REVIEW

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Associations of MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) Polymorphisms with Susceptibility to Bladder Cancer: A Systematic Review and Meta-Analysis

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Abstract

The effects of the MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms on bladder cancer risk have been evaluated in some studies. However, the results were conflicting and ambiguous. Therefore, we aimed to perform a comprehensive meta-analysis to investigate the association of these polymorphisms with risk of bladder cancer from all eligible case-control studies. PubMed, Web of science, Scopus, SID, CNKI and SciELO databases were searched to identify all relevant studies published up to 1 January, 2021. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to estimate the strength of associations. A total of 20 case-control studies including 11 studies with 3463 cases and 3927 controls on MTHFR rs1801133 (677C>T) and 9 studies with 3177 cases and 3502 controls on rs180113 (1298A>C) polymorphism were selected. Pooled data revealed that the MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms were not associated with risk bladder cancer in overall. Stratified analysis by ethnicity revealed that the MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms were associated with bladder cancer risk in Asians, but not in Caucasians. There was no publication bias. The current meta-analysis revealed that the MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms were not risk factor for development of bladder cancer globally. However, large sample size, well-designed, and population-based studies should be performed to verify the association of the MTHFR polymorphisms with bladder cancer risk.

Keywords: Bladder cancer- urinary neoplasms- folate-MTHFR- polymorphism- meta-analysis

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Introduction

Bladder cancer is the second most common genitourinary malignancy, after prostate cancer in human (Oeyen et al., 2019; Lenis et al., 2020). It is the sixth most prevalent malignancy in the United States and causes more than 16,000 deaths annually (Degeorge et al., 2017), which represents 4.4% of all new cancer diagnoses in the USA (Wong et al., 2018). More than 60% of all bladder cancer cases and half of all the 165,000 bladder cancer deaths occur in the less developed regions of the world (Antoni et al., 2017). Bladder cancer is more common in men than women, with a respective incidence of 9.6 among men and 2.4 among women per 100,000 person-year globally, respectively (Bouffioux, 1984). Bladder cancer is one of the most expensive cancer to care for from diagnosis to death due to the frequent procedures required for this malignancy monitoring and treatment (Andreassen et al., 2016).

The leading risk factor for development of bladder cancer is tobacco use, which actually accounts for more 50 percent of the cases and increases the chance of development of the disease by three times compared to not smoking individuals (Freedman et al., 2011; Mobley and Baum, 2015). Moreover, studies have found that occupational exposures (such as paint, textiles, rubber, leather, and dyes) and pollutants in drinking water (such as arsenic and chlorinated byproducts) constitute the

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second most important risk factor for development of bladder cancer (Gu and Wu, 2011; Aminian et al., 2014). Therefore, bladder cancer is an excellent model for studying genetic susceptibility and gene-environment interaction in cancer etiology (Gu and Wu, 2011). Since the major environmental risk factors for development of bladder cancer have been identified (Letaiová et al., 2012; Al-Zalabani et al., 2016) some efforts were made in the last few years to identify genetic variations in the pathways involved in the carcinogenesis processes, including metabolism of carcinogens, DNA repair, cell cycle checkpoints, apoptosis and inflammatory response (Grotenhuis et al., 2010; Gu and Wu, 2011). It is well-known that folate metabolism may be has an important role in development of several tumours through its involvement in both DNA methylation and nucleotide synthesis (Li et al., 2013). It has been shown that cigarette smoke exposure is associated with decreased serum levels of folate and vitamin B12 antioxidants (Tungtrongchitr et al., 2003; Wu et al., 2007). Folate and other B vitamins play important roles in the one-carbon metabolism pathway, which is associated with DNA methylation, synthesis and impaired DNA repair (Pan et al., 2019). The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) is involved in the circulation form of folate as it catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Maruti et al., 2009; Soleimani-Jadidi et al., 2020; Tabatabaei et al., 2020).

The candidate gene approaches revealed that functional polymorphisms at the MTHFR gene may be play an important role in development of bladder cancer (Mannino et al., 2003; Tungtrongchitr et al., 2003; Baghestani et al., 2018; Ahmadi et al., 2021). The human MTHFR gene is located on chromosome 1 at 1p36.3, composed of 11 exons and consists of 17 kb (Rosenberg et al., 2002). It was found that the rs1801133 (677C>T) in exon 4 and rs180113 (1298A>C) in exon 7 of the MTHFR gene resulted in amino acid substitution and a reduction of MTHFR activity (Liu et al., 2020). To date, several epidemiological studies have evaluated the association of MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms with susceptibility to bladder cancer (You et al., 2013; Xu and Zuo, 2020). However, these associations were still inconclusive. Although two meta-analyses have reported the association of MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms and bladder cancer risk (Wang et al., 2009; Xu et al., 2013; Akbari et al., 2015), they did not perform subgroup analysis by country of origin and source of controls. Thus, to comprehensively estimate the association of MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms with susceptibility to bladder cancer, we carried out this systematic review and meta-analysis.

Materials and Methods

Search Strategy

We carried out a comprehensive online literature search on electronic databases including PubMed, Scopus, EMBASE, Web of Knowledge, Cochrane Library, Google Scholar, Scientific Information Database (SID), WanFang, VIP, Chinese Biomedical Database (CBD), Scientific Electronic Library Online (SciELO) and China National Knowledge Infrastructure (CNKI) database to identify all relevant studies on the association of MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms with susceptibility to bladder cancer up to 1 January, 2021. We used the combination of following keywords and terms: (''Bladder Cancer'' OR ''Urinary Cancer'' OR ''Urinary Bladder Neoplasm'') AND ("Methylenetetrahydrofolate Reductase" OR ''MTFR'' OR "Methionine Synthase Reductase" OR "Folate Pathway'') and (''MTHFR 677C>T'' OR ''C677T'' OR ''rs1801133'' OR ''p. Ala222Val'' OR ''A222V'' OR ''g.11796321G>A'') AND ("MTHFR 1298A>C" OR "MTHFR Glu222Val" OR "rs1801131'') AND (''Gene'' OR ''Genotype'' OR ''Allele'' OR ''Polymorphism'' OR '' Single nucleotide polymorphisms'' OR ''SNP'' OR ''Variation'' OR ''Mutation''). Languages were limited to English, Portuguese, Farsi and Chinese. Moreover, the reference lists of retrieved studies including case-control studies, previous meta-analyses and reviews were manually searched to find other relevant publications.

