frequencies of MTHFR polymorphisms from expectation under HWE was assessed by a $\chi 2$ test in controls using the Pearson chi-square test and as previously, we associate the probability values which are less than 0.05 with significance of the test. All statistical tests for this meta-analysis were performed with Stata software (version 10.0).

Genotyping methods

We conduct genotyping for SNP of MTHFR gene polymorphisms using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), TaqMan, and DNA sequence.

Results

Study characteristics

All 24 abstracts retrieved through the search criteria in Pubmed, 9 were excluded (including duplication, review, meta-analysis, case-only which didn't contain cancer stage data and insufficient genotype). Finally, we identified 15 articles (27 case-control studies, 11 different first authors) to evaluate the association of C677T and/or A1298C polymorphisms in MTHFR gene with risk and/or prognosis for bladder cancer. There are two studies (Safarinejad et al., 2010; Sanyal et al., 2007) about classification of aggressiveness in bladder cancer but are not similar to our study design. In fact, Sanyal et al. (2007) considered pTa or G1+G2A as 'low risk' and ≥ pT1 or G2B+G3+G4 as 'high risk', whereas Safarinejad et al. (2010) classified tumor grade into two parts, 'low' (G1) and 'high' (G2+G3) and so these prognostic data were not included in our analysis. Table 1 shows the study characteristics of our research. The distribution of genotypes in the controls was consistent with HWE in all studies and therefore there were fourteen publications involving 7 212 cases and 7 801 controls. Control population including study participants didn't have history of malignant diseases. For the C677T polymorphism the T% in Asian (40.7%) was higher than in African (32.8%) or in European (34.5%) or even in Mixed populations (32.9%). In fact referring to the existing literature, one can find five studies of Europeans, four of Asians, four of Africans and two of Mixed populations. Six studied contained smokers data and five for non-smokers. Also we can notice that for the A1298C polymorphism there are C% in European (29.5%) and Mixed (27.2%) and these figures are higher than in Asian (21.9%) or African (25.9%) in cases. In addition, there are four studies of European, three of African, three of Asian and two of Mixed populations. Four studied contained smokers data and four for non-smokers, and two studies referred to

Table 1. Study Characteristics from Published Studies on the Relationship Between Two Polymorphisms in Mthfr Gene and Bladder Cancer

First Author (Year)	Race	Case/control		HWE of control		Mean±SD (Age Range)		StudyDesign
		C677T	A1298C	C677T	A1298C	Case	Control	
Rouissi (2011)	African	130/-	130/-	-	-	67.86±9.16(NA)	-	HB
Safarinejad (2010)	Asian	158/316	158/316	0.460	0.555	62.67±10.64(NA)	61.64±9.47(NA)	HB
Chung (2010)	Asian	150/300	-	0.256	-	65.32±1.08(NA)	66.2±0.73(NA)	HB
Cai (2009)	Asian	312/325	312/325	0.076	0.504	NA	NA	HB
Rouissi (2009)	African	185/191	185/191	0.494	0.478	67.45±9.7(NA)	67.45±9.7(NA)	HB
Ouerhani (2009)	African	90/110	-	0.417	-	68.74±8.39(NA)	64.26±10.64(NA)	HB
Wang (2009)	Asian	239/250	239/250	0.066	0.187	NA	NA	PB
Ouerhani (2007)	African	111/131	111/131	0.550	0.089	72.28±7.92(NA)	72.28±7.92(NA)	HB
Moore (2007)	European	1041/1049	1068/1078	0.481	0.467	66±10(NA)	65±10(NA)	PB
Karagas (2005)	European	350/543	350/542	0.702	0.333	NA	NA	PB
Lin (2004)	Mixed(Mexican)	17/17	17/17	0.582	0.679	65±NA(18-88)	64±NA(21-91)	PB
Lin (2004)	African	21/21	21/21	0.760	0.281	65±NA(18-87)	64±NA(21-90)	PB
Moore (2004)	Mixed(Argentine)	106/109	106/108	0.293	0.771	NA(20-80)	NA(20-80)	PB
Lin (2004)	European	410/410	410/409	0.900	0.351	65±NA(18-86)	64±NA(21-89)	PB
Sanyal (2004)	European	309/246	311/245	0.823	0.600	70±NA(33-96)	70±NA(33-97)	PB
Kimura (2001)	European	165/150	-	0.169	-	67.4±11.5(34-96)	62±11.4(16-90)	HB

Table 2. Total and Stratified Analysis of Two Polymorphisms in MTHFR gene on Bladder Cancer

