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Full Length Research Paper

Methylene-tetrahydrofolate reductase (MTHFR) gene polymorphisms and bladder cancer susceptibility: A meta-analysis that includes race, smoking status and tumor stage

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Epidemiological studies have investigated that functional polymorphisms in methylene-tetrahydrofolate reductase (MTHFR) gene may play an essential role in bladder carcinogenesis, but the several published studies have reported inconclusive results. The objective of the current study is to conduct an update analysis investigating the association between polymorphisms in MTHFR gene and the risk of bladder cancer. We searched the Pubmed database for all articles published up to March 31, 2011 that addressed the issue of bladder cancer and polymorphisms and variants or mutations of MTHFR. We perform a statistical analysis using STAT-A software. Two polymorphisms (C677T and A1298C) in 27 case-control studies from 15 articles that have been analyzed. The results indicated that individuals who carry the 677T allele (TC or TT+TC) have a 29% or 21% to develop bladder cancer compared to 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). 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 is found in two polymorphisms in the stratified analysis by smoking status. Interestingly, we indicate that individuals carrying 677T allele (TT+TC) have a higher percentage in invasive cases than superficial cases (OR: 1.38, 95%CI: 1.13-1.69). Results from the current new analysis suggest that C677T and A1298C polymorphisms in MTHFR gene are associated with bladder cancer risk and prognosis. Further evaluation based on more studies with larger groups of patients will be required in future research.

Key words: Methylenetetrahydrofolate reductase, polymorphism, prognosis, susceptibility, meta-analysis.

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 (5, 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 the countries of Europe, North America and Northern Africa whereas the lowest rates are found in Melanesia and Central Africa (Jemal et al., 2011).Bladder cancer has multifactorial etiology including interactions between genetic background and environmental factors and 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).

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Vitamin B12 is a strong antioxidant and high intakes of folate and B vitamins (B12, B6 and B2) are associated with significant decrease in bladder cancer risk (García-Closas et al., 2007). Cigarette smoke contains a range of xenobiotics including oxidants and free radical, and thus cigarette smoke exposure is associated with lower 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 play crucial and interrelated roles in folate pathway, DNA synthesis, and repair and methylation. MTHFR gene is 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 that were discovered are C677T and A1298C (Frosst et al., 1995; Weisberg 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 et al., 1998).

A number of studies indicated that there are two polymorphisms 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) did not show any significantly association between MTHFR two polymorphisms and bladder cancer. But in the last two years new studies were conducted to further investigate the issue. Considering the important role of MTHFR gene in bladder carcinogenesis we perform 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 and taking into account race, smoking status, 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.

METHODS

Identification of eligible studies

We conduct a literature search of the Pubmed database (http://www.ncbi.nlm.nih.gov/) using combinations of the following keywords polymorphism, variant or mutation, bladder cancer or carcinoma, and MTHFR or methylenetetrahydrofolate reductase. There was no language restriction in the search. All the 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: (i) evaluation of MTHFR C677T and/or A1298C polymorphisms and bladder cancer risk; (ii) case-control design; (iii) availability of genotype frequency; (iv) only full-text manuscripts are included; (v) genotype distributions of control consistent with Hardy–Weinberg equilibrium (HWE) and (vi) some date containing cancer stage (Rouissi et al., 2011).

Meanwhile, the following exclusion criteria are also applied: (i) no control population; (ii) no available genotype frequency; (iii) HWE of controls are less than 0.05; (iv) for studies with overlapping or repeating data, only the most recent or complete studies with the largest numbers of cases and controls are included.

Data collection

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, and study design and HWE of controls.

Statistical analysis

We use crude odds 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 identify 677T or 1298C as 'M', and C677 or A1298 as 'W'. We analyze 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 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) is conducted only under the dominant model based on studies whose stratification data on smoking are available. Furthermore, bladder cancer are classified into superficial (pTa and pT1) and muscle invasive (≥ pT2) stages. Thus in our study, cancer stage is also performed only in the dominant model similarly to smoking status. We evaluate the heterogeneity assumption with a chi-square-based *Q*-test. A *P*-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 is determined with the *Z*-test. In order to better evaluate the extent of heterogeneity between studies, we also use the I^2 test (Higgins et al., 2003). As a guide, I^2 values which are less than 25% are be considered 'low', values near 50% are 'moderate' and values greater that 75% are referred to as 'high'. If $P \leq 0.05$ or $I^2 \geq 50\%$ then we adopt a random-effects model (DerSimonian–Laird method), otherwise if $P > 0.05$, and $I^2 < 50\%$ then we choose a fixed-effects model (Mantel–Haenszel method) as described in previous studies (DerSimonian et al., 1986; Mantel et al., 1959). The funnel plot asymmetry and publication are assessed with Egger's test where a *P-value* < 0.05 was considered statistically significant (Egger et al., 1997). The departure of frequencies of MTHFR polymorphisms from expectation under HWE was assessed by χ^2 test in controls using the Pearson chi-square test and as previously we associate the *P*-values < 0.05 with significance of the test. All statistical tests for this meta-analysis were performed with Stata software (version 10.0).