Selection Criteria

Studies meeting the following criteria were included: 1) studies with case-control or cohort design; 2) studies evaluated the association of the MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms with bladder cancer risk; 3) genotype distributions in cases and healthy controls were available for calculating an odds ratio (OR) with 95% confidence interval (CI). The following were exclusion criteria: 1) animal studies or in vitro studies; 2) studies evaluated other polymorphisms at MTHFR gene; 3) case only studies; 4) linkage studies and family based studies (sibling, twins and trios-parents studies); 5) studies did not report genotype frequencies; 6) abstracts, posters, case reports, reviews, meta-analyses, commentaries, editorials, conference articles, and proceedings; 7) duplicates of previous published studies or studies with overlapping data. If more than one study was published by the same author(s) using repeated or overlapped data, the most complete one or more recently published study was selected.

Data Extraction

Two authors carefully reviewed and extracted data from all eligible studies according to the inclusion criteria. If any disagreement appeared, a third author was consulted to resolve the dispute and the final consensus was made by the majority of the votes. The following data were extracted from each study: the name of first author, year of publication, country of origin, ethnicity (Caucasian, Asian, African, Mixed populations), source of controls (hospital based or population based), genotyping methods, sample size, alleles and genotypes frequencies for MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms in cases and controls, Minor Allele Frequency (MAFs) and Hardy-Weinberg equilibrium (HWE) in healthy controls. The ''mixed'' group means mixed or unknown populations.

Quality score assessment

The Newcastle-Ottawa Score (NOS) were performed to assess the quality of included studies in the meta-analysis and to assess the various aspects of the methodology used by the observational research, which are relevant to the quality of the study. This standard assessed 3 sections (selection of cases, comparability of groups, and determination of exposure) and 8 items. In the selection and exposure categories, a quality research item received 1 star, and a comparable category could receive at most 2 stars. The quality assessment values ranged from 0 stars (worst) to 9 stars (best), and studies with a score ≥7 were defined as high quality. Generally, the study which scored at least 5 points was considered to be included in meta-analysis and any discrepant opinions were resolved by discussion and consensus.

Statistical Analysis

Crude odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated to evaluate the strength of MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms with susceptibility to bladder cancer. The statistical significance of pooled ORs was assessed by the Z test, in which p-value less than 0.05 was considered as statistically significant. The associations was estimated under all five genetic models, i.e., allele (B vs. A), homozygote (BB vs. AA), heterozygote (BA vs. AA), dominant (BB+BA vs. AA), and the recessive (BB vs. BA+AA). A test of between-studies heterogeneity was conducted using Cochran's Q test, in which $P \leq 0.01$ indicated a significant heterogeneity. In addition, I^2 statistic was used to quantify the proportion of the between-study heterogeneity (range of 0 to 100%: $I^2 \leq 50\%$, no heterogeneity; $I^2 \geq 50\%$, presence of heterogeneity). Thus, when the heterogeneity was absent the fixed-effect model (Mantel-Haenszel method) was used to calculate the overall or pooled OR; otherwise, the random-effects model (DerSimonian and Laird method) was applied. To explore sources of between-study heterogeneity, we have performed subgroup analysis by ethnicity, country, source of controls and HWE status. We used the Chi-squared test to evaluate Hardy-Weinberg equilibrium (HWE) in controls, and we considered $p < 0.05$ as a significant deviation from HWE (HWE-violating) (Bahrami et al., 2020). Sensitivity analyses were performed to assess the stability of the results by sequential removing of each study. The Begg's visual inspection of funnel plot and the Egger's regression tests were used to evaluate publication bias in the literature, in which $P<0.05$ was considered statistically significant. All of the statistical calculations were performed using Comprehensive Meta-Analysis (CMA) software version 2.0 (Biostat, USA). Two-sided P-values < 0.05 were considered statistically significant.

