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Original Article

Association between methylenetetrahydrofolate reductase gene rs1801133 C/T polymorphism and urinary cancer risk

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Abstract: A single nucleotide polymorphism (SNP) named *rs1801133 C/T* may decrease methylenetetrahydrofolate reductase (MTHFR) activity, which may affect DNA methylation, synthesis, and repair for cancer development. However, the data from previously published studies were inconsistent and had low statistical power for urinary cancer. The objective of our current study was to conduct an update analysis of the association between the *MTHFR rs1801133 C/T* polymorphism and the risk of urinary cancer. We performed a meta-analysis of 40 case-control studies. We assessed the strength of the association by using odds ratios (OR) with 95% confidence intervals (CI). On one hand, we found that the *MTHFR rs1801133 C/T* polymorphism was associated with decreased prostate cancer risk among Asians and a source of benign prostatic hyperplasia (BPH) patients, with decreased bladder cancer risk among a mixed population. On the other hand, we found that the *MTHFR rs1801133 C/T* polymorphism might increase renal cell carcinoma risk. Results from this update analysis suggested that *MTHFR rs1801133 C/T* is associated with urinary cancer.

Keywords: MTHFR, urinary cancer, polymorphism, risk, meta-analysis

Introduction

Methylenetetrahydrofolate reductase (MTHFR) is a central enzyme in the folate pathway that plays crucial and interrelated roles in DNA biosynthesis, methylation, and genomic integrity. MTHFR catalyzes the irreversible conversion of 5,10-methylenetrahydrofolate to 5-methylenetrahydrofolate, which provides one-carbon groups for the methylation of homocysteine to methionine via S-adenosyl methionine (SAM), the universal donor of one-carbon groups. Insufficient DNA methylation or hypomethylation can lead to genomic instability and activation of oncogenes [1-3]; hence, this gene could influence cancer development.

MTHFR is located on the short arm of chromosome 1 (1p36.3) [4]. A common, functional, single nucleotide polymorphism (SNP), *C677T/rs1801133 C/T*, is located in the amino terminal catalytic domain and can lead to a thermolabile enzyme with 35-50% reduced activity [5], which acts as a protective factor against cancer development. Many studies have indicated that *MTHFR rs1801133 C/T* is involved in the etiology of urinary cancer. However, the results from those studies are conflicting. Considering the important role of *MTHFR* in cancer carcinogenesis, we performed an update analysis on all eligible case-control studies to estimate the urinary cancer risk associated with *MTHFR rs1801133 C/T*.

Methods

Identification of eligible studies and search criterion

A literature search of Pubmed, and the Chinese databases, CNKI and WANFANG (updated on Aug 10, 2015), was conducted using combinations of the following keywords: 'polymorphism', or 'variant' or 'mutation' and 'bladder cancer' or 'prostate cancer' or 'renal' and 'MTHFR' or 'methylenetetrahydrofolate reduc-