Variables	N	Case/Control	M-allele vs. W-allele				MW vs. WW				MM+MW vs. WW			
			OR (95%CI)	Ph	P	I2	OR (95%CI)	Ph	P	I2	OR (95%CI)	Ph	P	I2
C677T														
Total	15	3664/4168	1.01(0.92-1.12)	0.032	0.829	44.7	1.02(0.97-1.07)	0.076	0.422	36.7	1.04(0.90-1.19)	0.029	0.599	45.2
Race														
Asia	4	859/1191	1.14(0.91-1.44)	0.026	0.260	67.6	1.06(0.98-1.15)	0.396	0.163	0.0	1.07(1.00-1.14)	0.155	0.062	42.7
Europe	5	2275/2398	0.98(0.93-1.03)	0.342	0.455	11.2	1.00(0.95-1.07)	0.271	0.907	22.5	0.99(0.95-1.04)	0.238	0.796	27.6
Africa	4	407/453	0.97(0.85-1.11)	0.645	0.623	0.0	1.10(0.96-1.25)	0.275	0.180	22.7	1.04(0.93-1.17)	0.340	0.508	10.6
Mixed	2	123/126	0.84(0.67-1.06)	0.254	0.137	23.0	0.71(0.55-0.93)	0.300	0.012	7.0	0.79(0.64-0.97)	0.259	0.022	21.6
Smoking														
Yes	6	966/1039	-				-				0.99(0.92-1.08)	0.567	0.897	0.0
No	5	242/532	-				-				1.02(0.89-1.16)	0.286	0.827	20.1
A1298C														
Total	12	3288/3633	1.10(0.95-1.28)	0.000	0.204	66.8	1.18(0.99-1.42)	0.004	0.072	60.0	1.17(0.96-1.41)	0.001	0.112	66.6
Race														
Asia	3	709/891	1.27(0.73-2.21)	0.000	0.403	89.8	1.36(0.71-2.59)	0.000	0.350	88.1	1.39(0.69-2.81)	0.000	0.359	90.5
Europe	4	2139/2274	0.98(0.92-1.05)	0.987	0.645	0.0	1.02(0.96-1.09)	0.774	0.497	0.0	1.01(0.95-1.07)	0.900	0.834	0.0
Africa	3	317/343	1.24(1.02-1.51)	0.745	0.030	0.0	1.35(1.10-1.66)	0.509	0.004	0.0	1.29(1.08-1.55)	0.609	0.006	0.0
Mixed	2	123/125	1.00(0.75-1.33)	0.306	0.987	4.6	1.00(0.75-1.35)	0.595	0.990	0.0	1.00(0.77-1.30)	0.429	1.000	0.0
Smoking														
Yes	4	763/706	-				-				1.05(0.95-1.16)	0.605	0.367	0.0
No	4	230/468	-				-				1.03(0.88-1.19)	0.070	0.751	57.5

Table 3. Relationship Between C677T Polymorphism in MTHFR Gene and Bladder Cancer Prognosis

MTHFR	Genotype	Invasive case	Superficial case	OR(95%CI)	Q' test	P	Z' test
C677T	CC	26	101				
	TT+TC	59	102	1.38(1.13-1.69)	P=0.324	0.002	P=3.10

cancer stage involving in two polymorphisms (Khedhiri et al., 2010; Safarinejad et al., 2010). Among these studies, eight were hospital-based and also eight were population-based.

Meta-analysis

a) C677T polymorphism

Overall there are not obvious significantly relationships between C677T polymorphism and bladder cancer risk in three available genotype models (T-allele vs. C-allele: OR = 1.01, 95%CI = 0.92-1.12, Ph = 0.032, P = 0.829, I2 = 44.7; TC vs. CC: OR = 1.02, 95%CI = 0.97-1.07, Ph = 0.076, P = 0.422, I2 = 36.7; TT+TC vs. CC: OR = 1.04, 95%CI = 0.90-1.19, Ph = 0.029, P = 0.599, I2 = 45.2). However the stratified analysis by race shows that C677T polymorphism is strongly associated with decreased bladder cancer risk under heterozygote comparison (OR = 0.71, 95%CI = 0.55-0.93, Ph = 0.300, P = 0.012, I2 = 7.0) and dominant model (OR = 0.79, 95%CI = 0.64-0.97, Ph = 0.259, P = 0.022, I2 = 21.6) in Mixed populations. In the subgroup analysis by smoking status there is also no association in smoker or non-smoker with bladder cancer (Table 2). Interestingly, 677T allele (TT+TC) has a significantly higher percentage value than C677 allele (CC) in the subgroup of invasive cases (OR = 1.38, 95% CI=1.13-1.69, Ph = 0.324, P=0.002) as shown in Table 3.

b) A1298C polymorphism

Our analysis shows no relationship is found in all the three models between this polymorphism and bladder cancer risk (C-allele vs. A-allele, OR = 1.10, 95%CI = 0.95-1.28, Ph = 0.000, P = 0.204, I2 = 66.8; CA vs. AA, OR = 1.18, 95%CI = 0.99-1.42, Ph = 0.004, P = 0.072, I2 = 60.0; CC+CA vs. AA, OR = 1.17, 95%CI = 0.96-1.41, Ph = 0.001, P = 0.112, I2 = 66.6). Specifically, 1298C allele is shown to be a significantly increased bladder cancer risk in African race (C-allele vs. A-allele, OR = 1.24, 95%CI = 1.02-1.51, Ph = 0.745, P = 0.030, I2 = 0.0; CA vs. AA, OR = 1.35, 95%CI = 1.10-1.66, Ph = 0.509, P = 0.004, I2 = 0.0; CC+CA vs. AA, OR = 1.29, 95%CI = 1.08-1.55, Ph = 0.609, P = 0.006, I2 = 0.0). Moreover, the results in Table 2 indicate a similar conclusion in the subgroup of smoking status as with C677T polymorphism.