Results

Characteristics of Selected Studies

As shown in Figure 1, our initial search yielded 315

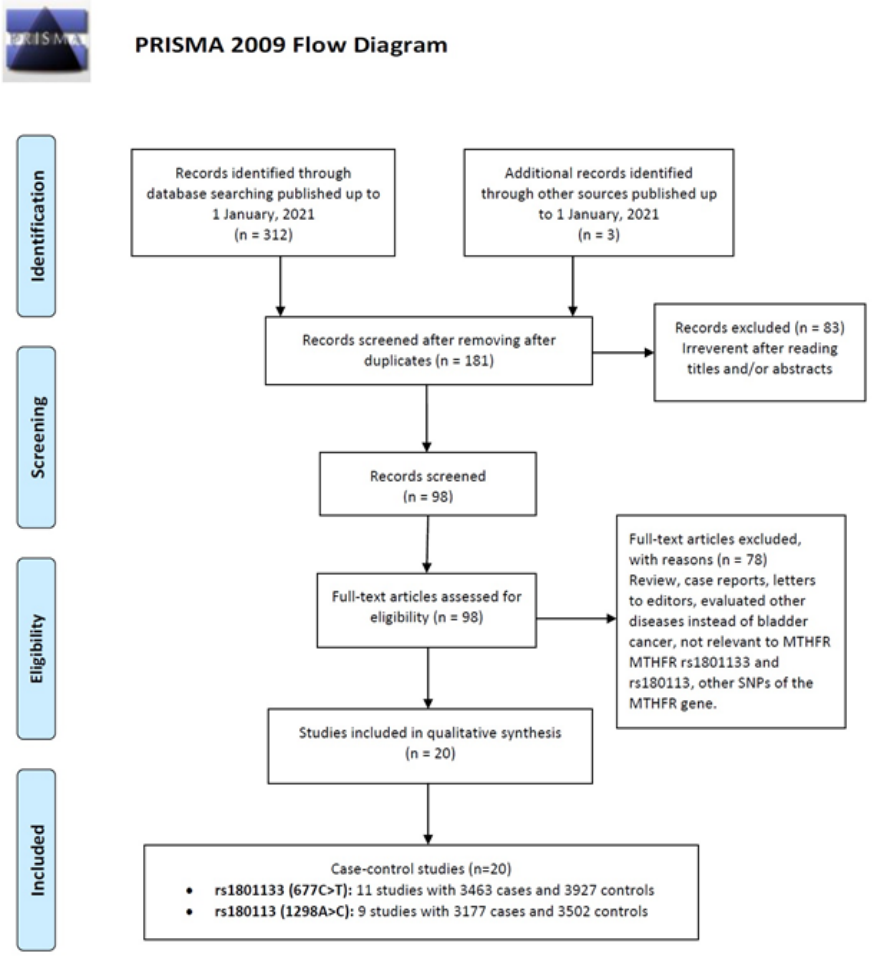


Figure 1. Flow Chart for the Process of Selecting Eligible Studies.

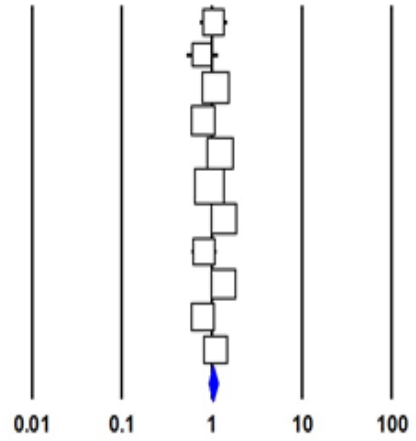
Table 1. Main Characteristics of Studies Included in the Meta-Analysis

First Author/Year	Country (Ethnicity)	Genotyping Method	SOC	Case/Control	Cases					Controls					MAFs	HWE	NOS
					Genotypes			Allele		Genotypes			Allele				
rs1801133					CC	CT	TT	C	T	CC	CT	TT	C	T			
Kimura 2001	Germany(Caucasian)	PCR-RFLP	HB	165/150	70	80	15	220	110	65	73	12	203	97	0.323	0.169	7
Moore 2004	Argentina(Mixed)	PCR-RFLP	PB	106/109	45	42	19	132	80	32	59	18	123	95	0.436	0.292	7
Lin 2004	USA(Caucasian)	PCR-RFLP	HB	448/448	199	197	52	595	301	218	177	53	613	283	0.316	0.069	9
Sanyal 2004	Sweden(Caucasian)	PCR-RFLP	HB	309/246	173	113	23	459	159	121	102	23	344	148	0.301	0.822	7
Karagas 2005	USA(Caucasian)	PCR-RFLP	PB	350/543	140	171	39	451	249	227	245	71	699	387	0.356	0.701	9
Moore 2007	Spain(Caucasian)	TaqMan	HB	1041/1049	418	478	145	1314	768	402	486	161	1290	808	0.385	0.48	7
Cai 2009	China(Asian)	PCR-RFLP	HB	312/325	82	169	61	333	291	113	170	42	396	254	0.391	0.075	7
Rouissi 2009	Tunisia(African)	PCR-RFLP	PB	185/191	87	86	12	260	110	81	90	20	252	130	0.34	0.494	7
Wang 2009	China(Asian)	PCR-RFLP	HB	239/250	66	128	45	260	218	88	132	30	308	192	0.384	0.066	9
Chung 2010	China(Asian)	PCR-RFLP	HB	150/300	80	57	13	217	83	141	123	36	405	195	0.325	0.256	7
Safarinejad 2011	Iran(Asian)	PCR-RFLP	HB	158/316	67	74	17	208	108	144	142	30	430	202	0.32	0.555	7
Total				3463/3927	1427	1595	625	5044	2477	1632	1799	562	5063	2791	0.366	0.064	8
rs180113					AA	AC	CC	A	C	AA	AC	CC	A	C			
Moore 2004	Argentina(Mixed)	PCR-RFLP	PB	106/108	52	45	9	149	63	55	45	8	155	61	0.282	0.77	7
Lin 2004	USA(Caucasian)	PCR-RFLP	HB	448/447	219	199	30	637	259	213	197	37	623	271	0.303	0.361	9
Sanyal 2004	Sweden(Caucasian)	PCR-RFLP	HB	311/245	145	133	33	423	199	110	111	24	331	159	0.324	0.6	7
Karagas 2005	USA(Caucasian)	PCR-RFLP	PB	350/542	173	146	31	492	208	267	220	55	754	330	0.304	0.333	9
Moore 2007	Spain(Caucasian)	TaqMan	HB	1068/1078	537	457	74	1531	605	557	429	92	1543	613	0.284	0.467	7
Cai 2009	China(Asian)	PCR-RFLP	HB	312/325	215	91	6	521	103	226	92	7	544	106	0.163	0.504	7
Rouissi 2009	Tunisia(African)	PCR-RFLP	PB	185/191	97	78	10	272	98	121	60	10	302	80	0.309	0.478	7
Wang 2009	China(Asian)	PCR-RFLP	HB	239/250	169	67	3	405	73	171	75	4	417	83	0.166	0.186	9
Safarinejad 2011	Iran(Asian)	PCR-RFLP	HB	158/316	48	85	25	181	135	178	115	23	471	161	0.255	0.46	8
Total				3177/3502	1655	1301	221	4611	1743	1898	1344	260	5140	1864	0.266	0.3	

PCR-RFLP, Polymerase Chain Reaction Restriction Fragment Length Polymorphism; SOC, source of controls; HB, Hospital Based; PB, Population Based; MAFs, Minor Allele Frequencies; HWE, Hardy-Weinberg Equilibrium; NOS, Newcastle-Ottawa Score.