Table 1. Study characteristics of all included studies about urinary cancer

First author	Year	Origin	Ethnicity	Design	Case	Control	Source of control	HWE in control	Genotype method
Prostat cancer									
Cai	2010	China	Asian	HB	217	220	BPH	0.572	PCR-RFLP
Mandal	2012	India	Asian	HB	195	250	Healthy	0.104	PCR-RFLP
Wu	2010	Taiwan	Asian	HB	218	436	Healthy	0.763	PCR-RFLP
Fard-Esfahani	2012	Iran	Caucasian	HB	67	75	BPH	0.071	ARMS-PCR
Singal	2004	USA	Caucasian	HB	81	42	BPH	0.280	PCR-RFLP
Muslumanoglu	2009	Turkey	Caucasian	HB	93	157	BPH	0.810	PCR-RFLP
Ghasemi	2014	Iran	Caucasian	HB	30	40	Healthy	0.608	ARMS-PCR
Safarinejad	2010	Iran	Caucasian	HB	174	348	Healthy	0.938	PCR-RFLP
Kimura	2000	Germany	Caucasian	PB	132	150	Healthy	0.169	PCR-RFLP
Johansson	2007	Sweden	Caucasian	PB	2677	1541	Healthy	0.468	TaqMan
Reljic	2007	Croatia	Caucasian	PB	269	102	Healthy	0.137	PCR-RFLP
Heijmans	2003	The Netherlands	Caucasian	PB	21	772	Healthy	0.690	PCR-RFLP
Collin	2009	UK	Caucasian	PB	1599	2084	Healthy	0.259	PCR-RFLP
Cicek	2004	USA	Mixed	PB	439	479	Healthy	0.139	PCR-RFLP
Jackson	2013	Jamaica	Caucasian	PB	202	206	Healthy	NA	real-time PCR
Vidal	2012	USA	Mixed	HB	55	192	Healthy	NA	MALDI-TOF-MS
Kobayashi	2012	Canada	Caucasian	HB	43	170	Healthy	0.042	PCR-RFLP
Küçükhüseyin	2011	Turkey	Caucasian	HB	55	50	Healthy	0.017	PCR-RFLP
Marchal	2008	Spain	Caucasian	HB	182	204	Healthy	0.022	TaqMan
Vogel	2013	Norway	Caucasian	PB	2522	2607	Healthy	0.000	MALDI-TOF-MS
López-Cortés	2013	USA	Caucasian	PB	104	110	Healthy	0.001	PCR-RFLP
Bladder cancer									
Rouissi	2009	Tunisia	African	HB	185	191	-	0.494	PCR-RFLP
Ouerhani	2007	Tunisia	African	HB	111	131	-	0.550	PCR-RFLP
Chung	2010	China-Taiwan	Asian	HB	150	300	-	0.256	PCR-RFLP
Cai	2009	China	Asian	HB	312	325	-	0.076	PCR-RFLP
Safarinejad	2011	Iran	Caucasian	HB	158	316	-	0.555	PCR-RFLP
Kimura	2001	Germany	Caucasian	HB	165	150	-	0.169	PCR-RFLP
Izmirli	2011	Turkey	Caucasian	HB	54	50	-	0.250	PCR-RFLP
Lin	2004	USA	African	PB	21	21	-	0.760	PCR-RFLP
Wang	2009	China	Asian	PB	239	250	-	0.066	PCR-RFLP
Moore	2007	Spain	Caucasian	PB	1041	1049	-	0.481	TaqMan
Lin	2004	USA	Caucasian	PB	410	410	-	0.900	PCR-RFLP
Sanyal	2004	Germany	Caucasian	PB	309	246	-	0.823	PCR-RFLP
Beebe-Dimmer	2012	USA	Caucasian	PB	219	273	-	0.928	Taqman
Karagas	2005	USA	Caucasian	PB	350	543	-	0.702	PCR-RFLP
Lin	2004	USA	Mixed	PB	17	17	-	0.582	PCR-RFLP
Moore	2004	USA	Mixed	PB	106	109	-	0.293	TaqMan
Renal cancer									
Ajaz	2012	Pakistan	Asian	HB	162	177	-	0.767	PCR-RFLP
Safarinejad	2012	Iran	Caucasian	PB	152	304	-	0.910	PCR-RFLP
Moore	2008	France	Caucasian	HB	818	1088	-	0.011	PCR-RFLP

HB: hospital-based; PB: population-based; PCR-RFLP: polymerase chain reaction and restrictive fragment length polymorphism; ARMS-PCR: amplification refractory mutation system-polymerase chain reaction; MALDI-TOF-MS: matrix-assisted laser-desorption/ionization time-off-light mass spectrometry; HWE: Hardy-Weinberg equilibrium.

tase'. There was no language restriction. All studies that evaluated the associations between *rs1801133 C/T* and urinary cancer risk were retrieved. Studies were included in our