Sensitivity analysis

We deleted each study involved in our meta-analysis to reflect the influence of the individual data-set to the pooled OR and the corresponding pooled OR was not materially altered indicating that our results were statistically robust.

Publication bias diagnosis

The Begg's funnel plot and Egger's test were performed to access the publication bias of literatures. The shape of the funnel plot did not reveal obvious asymmetry and the Egger's test suggested the absence of publication bias in each MTHFR polymorphism.

Discussion

Folic acid is essential for normal DNA synthesis and normal cellular methylation reactions. The 5, 10-methyltetrahydrofolate reductase (MTHFR) enzyme catalyzes the synthesis of 5-methylenetetrahydrofolate and the methyl donor for the B12-dependent remethylation of homocysteine to methionine. Methionine is the precursor for S-adenosyl-methionine (SAM) which is the major cellular methyl donor for DNA and RNA proteins and phospholipids methylation. Hence, all these pathways might be affected by the MTHFR C677T or A1298C functional polymorphism which could both reduce enzyme activity. With T allele of MTHFR at 677 position and C allele of MTHFR at 1298 position reported to influence MTHFR gene expression, there have been a number of investigations carried in the recent years to further explore this issue and provide more insights. However, the results from these studies were ambiguous because of their small sample size and unified ethnicity. Furthermore, the recent meta-analysis study which has focused on this point does not include most of the related studies (Ouerhani et al., 2007; Kimura et al., 2001).

To provide further insights and to shed more lights on this debated subject, an update meta-analysis is needed to achieve a more reliable and comprehensive conclusion on both variants. Although no significant associations were observed between C677T polymorphism and the susceptibility to bladder cancer risk in our analysis, there are fairly significant relationships that we can detect specifically in Mixed populations rather than just in European, Asian and African populations. This result may be explained by the fact that allele and genotype distribution of MTHFR C677T locus is different across various races. In fact, the Brazilian population holds the lowest frequency ever reported for the 677TT genotype (9%) and the highest (19.1%) is in the Chinese population (Hefenstein et al., 2005; Sun et al., 2004) and MTHFR C677T locus is also different in different Caucasians as shown in Dong et al., 2010. In addition, the frequency of MTHFR 677T allele is seen remarkably different between African and Afro-American populations (Gueant et al, 2007). Grade status and T stage could be considered as prognostic factors in bladder cancer where superficial low-grade tumors (G1 or pTa and pT1) are characterized by frequent recurrences. In contrast, high-grade tumors (G2 and G3 or ≥ pT2) represent a significant risk of future tumors progression and death for this disease. In our present study, we find that individuals who carried 677T allele have a high percentage in ≥ pT2 and this clearly shows that C677T polymorphism is partly related to bladder cancer outcome. As for the A1298C polymorphism, although this 1298C allele could reduce enzymatic activity as wild type MTHFR and influence its expression, we could not find any statistically significant

relationship with bladder cancer in our analysis.

In order to relate our finding to our results in the literature, one can notice that Safarinejad et al. (2010) found that 1298C allele (CA+CC) was significantly associated with increased risk of bladder cancer in Asians, however in our present analysis with includes more subjects we did not detect any relationship between Asians and A1298C polymorphism. A new association between Africans and A1298C polymorphism was observed in all three genetic models which also confirms our previous results reported in Rouissi et al., 2009. Furthermore, past studies have reported that smokers are two to three times more likely to develop bladder cancer, 50% of which are directly attributable to cigarette smoking (Hoover et al., 1971; Wynder et al., 1977) . To our knowledge smoking is a major risk factor to bladder cancer but to our regret any significantly increased association was only found between two MTHFR polymorphisms and bladder cancer.

Some potential limitations of our meta-analysis should be taken in consideration. Firstly, there were only two Mixed population studies about two polymorphisms and also we think that new studies should focus on this race factor. Secondly, incomplete data and diversity of genotyping methods may influence the overall effects to some extent and therefore more studies are needed to deal with this issue and pay more attention to gene-gene and gene-environment interactions. Thirdly, there are four studies which referred to tumor stage but only two studies have dealt with tumor stage and C677T polymorphism implication. Additional studies are needed to shed more light and to provide more explanation on this type of relationship and to strengthen the subsequent meta-analysis. Our meta-analysis provides three advantages which may be described as follows: (i) the quality of case–control studies included in the current meta-analysis is satisfactory based on our selection criteria; (ii) the HWE of controls are all more than 0.05; and (iii) there is no publication bias in all the genetic models which suggests that the results are relatively stable.

In conclusion, the present update-analysis found new evidence that MTHFR C677T or A1298C polymorphism had different effects on bladder cancer in different races. Moreover, C677T polymorphism was related to bladder cancer prognosis and could be considered as a pooled marker. We can expect further prospective studies with larger number of worldwide individuals to examine the associations between these two polymorphisms in MTHFR and bladder cancer risk.

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The authors declare that they have no competing interests.

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