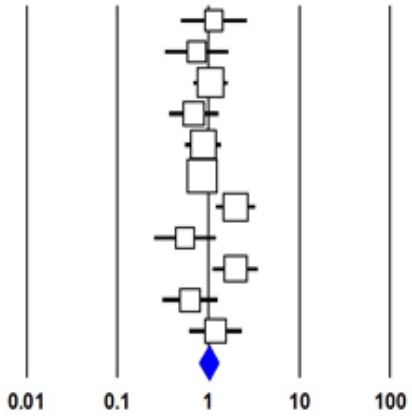
A

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value	Relative weight
Kimura 2001	1.046	0.750	1.460	0.267	0.790	7.08
Moore 2004	0.785	0.534	1.154	1.232-	0.218	5.97
Lin 2004	1.096	0.899	1.335	0.907	0.364	10.95
Sanyal 2004	0.805	0.618	1.048	1.609-	0.108	8.89
Karagas 2005	1.246	1.016	1.527	2.115	0.034	10.75
Moore 2007	0.933	0.823	1.058	1.084-	0.278	13.32
Cai 2009	1.362	1.091	1.702	2.723	0.006	10.14
Rouissi 2009	0.820	0.603	1.115	1.264-	0.206	7.71
Wang 2009	1.345	1.043	1.735	2.281	0.023	9.16
Chung 2010	0.794	0.585	1.078	1.478-	0.139	7.76
Safarinejad 2011	1.105	0.830	1.472	0.685	0.493	8.26
	1.028	0.912	1.159	0.450	0.653	



B

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value	Relative weight
Kimura 2001	1.161	0.506	2.664	0.352	0.725	5.77
Moore 2004	0.751	0.341	1.651	0.713-	0.476	6.20
Lin 2004	1.075	0.700	1.649	0.330	0.741	11.75
Sanyal 2004	0.699	0.375	1.304	1.125-	0.261	8.28
Karagas 2005	0.891	0.571	1.388	0.511-	0.609	11.43
Moore 2007	0.866	0.666	1.127	1.071-	0.284	15.33
Cai 2009	2.001	1.232	3.251	2.804	0.005	10.62
Rouissi 2009	0.559	0.257	1.215	1.469-	0.142	6.31
Wang 2009	2.000	1.141	3.507	2.420	0.016	9.25
Chung 2010	0.636	0.319	1.270	1.282-	0.200	7.33
Safarinejad 2011	1.218	0.628	2.361	0.584	0.559	7.72
	1.018	0.801	1.293	0.144	0.886	



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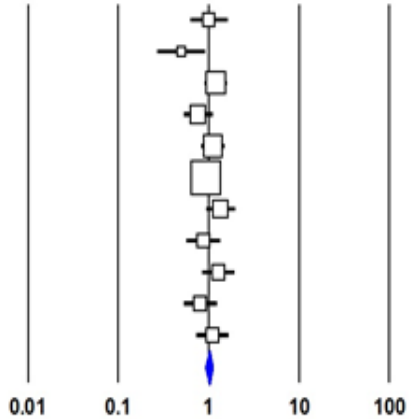
Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value	Relative weight
Kimura 2001	1.018	0.640	1.617	0.074	0.941	4.62
Moore 2004	0.506	0.277	0.924	2.218-	0.027	2.74
Lin 2004	1.219	0.922	1.612	1.390	0.164	12.70
Sanyal 2004	0.775	0.544	1.104	1.411-	0.158	7.90
Karagas 2005	1.132	0.849	1.508	0.844	0.399	12.03
Moore 2007	0.946	0.785	1.140	0.585-	0.558	28.62
Cai 2009	1.370	0.960	1.954	1.737	0.082	7.87
Rouissi 2009	0.890	0.583	1.358	0.542-	0.588	5.55
Wang 2009	1.293	0.866	1.931	1.255	0.209	6.17
Chung 2010	0.817	0.538	1.239	0.951-	0.341	5.71
Safarinejad 2011	1.120	0.748	1.677	0.550	0.582	6.09
	1.016	0.919	1.122	0.307	0.759	