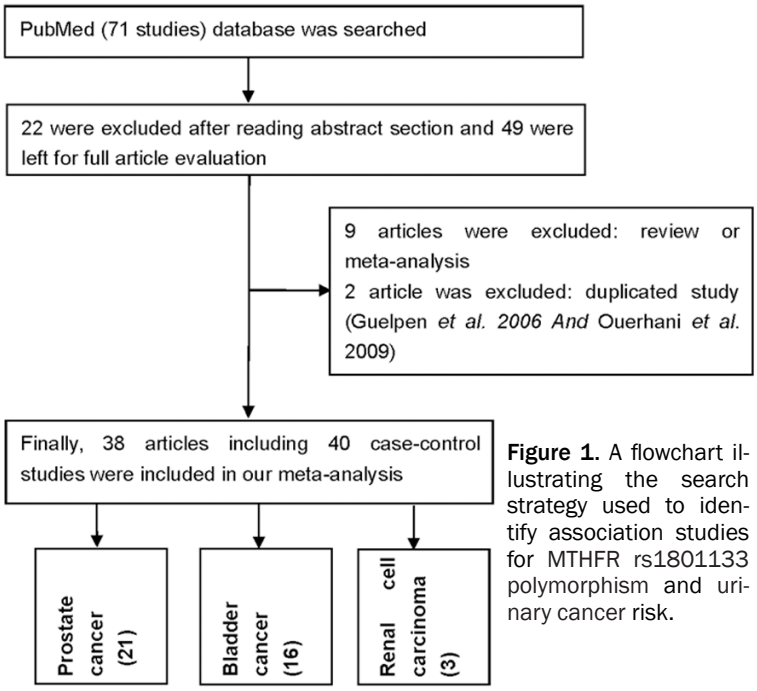

Figure 1. A flowchart illustrating the search strategy used to identify association studies for MTHFR rs1801133 polymorphism and urinary cancer risk.

meta-analysis only if they met the following criteria: (1) evaluation of *MTHFR rs1801133 C/T* and urinary cancer risk; (2) case-control design; (3) available genotype frequency; and (4) inclusion of full-text manuscripts. Meanwhile, the following exclusion criteria were also used: (1) studies with overlapping or repeating data, the most recent or complete study with the largest numbers of cases and controls was included. (2) Studies that have not yet been published.

Data extraction

Information was carefully extracted from all eligible publications by two authors (Tai-Mao Jiang, Qi-Xing Shi), 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, cancer type, sample size (cases/controls), study design (hospital-based, HB, or population-based, PB), source of control for prostate cancer subgroup, Hardy-Weinberg equilibrium (HWE) of controls and genotype method.

Statistical analysis

Crude risk ratios (OR) with 95% confidence intervals (CI) were used to measure the strength of the association between *rs1801133 C/T* and urinary cancer. We analyzed this relationship by using four different genetic models: allelic contrast (T-allele vs. C-allele), heterozygote comparison (TC vs. CC), dominant genetic model (MM+MW vs. WW), recessive genetic model (TT vs. TC+CC), and homozygous comparison (TT vs. CC). Different ethnic descents were categorized as Caucasian, Asian, African, or Mixed (if study population was not a pure race). We divided the control group into four classes based on source: HB, PB, benign prostatic hyperplasia (BPH), and healthy man.

Heterogeneity assumption was evaluated with a chi-square-based Q-test. The statistical significance of the summary OR was determined with the Z-test. When P for the heterogeneity test (P_h) > 0.10, the pooled OR of each study was calculated by using the fixed-effects model; otherwise, the random-effects model was used [6, 7]. The funnel plot asymmetry and publication bias were assessed using both Egger's test and Begg's test, and $P < 0.05$ was considered statistically significant [8, 9]. The departure of frequencies of *MTHFR rs1801133 C/T* from expected values under HWE was assessed in controls by using the Pearson chi-square test, and $P < 0.05$ was considered significant. All statistical tests for this meta-analysis were performed using Stata (version 11.0; StataCorp LP, College Station, TX).

Results

Study characteristics

After reviewing the title, abstract, and full text, we excluded meta-analyses, reviews, case-only studies, and other gene polymorphisms. Then, 40 different papers were included for the final analysis. For prostate cancer, data in Guelpen *et al.* [10] duplicated some of the information in Johansson *et al.* [11], so we included the larger study from Johansson *et al*. Moreover, for bladder cancer, Ouerhani *et al*. [12, 13] published two papers in 2007 and 2009 that contained duplicated data, so we included the larger num-

Table 2. Total and stratified analysis of MTHFR rs1801133 C/T polymorphism and each urinary cancer