Table 1. Results from published studies on the relationship between two polymorphisms in MTHFR gene and bladder cancer risk.

First author (year)	Race	Case/control		HWE of control		Mean±SD (age range)		Study design
		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

NA: Not available; HB: hospital-based; PB: population-based; SD: standard deviation.

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

In the total of 24 abstracts retrieved through the search criteria in Pubmed we exclude 9 that didn't contain cancer stage data and insufficient genotype. So we identify 15 articles which include 27 case-control studies and 11 different first authors (Wang et al., 2009; Rouissi et al., 2011; Safarinejad et al., 2010; Chung et al., 2010; Cai et al., 2009; Rouissi et al., 2009; Ouerhani et al., 2009; Ouerhani et al., 2007; Moore et al., 2007; Karagas et al., 2005; Lin et al., 2004; Moore et al., 2004; Sanyal et al., 2004; Kimura et al., 2001; Sanyal et al., 2007) 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 they are not consisting with our study design. In fact in one study, Sanyal et al. (2007) consider pTa or G1+G2A as 'low risk' and ≥ pT1 or G2B+G3+G4 as 'high risk', whereas in the second study Safarinejad et al., (2010) classified tumor grade into two parts, 'low' (G1) and 'high' (G2+G3). The difference in our study is that we did not include these prognostic data, and Table 1 shows in detail the characteristics of our study. We notice that the distribution of genotypes in the controls is consistent with HWE in all studies and there are were fourteen publications involving 7 212 cases and 7 801 controls. Control population including all consisted of study participants do not have history of malignant diseases. For the C677T polymorphism, T% in Asian (40.7%) was higher than in African (32.8%), European (34.5%) and even in Mixed populations (32.9%) in cases. In fact referring to the literature, one can find five studies of European, four of Asian, four of African

and two of Mixed population, and also six studies include smoker data and five for non-smoker. 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%) cases. In addition, there are four studies of European, three of African, three of Asian and two of Mixed population for which four studies have included smoker data and the same number of studies considered non-smoker data, and only two studies refer to cancer stage and use two polymorphisms (Rouissi et al., 2011; Safarinejad et al., 2010). Finally, among all the studies searched we find eight that are hospital-based and also eight that are population-based.

Meta-analysis

C677T polymorphism

Overall, we find no obvious significant 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, P_h = 0.032, P = 0.829, I^2 = 44.7; TC vs. CC: OR = 1.02, 95%CI = 0.97-1.07, P_h = 0.076, P = 0.422, I^2 = 36.7; TT+TC vs. CC: OR = 1.04, 95%CI = 0.90-1.19, P_h = 0.029, P = 0.599, I^2 = 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, P_h = 0.300, P = 0.012, I^2 = 7.0) and dominant model (OR = 0.79, 95%CI = 0.64-0.97, P_h = 0.259, P = 0.022, I^2 = 21.6) in Mixed populations. In the subgroup analysis by smoking status there is also no association between smoker or non-smoker and bladder cancer as shown in Table 2. Interestingly, we find that 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, P_h = 0.324, P = 0.002). Table 3 illustrates the result.

A1298C polymorphism

Our analysis shows no relationship can be 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, P_h = 0.000, P = 0.204, I^2 = 66.8; CA vs. AA: OR = 1.18, 95%CI = 0.99-1.42, P_h = 0.004, P = 0.072, I^2 = 60.0; CC+CA vs. AA: OR = 1.17, 95%CI = 0.96-1.41, P_h = 0.001, P = 0.112, I^2 = 66.6). In addition, we find that 1298C allele has a significant role in increasing bladder cancer risk in African race (C-allele vs. A-allele: OR = 1.24, 95%CI = 1.02-1.51, P_h = 0.745, P = 0.030, I^2 = 0.0; CA vs. AA: OR = 1.35, 95%CI = 1.10-1.66, P_h = 0.509, P = 0.004, I^2 = 0.0; CC+CA vs. AA: OR = 1.29, 95%CI = 1.08-1.55, P_h = 0.609, P = 0.006, I^2 = 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 remove each individual study involved in our meta-analysis in order to reflect the influence of the specific data of that study on the pooled OR. We find that the new pooled OR does not change beyond a small margin, so we may conclude that the study results are statistically robust.