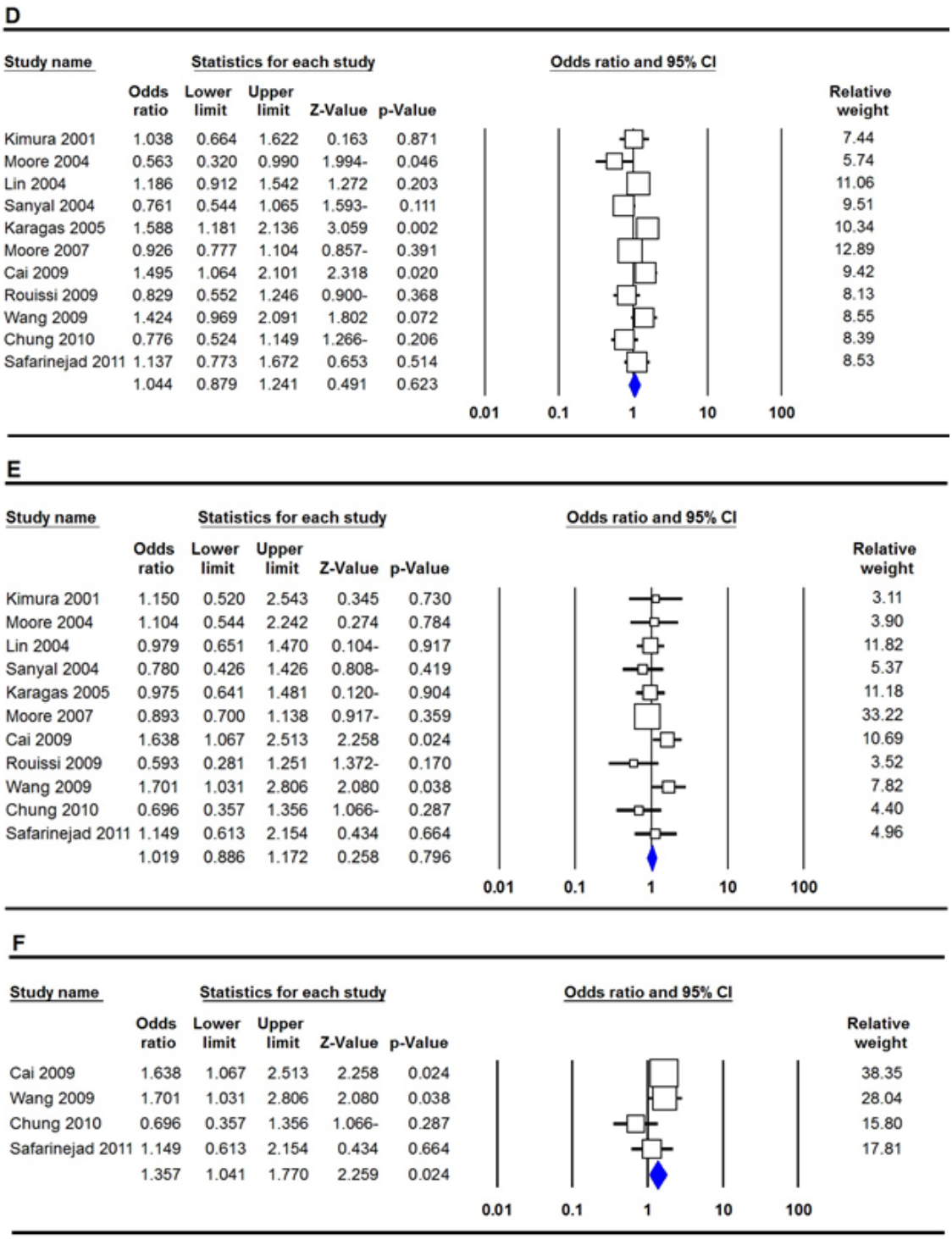


Figure 2. Forest Plot for Association between MTHFR rs1801133 (677C>T) polymorphism and Bladder Cancer Risk. A: allele model (T vs. C); B: homozygote model (TT vs. CC); C: heterozygote model (TC vs. CC); D: dominant model (TT+TC vs. CC); E: recessive model (TT vs. TC+CC); and F: Asians (recessive model: TT vs. TC+CC).

studies on MTHFR polymorphisms and bladder cancer, with duplicate studies removed resulting in 181 studies remaining. Among them, 83 studies were excluded based on titles and abstracts. Following the inclusion exclusion criteria 78 studies were excluded to case reports, review, previous meta-analyses, and other polymorphisms of MTHFR gene or lack of the relevant data. Finally, a total of 20 case-control studies from eleven independent papers (Kimura et al., 2001; Sanyal et al., 2004; Moore et al., 2004, 2007; Lin et al., 2004; Karagas et al., 2005; Cai et al., 2009; Wang et al., 2009; Rouissi et al., 2009; Chung et al., 2010; Safarinejad et al., 2011; Amooee et al., 2019) were selected. The main characteristics of each study identified are listed in Table 1. Of them, eleven case-control studies with 3,463 cases and 3,927 controls were on MTHFR rs1801133 (677C>T) and nine case-control studies with 3,177 cases and 3,502 controls were on MTHFR rs180113 (1298A>C). For the MTHFR rs1801133 (677C>T), five studies were conducted on Caucasians, four on Asians and one study on African and mixed population. For

Table 2. Summary Risk Estimates for Association between MTHFR rs1801133 (677C>T) Polymorphism and Bladder Cancer Risk

Subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio		Publication Bias			
			I^2 (%)	P_H	OR	95% CI	Z_{test}	P_{OR}	P_{Beggs}	P_{Eggers}
Overall	T vs. C	Random	62.67	0.003	1.028	0.912-1.159	0.450	0.653	0.640	0.846
	TT vs. CC	Random	52.97	0.019	1.018	0.801-1.293	0.144	0.886	0.755	0.960
	TC vs. CC	Fixed	37.10	0.103	1.016	0.919-1.122	0.307	0.759	0.212	0.557
	TT+TC vs. CC	Random	65.24	0.001	1.044	0.879-1.241	0.491	0.623	0.275	0.841
	TT vs. TC+CC	Fixed	29.94	0.161	1.019	0.886-1.172	0.258	0.796	0.876	0.895
Ethnicity										
Caucasian	T vs. C	Fixed	56.16	0.058	1.004	0.922-1.093	0.087	0.931	1.000	0.761
	TT vs. CC	Fixed	0.00	0.785	0.902	0.750-1.087	-1.084	0.278	1.000	0.734
	TC vs. CC	Fixed	19.51	0.290	1.007	0.891-1.139	0.116	0.907	0.806	0.945
	TT+TC vs. CC	Random	70.99	0.008	1.068	0.843-1.353	0.542	0.588	1.000	0.719
	TT vs. TC+CC	Fixed	0.00	0.937	0.923	0.775-1.098	-0.909	0.364	0.806	0.555
Asian	T vs. C	Random	67.64	0.026	1.143	0.906-1.443	1.126	0.260	0.089	0.119
	TT vs. CC	Random	65.33	0.034	1.381	0.834-2.285	1.255	0.209	0.089	0.126
	TC vs. CC	Fixed	22.33	0.277	1.149	0.945-1.398	1.392	0.164	0.089	0.239
	TT+TC vs. CC	Fixed	58.16	0.067	1.195	0.991-1.440	1.864	0.062	0.089	0.316
	TT vs. TC+CC	Fixed	46.83	0.130	1.357	1.041-1.770	2.259	0.024	0.089	0.120
Country										
Chinese	T vs. C	Random	77.86	0.011	1.149	0.836-1.579	0.856	0.392	0.292	0.269
	TT vs. CC	Random	75.70	0.016	1.416	0.723-2.775	1.014	0.311	0.296	0.276
	TC vs. CC	Fixed	47.94	0.146	1.159	0.926-1.450	1.286	0.198	0.296	0.437
	TT+TC vs. CC	Random	71.78	0.029	1.190	0.795-1.784	0.845	0.398	0.296	0.517
	TT vs. TC+CC	Fixed	62.37	0.070	1.316	0.804-2.154	1.091	0.275	0.296	0.269
Source of Controls										
HB	T vs. C	Random	63.94	0.007	1.046	0.911-1.199	0.636	0.525	0.901	0.647
	TT vs. CC	Random	60.66	0.013	1.120	0.834-1.503	0.752	0.452	1.000	0.560
	TC vs. CC	Fixed	28.16	0.203	1.033	0.924-1.155	0.570	0.569	0.901	0.647
	TT+TC vs. CC	Random	54.00	0.033	1.057	0.893-1.249	0.643	0.520	1.000	0.527
	TT vs. TC+CC	Fixed	41.95	0.099	1.045	0.895-1.220	0.556	0.578	1.000	0.620
PB	T vs. C	Random	72.83	0.025	0.954	0.690-1.320	-0.282	0.778	1.000	0.186
	TT vs. CC	Fixed	0.00	0.589	0.786	0.556-1.110	-1.367	0.172	1.000	0.352
	TC vs. CC	Fixed	65.05	0.067	0.951	0.762-1.186	-0.448	0.654	0.296	0.107
	TT+TC vs. CC	Random	84.75	0.001	0.937	0.510-1.721	-0.210	0.834	0.296	0.153
	TT vs. TC+CC	Fixed	0.00	0.437	0.911	0.658-1.260	-0.564	0.573	1.000	0.694