Variables	N	Case/Control	T-allele vs. C-allele		TC vs. CC		TT vs. TC+CC		TT vs. CC	
			OR (95% CI)	P_h	OR (97% CI)	P_h	OR (99% CI)	P_h	OR (95% CI)	P_h
Total	40	14354/16185	0.99 (0.93-1.07)	0.000	1.03 (0.93-1.13)	0.000	0.95 (0.84-1.08)	0.001	0.96 (0.83-1.12)	0.000
HWE	34	10630/11956	0.99 (0.92-1.07)	0.000	1.01 (0.92-1.10)	0.011	0.98 (0.84-1.13)	0.002	0.98 (0.83-1.16)	0.000
Prostate cancer										
Total	21	9375/10235	0.93 (0.84-1.03)	0.000	0.94 (0.82-1.09)	0.000	0.87 (0.70-1.09)	0.000	0.85 (0.67-1.07)	0.000
HWE	16	6469/7094	0.93 (0.82-1.05)	0.000	0.92 (0.81-1.05)	0.067	0.94 (0.71-1.24)	0.001	0.89 (0.66-1.20)	0.000
Ethnicity										
Caucasian	16	8251/8658	0.97 (0.87-1.09)	0.001	0.97 (0.83-1.14)	0.000	0.96 (0.74-1.24)	0.001	0.95 (0.74-1.23)	0.002
Asian	3	630/906	0.81 (0.57-1.14)	0.029	0.84 (0.54-1.29)	0.054	0.60 (0.42-0.87)	0.546	0.53 (0.35-0.80)	0.387
Mixed	2	494/671	NA		NA	NA	NA	NA	NA	
Source of control										
HB	12	1410/2184	0.80 (0.71-0.89)	0.244	0.85 (0.65-1.11)	0.004	0.57 (0.44-0.75)	0.530	0.54 (0.40-0.72)	0.742
PB	9	7965/8051	1.03 (0.91-1.17)	0.000	1.00 (0.85-1.18)	0.001	1.08 (0.85-1.36)	0.004	1.06 (0.82-1.38)	0.002
BPH	4	458/494	0.78 (0.64-0.95)	0.677	0.80 (0.60-1.07)	0.661	0.63 (0.43-0.94)	0.208	0.56 (0.36-0.89)	0.262
Healthy man	17	8917/9741	0.96 (0.85-1.07)	0.000	0.97 (0.83-1.14)	0.000	0.91 (0.72-1.15)	0.001	0.89 (0.70-1.14)	0.001
Bladder cancer										
Total	16	3847/4381	1.02 (0.92-1.13)	0.010	1.05 (0.93-1.20)	0.087	0.99 (0.87-1.13)	0.147	1.01 (0.81-1.25)	0.035
Ethnicity										
Caucasian	8	2706/3037	0.99 (0.92-1.07)	0.130	1.04 (0.93-1.16)	0.310	0.92 (0.79-1.09)	0.777	0.94 (0.79-1.12)	0.584
Asian	3	701/875	1.15 (0.84-1.58)	0.011	1.16 (0.93-1.45)	0.146	1.32 (0.80-2.15)	0.070	1.42 (0.72-2.78)	0.016
Mixed	2	123/126	0.75 (0.51-1.09)	0.286	0.47 (0.26-0.84)	0.453	NA	NA	NA	
African	3	317/343	0.91 (0.72-1.14)	0.627	1.09 (0.79-1.51)	0.310	0.60 (0.35-1.02)	0.822	0.64 (0.36-1.12)	0.798
Source of control										
HB	7	1135/1463	1.07 (0.88-1.31)	0.014	1.11 (0.94-1.31)	0.294	1.00 (0.69-1.47)	0.083	1.06 (0.67-1.67)	0.028
PB	9	2712/2918	0.99 (0.91-1.07)	0.103	1.01 (0.85-1.21)	0.063	0.95 (0.81-1.11)	0.362	0.96 (0.81-1.13)	0.209
Renal cell carcinoma										
Total	3	1132/1569	1.24 (1.10-1.40)	0.317	1.40 (1.19-1.65)	0.368	1.15 (0.89-1.48)	0.550	1.36 (1.04-1.78)	0.297
HWE	2	314/481	1.30 (1.03-1.32)	0.149	1.44 (1.05-1.97)	0.161	1.28 (0.77-2.14)	0.333	1.64 (0.94-2.85)	0.178

P_h: value of *Q*-test for heterogeneity test; NA: not available.