Publication bias diagnosis

We perform the Begg's funnel plot and Egger's test in order to assess the literature publication bias. It should be noted that the shape of the funnel plot does not reveal obvious asymmetry and also the Egger's test suggests no 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, RNA, and for proteins and phospholipids methylation. Hence, all these pathways might be affected by the MTHFR C677T or A1298C functional polymorphism which both could reduce enzyme activity. With T allele of MTHFR at 677 positions and C allele of MTHFR at 1298 position reported to influence MTHFR gene expression there have been a number of investigations carried on in recent years. However, the results from these studies are ambiguous because of for 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 (Wang et al., 2009; Ouerhani et al., 2009; Ouerhani et al., 2007; Moore et al., 2007; Karagas et al., 2005; Lin et al., 2004; Moore et al., 2004; Sanyal et al., 2004; Kimura et al., 2001).

In order to provide further insights and to shed more lights on this debated subject we need an updated meta-analysis to achieve a more reliable and comprehensive conclusion on both variants. Although in our analysis we do not find overall significant associations between C677T polymorphism and the susceptibility to bladder cancer risk, our results point to fairly significant relationships that are detected in mixed populations

Table 2. Pooled and stratified analysis of two polymorphisms in MTHFR gene on bladder cancer.

C677T	Variable	N	Case/ Control	M-allele vs. W-allele				MW vs. WW				MM+MW vs. WW			
				OR(95%CI)	P_h	P	I^2	OR(95%CI)	P_h	P	I^2	OR(95%CI)	P_h	P	I^2
	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														
	Asian	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
	European	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
	African	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														
	Smoker	6	966/1039	-				-				0.99(0.92-1.08)	0.567	0.897	0.0
	Non- smoker	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														
	Asian	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
	European	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
	African	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														
	Smoker	4	763/706	-				-				1.05(0.95-1.16)	0.605	0.367	0.0
	Non-smoker	4	230/468	-				-				1.03(0.88-1.19)	0.070	0.751	57.5

rather than in just Europeans, Asians and Africans 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, statistical results from the literature show that Brazilian population holds the lowest frequency ever reported for the 677TT genotype (9%) and the highest (19.1%) is in the Chinese population (Helfenstein et al. 2005; Sun et al., 2004); and also it is found that MTHFR C677T locus differs across Caucasians (Dong et al., 2010). In addition, the frequency of MTHFR 677T allele is seen remarkable differently between African and Afro-American populations (Guéant 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 and in contrast, high-grade tumors (G2 and G3 or ≥ pT2) a 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 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 a significant relationship that connects it to bladder cancer.

In order to relate our findings with the results in the literature, we note that (Safarinejad et al., 2010) found that 1298C allele (CA+CC) was

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

significantly associated with increased risk of bladder cancer in Asians, however in our analysis which includes more subjects we did not find any relationship between Asians and A1298C polymorphism. However, we found an association between Africans and A1298C polymorphism in the three genetic models, and this confirms our previous results reported in 2009 (Rouissi et al., 2009).

Furthermore, previous studies have reported that smokers are two to three times more likely to develop bladder cancer and 50% of the cases are directly attributed to cigarette smoking (Hoover and Cole 1971). To our knowledge, smoking is a major risk factor to bladder cancer but to our regret, the significant positive association is only found between two MTHFR polymorphisms and bladder cancer.

We recognize some potential limitations in our meta-analysis which should be taken into consideration. Firstly, there are only two mixed population studies about two polymorphisms and we also 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 these problems and to pay closer attention to gene-gene and gene-environment interactions. Thirdly, there are four studies which refer to tumor stage but only two addressed the issue of tumor stage and C677T polymorphism. Additional studies are required to shed further light and to provide more explanation on this type of relationship and to strengthen the subsequent meta-analysis. Despite these challenges, 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 were all more than 0.05; and (iii)there is publication bias in all genetic models, which suggests that results were stable.

Conclusion

The present up to date analysis reveals new evidence that MTHFR C677T and A1298C polymorphism have effects on bladder cancer that differs across population races. Moreover, it is found that C677T polymorphism is related to bladder cancer prognosis and could be considered as a pooled marker. We can expect future 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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