HB, Hospital Based; PB, Population Based

the MTHFR rs180113 (1298A>C), four studies were conducted on Caucasians, three on Asians and one study on African and mixed population. The eligible studies were published between 2001 and 2011. In term of study design, there were 14 hospital-based (HB) and six population-based (PB). Genotyping methods were conducted using restrictive fragment length polymorphism (PCR-RFLP) and TaqMan. The alleles, genotypes and minor allele frequencies (MAFs) distributions for both MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms in the cases and controls are present in Table 1. The distribution of genotypes in the controls was in agreement with Hardy-Weinberg equilibrium (HWE) for all selected studies (Table 1). The NOS score of eligible articles ranged from 7 to 9, which indicated that all included studies were of high quality (Table 1).

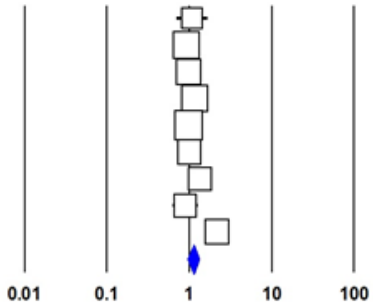
Quantitative Data Synthesis

MTHFR rs1801133 (677C>T) Polymorphism

The summary of association between the MTHFR rs1801133 (677C>T) polymorphism and bladder cancer risk are shown in Table 2. Overall, the combined data did not show a significant association between MTHFR rs1801133 (677C>T) polymorphism and increased risk of bladder cancer globally under all five genetic models, i.e., allele (T vs. C: OR = 1.028, 95% CI 0.912-1.159, p=0.653, Fig 2A), homozygote (TT vs. CC: OR = 1.018, 95% CI 0.801-1.293, p=0.886, Fig 2B), heterozygote (TC vs. CC: OR = 1.016, 95% CI 0.919-1.122, p=759, Figure 2C), dominant (TT+TC vs. CC: OR = 1.044, 95%

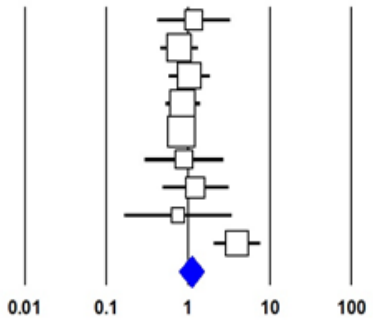
A

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value	Relative weight
Moore 2004	1.088	0.717	1.652	0.397	0.691	7.89
Lin 2004	0.938	0.765	1.149	0.621-	0.535	12.90
Sanyal 2004	0.995	0.772	1.281	-0.041	0.967	11.62
Karagas 2005	1.185	0.959	1.465	1.575	0.115	12.68
Moore 2007	0.992	0.868	1.134	0.114-	0.910	14.52
Cai 2009	1.015	0.754	1.365	0.096	0.924	10.55
Rouissi 2009	1.360	0.970	1.907	1.785	0.074	9.57
Wang 2009	0.906	0.643	1.276	0.567-	0.571	9.45
Safarinejad 2011	2.182	1.640	2.904	5.350	0.000	10.81
	1.132	0.960	1.335	1.479	0.139	



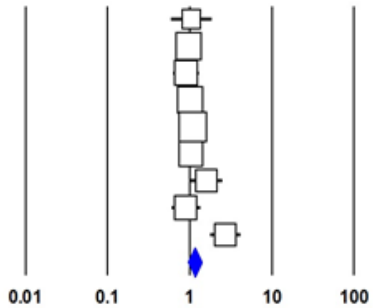
B

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value	Relative weight
Moore 2004	1.190	0.427	3.317	0.332	0.740	7.56
Lin 2004	0.789	0.470	1.323	0.900-	0.368	14.40
Sanyal 2004	1.043	0.583	1.865	0.142	0.887	13.31
Karagas 2005	0.870	0.538	1.406	0.569-	0.569	15.04
Moore 2007	0.834	0.601	1.158	1.082-	0.279	17.66
Cai 2009	0.901	0.298	2.724	0.185-	0.853	6.84
Rouissi 2009	1.247	0.499	3.119	0.473	0.636	8.67
Wang 2009	0.759	0.167	3.442	0.358-	0.721	4.32
Safarinejad 2011	4.031	2.105	7.720	4.204	0.000	12.21
	1.107	0.778	1.576	0.566	0.571	