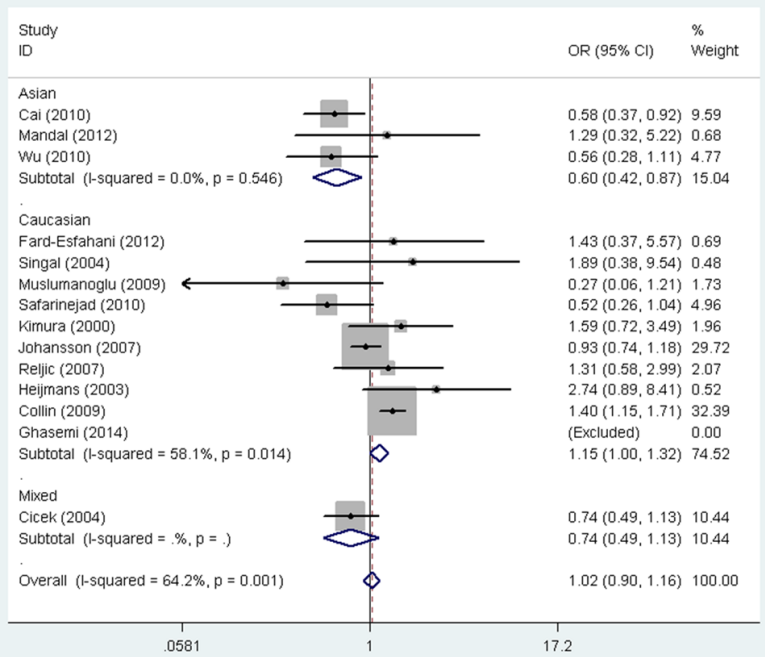

Figure 2. Forest plot of prostate cancer risk associated with the MTHFR rs1801133 polymorphism (TT vs. TC+CC) by ethnicity subgroup. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

bers from Ouerhani *et al.* [12] in our analysis. Finally, we identified 38 different papers describing 40 case-control studies (21 case-control studies for prostate cancer, 16 for bladder cancer, and three for renal cell carcinoma, **Table 1**; **Figure 1**) [11, 12, 14-49] to evaluate the association of *MTHFR rs1801133 C/T*. Study characteristics are shown in **Table 1**. The distribution of genotypes in the controls was consistent with HWE in all studies, except for six papers. None of the control populations had a history of malignant diseases. Genotyping was conducted using polymerase chain reaction and restrictive fragment length polymorphism (PCR-RFLP), amplification refractory mutation system-polymerase chain reaction (ARMS-PCR), and matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

Quantitative synthesis

Total urinary cancer: In the total analysis, no relationship was found in any model between *MTHFR rs1801133 C/T* and urinary cancer risk. At the same time, if we excluded six papers that were not consistent with HWE, no association was detected (**Table 2**).

Prostate cancer: Overall, there were no significant relationships between *MTHFR rs1801133 C/T* and prostate cancer risk in any of the available genotype models. Moreover, to avoid publishing bias, five papers that were not consistent with HWE were excluded, so 16 case-control studies were left for analysis, and, to our regret, no association was detected. However, based on ethnicity-stratified analysis, *rs1801133 C/T* was strongly associated with decreased prostate cancer risk under the recessive genetic model (OR = 0.60; 95% CI = 0.42-0.87; Pheterogeneity = 0.546, **Figure 2**) and homozygous comparison (OR = 0.53; 95% CI = 0.35-0.80; Pheterogeneity = 0.387) in Asian populations, but not in Caucasian or Mixed populations. In addition, in the source of control subgroup, the *MTHFR rs1801133* T allele was a protective factor for prostate cancer, if the controls were from HB (T-allele vs. C-allele: OR = 0.80, 95% CI = 0.71-0.89, Pheterogeneity = 0.244; TT vs. TC+CC: OR = 0.57, 95% CI = 0.44-0.75, Pheterogeneity = 0.530; TT vs. CC: OR = 0.54, 95% CI = 0.40-0.72, Pheterogeneity = 0.742) or were BPH patients (T-allele vs. C-allele: OR = 0.78, 95% CI = 0.64-0.95, Pheterogeneity = 0.677, **Figure 3**; TT vs. TC+CC: OR = 0.63, 95% CI = 0.43-0.94, Pheterogeneity = 0.208; TT vs. CC: OR = 0.56, 95% CI = 0.36-0.89, Pheterogeneity = 0.262) (**Table 2**).

Bladder cancer: Detailed results of the meta-analysis are shown in **Table 2**. No statistically significant association was detected between *MTHFR rs1801133 C/T* and bladder cancer risk in the total group or in the subgroup of source of control. Interestingly, in the ethnicity subgroup analysis, there was a decreased risk of bladder cancer in the Mixed population (heterozygote comparison: OR = 0.47, 95% CI = 0.26-0.84, Pheterogeneity = 0.453, **Figure 4**), but not in Caucasians, Asians, or Africans.

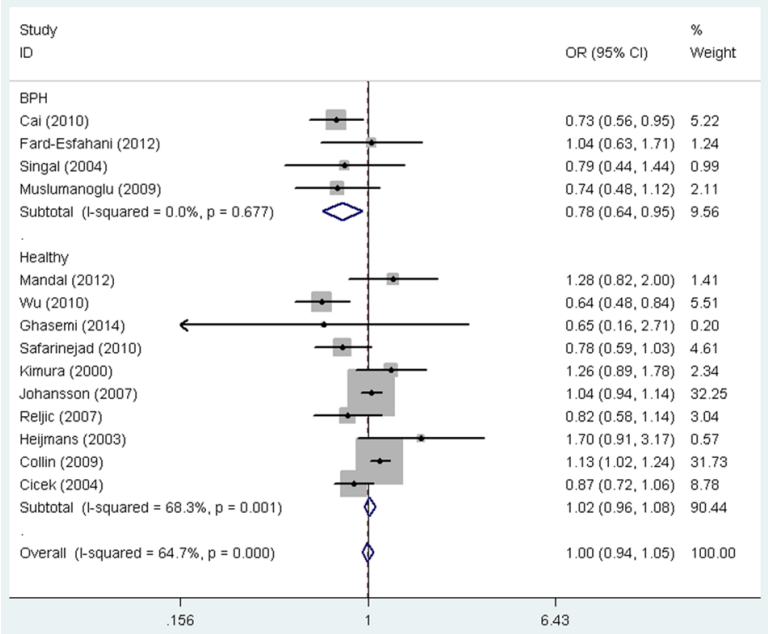

Figure 3. Forest plot of prostate cancer risk associated with the MTHFR rs1801133 polymorphism (T-allele vs. C-allele) by source of control subgroup. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

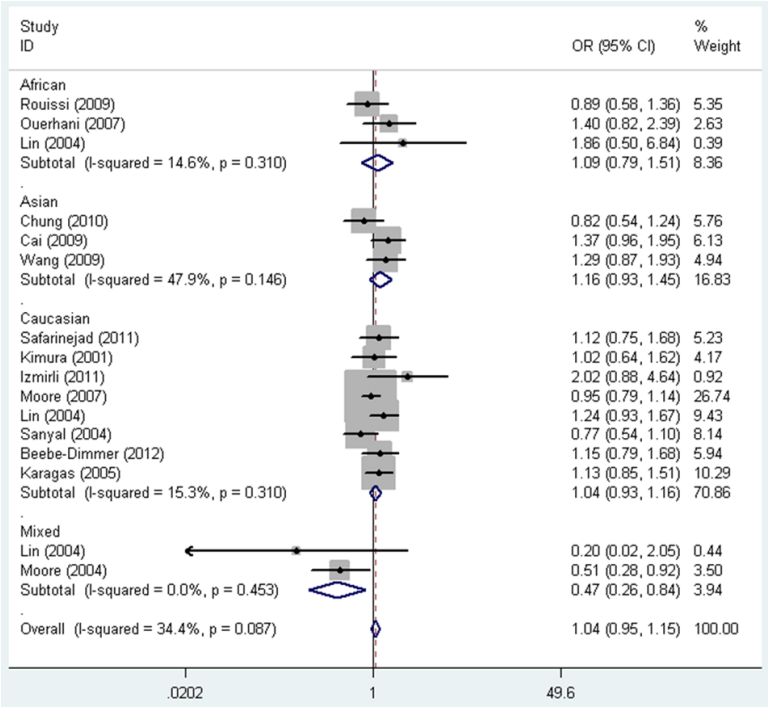

Figure 4. Forest plot of bladder cancer risk associated with the MTHFR rs1801133 polymorphism (TC vs. CC) by ethnicity subgroup. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

Renal cell carcinoma: In the total analysis, an increased relationship was found between *MTHFR rs1801133 C/T* and renal cell carcinoma risk in the total group (T-allele vs. C-allele: OR = 1.24, 95% CI = 1.10-1.40, Pheterogeneity = 0.317; TC vs. CC: OR = 1.40, 95% CI = 1.19-1.65, Pheterogeneity = 0.368; TT vs. CC: OR = 1.36, 95% CI = 1.04-1.78, Pheterogeneity = 0.297, **Figure 5**) and in all HWE studies (T-allele vs. C-allele: OR = 1.30, 95% CI = 1.03-1.32, Pheterogeneity = 0.149; TC vs. CC: OR = 1.44, 95% CI = 1.05-1.97, Pheterogeneity = 0.161) (**Table 2**).

Sensitivity analysis and publication bias diagnosis: We deleted each study involved in our meta-analysis to reflect the influence of the individual data-set on the pooled OR; the corresponding pooled OR was not significantly altered, indicating that our results were statistically robust. Begg's funnel plot and Egger's test were performed to access the publication bias of the literature. The shape of the funnel plot did not reveal obvious asymmetry and the Egger's test suggested the absence of publication bias [for example (TC vs. CC) (z = 0.62, *P* = 0.537 for Begg's test; t = -0.04, *P* = 0.970 for Egger's test, **Figures 6**, **7**)].