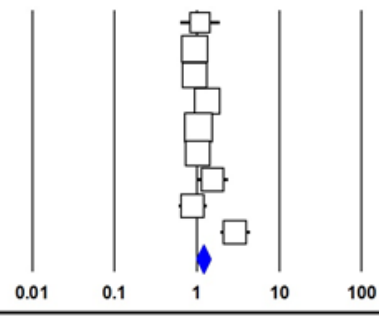
C

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value	Relative weight
Moore 2004	1.058	0.604	1.853	0.196	0.845	7.04
Lin 2004	0.982	0.748	1.291	0.127-	0.899	13.11
Sanyal 2004	0.909	0.638	1.294	0.529-	0.597	11.10
Karagas 2005	1.024	0.771	1.360	0.165	0.869	12.84
Moore 2007	1.105	0.925	1.319	1.104	0.270	15.50
Cai 2009	1.040	0.737	1.468	0.222	0.825	11.31
Rouissi 2009	1.622	1.055	2.492	2.205	0.027	9.39
Wang 2009	0.904	0.611	1.338	0.505-	0.614	10.20
Safarinejad 2011	2.741	1.793	4.190	4.655	0.000	9.50
	1.156	0.955	1.399	1.486	0.137	



D

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value	Relative weight
Moore 2004	1.097	0.643	1.873	0.340	0.734	7.77
Lin 2004	0.956	0.736	1.243	0.334-	0.738	12.77
Sanyal 2004	0.954	0.682	1.336	0.272-	0.786	11.29
Karagas 2005	1.348	1.016	1.788	2.067	0.039	12.35
Moore 2007	1.055	0.889	1.253	0.614	0.539	14.45
Cai 2009	1.030	0.736	1.442	0.172	0.864	11.28
Rouissi 2009	1.568	1.038	2.368	2.139	0.032	9.82
Wang 2009	0.897	0.610	1.318	0.555-	0.579	10.32
Safarinejad 2011	2.956	1.971	4.434	5.239	0.000	9.95
	1.203	0.978	1.481	1.746	0.081	



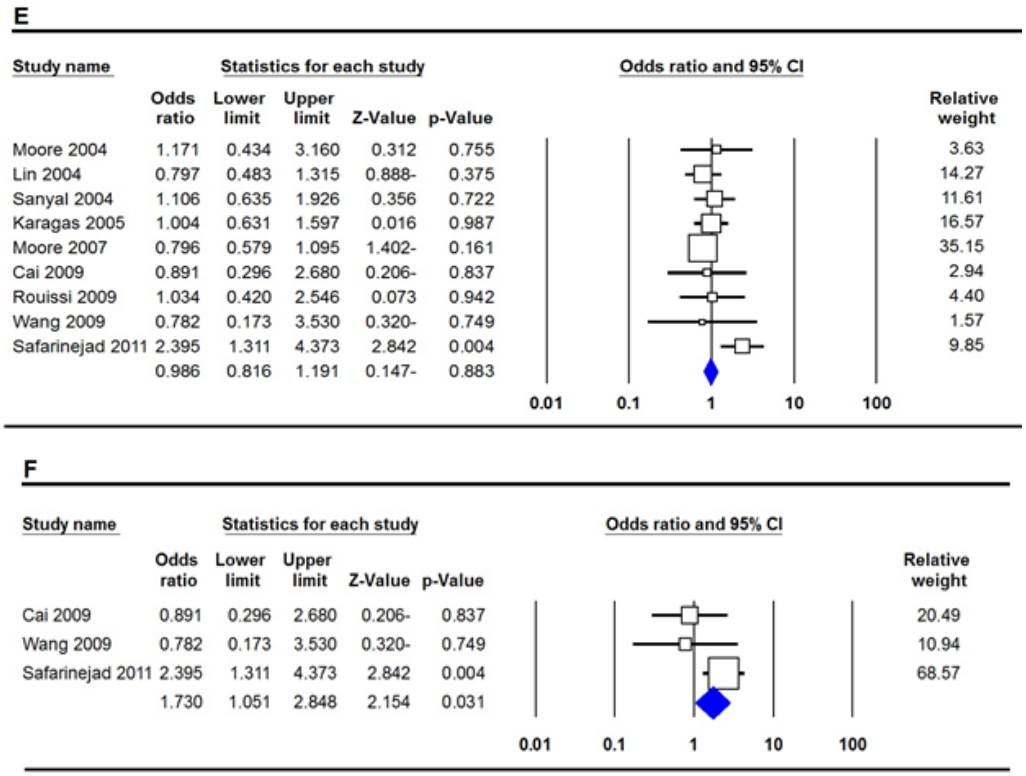
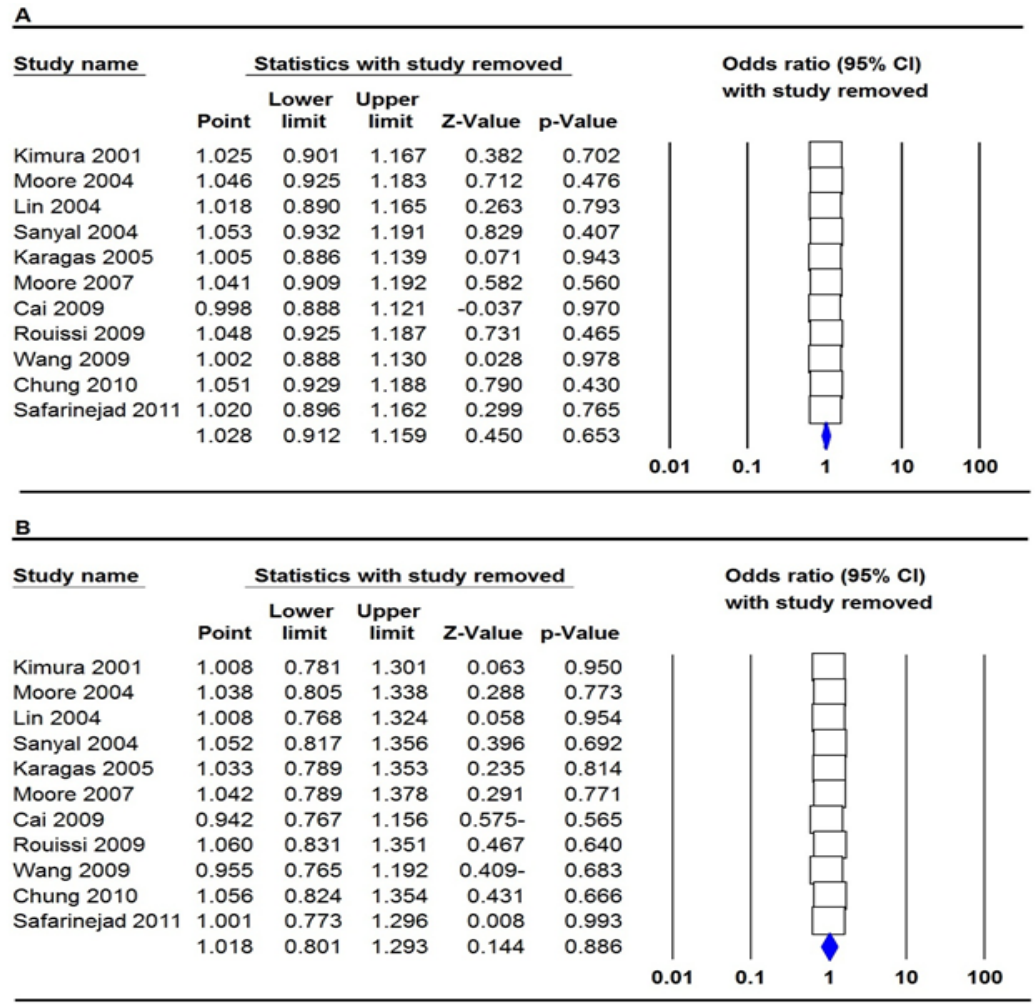