Discussion

Reduced MTHFR activity may decrease the methylation of homocysteine to methionine and, in turn, the level of SAM, resulting in DNA hypomethylation. In addition, reduced MTHFR can lead to uracil misincorporation into DNA, diminished DNA repair, and

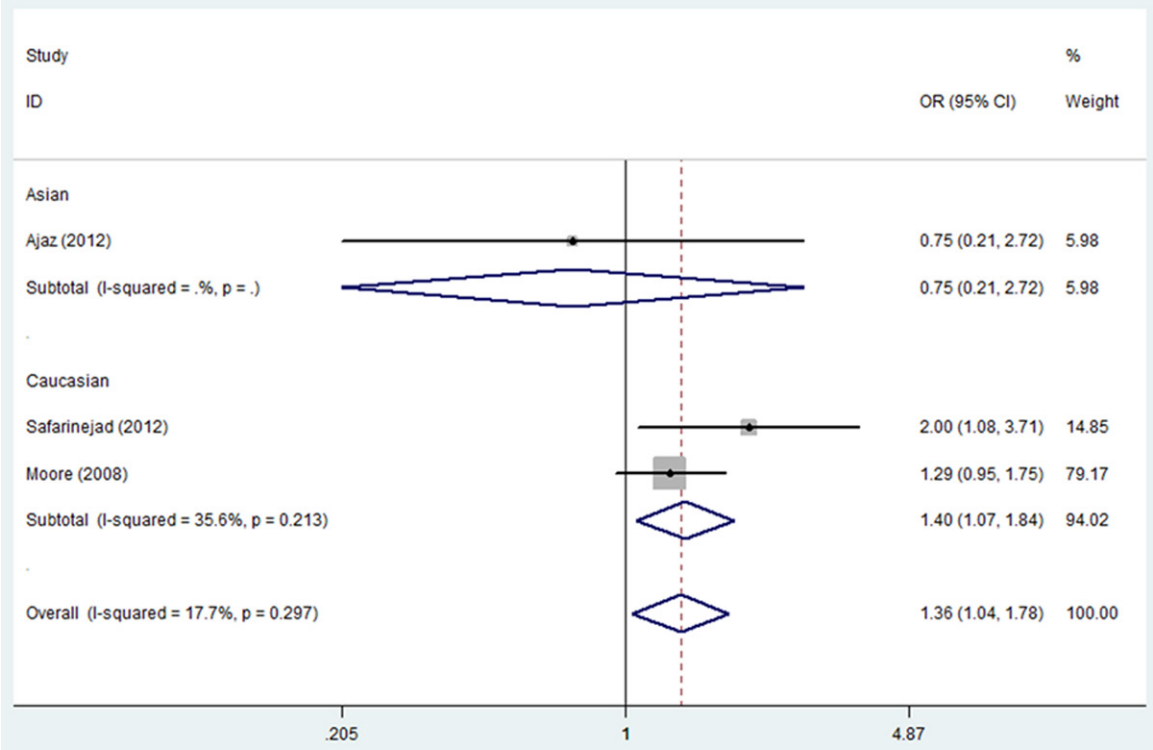

Figure 5. Forest plot of renal cell carcinoma risk associated with the MTHFR rs1801133 polymorphism (TT vs. CC) in total. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

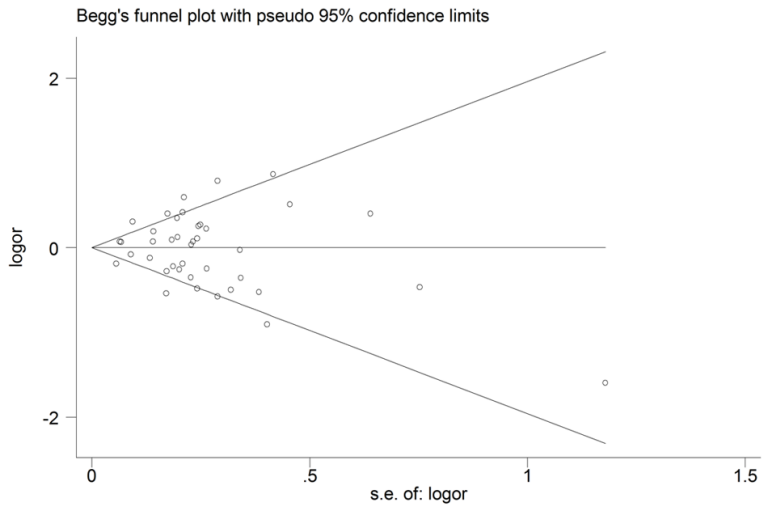

Figure 6. Begg's funnel plot for publication bias test (TC vs. CC). Each point represents a separate study for the indicated association. Log [OR], natural logarithm of OR. Horizontal line, mean effect size.

increased frequency of chromosomal breaks and damage. The reduced activity of MTHFR may increase the amount of damage that occurs in malignant tumor cells, since they have a higher requirement for DNA synthesis that should make them more susceptible to folate deficiency and the resultant DNA damage. Hence, the reduced MTHFR activity may protect the cells against carcinogenesis. In recent years, interest in the genetic susceptibility to cancers has led to increased focus on polymorphisms of genes involved in tumorigenesis. The mutant T-allele of the *MTHFR rs1801133* polymorphism has been reported to reduce the MTHFR enzymatic activity of the wild type C-allele [5], which could decrease cancer risk. For example, Liu et al. [50] reported that reduced levels of MTHFR mRNA had a decreased association with the risk for esophageal cancer. This result may be explained by the hypothesis that reduced mRNA levels lead to a

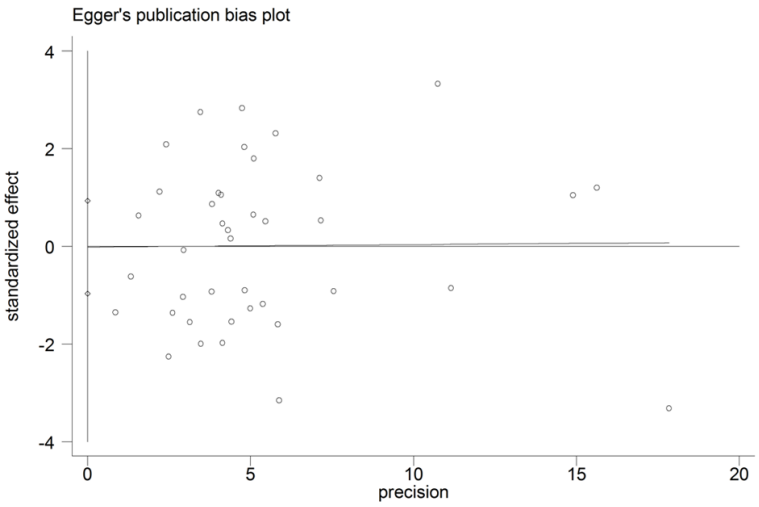

Figure 7. Egger's publication bias plot (TC vs. CC). Each point represents a separate study for the indicated association. Horizontal line, mean effect size.

decreased supply of de novo methionine methyl groups, which subsequently leads to hypomethylation of genomic DNA.

To the best of our knowledge, this is the first meta-analysis to explore the association between *MTHFR rs1801133* and urinary cancer risk, involving approximately 14,354 cancer cases and 16,185 controls. We found that this polymorphism may have a decreased association with not only prostate cancer risk in Asians, but also with bladder cancer risk in Mixed populations. The polymorphism may act as a protective factor in both types of urinary cancer, possibly through the mechanism described above. However, *MTHFR rs1801133* may become a risk factor, and could have an increased association with, renal cell carcinoma, which is in contrast to the functional polymorphism.

A number of factors may have influenced our results. First, differences in the distribution of various ethnicities between cases and controls may be a source of variability when pooling studies. Second, cancer is a multifactorial disease that results from complex interactions between many genetic and environmental factors; this means that there will not be a single gene or single environmental factor that has a large effect on cancer susceptibility [51]. Environmental factors, such as tobacco smoke, dietary factors, infectious agents, add to the carcinogenic load to which humans are exposed.

In interpreting the current results, some limitations should be considered. First, sample sizes varied widely in the different studies (range of the number of cases/controls 17/17 to 2677/2607), which may increase the publication bias. There were only three case-control studies regarding renal cell carcinoma, additional studies should consider this association. Second, few studies used Mixed, Asian, or African populations. New, additional studies should focus on these races. Third, additional studies are needed to address the effects of race and sample size on the predicted associations, and more attention should be placed on gene-gene and gene-environment interactions.

In conclusion, the present update analysis found novel evidence that the *MTHFR rs1801133* polymorphism has different effects on the risk of different urinary cancers. Further studies, with larger numbers, are expected to examine associations between this polymorphism in MTHFR and urinary cancer risk.

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Disclosure of conflict of interest

None.

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