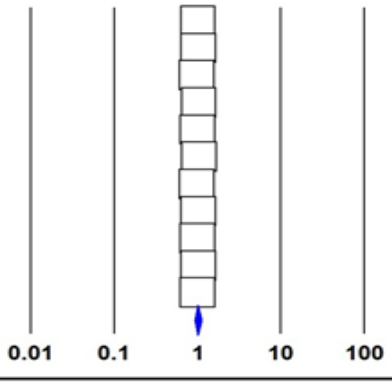
Figure 3. Forest Plot for Association between MTHFR rs180113 (1298A>C) Polymorphism and Bladder Cancer Risk. A: allele model (A vs. C); B: homozygote model (AA vs. CC); C: heterozygote model (AC vs. CC); D: dominant model (AA+AC vs. CC); E: recessive model (AA vs. AC+CC) and F: Asians (recessive model: TT vs. TC+CC).



C

Study name	Statistics with study removed				
	Point	Lower limit	Upper limit	Z-Value	p-Value
Kimura 2001	1.016	0.917	1.125	0.298	0.766
Moore 2004	1.036	0.936	1.146	0.684	0.494
Lin 2004	0.989	0.889	1.100	0.202-	0.840
Sanyal 2004	1.040	0.937	1.153	0.733	0.464
Karagas 2005	1.001	0.900	1.113	0.015	0.988
Moore 2007	1.045	0.929	1.176	0.734	0.463
Cai 2009	0.990	0.892	1.098	0.188-	0.851
Rouissi 2009	1.024	0.924	1.134	0.447	0.655
Wang 2009	1.000	0.902	1.108	-0.005	0.996
Chung 2010	1.029	0.929	1.140	0.550	0.582
Safarinejad 2011	1.009	0.911	1.119	0.177	0.860
	1.016	0.919	1.122	0.307	0.759

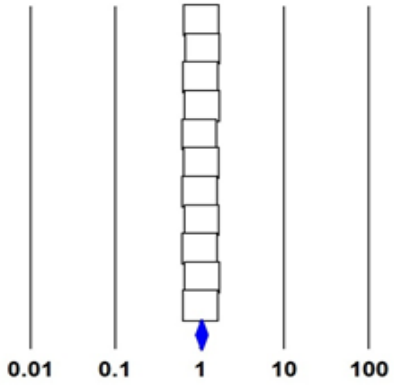
Odds ratio (95% CI) with study removed



D

Study name	Statistics with study removed				
	Point	Lower limit	Upper limit	Z-Value	p-Value
Kimura 2001	1.043	0.866	1.256	0.447	0.655
Moore 2004	1.084	0.917	1.282	0.948	0.343
Lin 2004	1.026	0.846	1.244	0.260	0.795
Sanyal 2004	1.080	0.904	1.291	0.849	0.396
Karagas 2005	0.998	0.848	1.175	-0.026	0.979
Moore 2007	1.059	0.869	1.291	0.567	0.570
Cai 2009	1.007	0.845	1.200	0.080	0.937
Rouissi 2009	1.065	0.887	1.279	0.675	0.499
Wang 2009	1.014	0.847	1.214	0.156	0.876
Chung 2010	1.073	0.896	1.285	0.766	0.444
Safarinejad 2011	1.034	0.857	1.248	0.352	0.725
	1.044	0.879	1.241	0.491	0.623

Odds ratio (95% CI) with study removed



E

Study name	Statistics with study removed				
	Point	Lower limit	Upper limit	Z-Value	p-Value
Kimura 2001	1.015	0.880	1.170	0.201	0.841
Moore 2004	1.015	0.880	1.171	0.208	0.835
Lin 2004	1.024	0.882	1.189	0.313	0.754
Sanyal 2004	1.034	0.896	1.194	0.458	0.647
Karagas 2005	1.024	0.883	1.188	0.317	0.751
Moore 2007	1.088	0.917	1.291	0.963	0.335
Cai 2009	0.962	0.830	1.116	0.507-	0.612
Rouissi 2009	1.039	0.901	1.198	0.525	0.599
Wang 2009	0.975	0.843	1.128	0.336-	0.737
Chung 2010	1.037	0.898	1.196	0.493	0.622
Safarinejad 2011	1.012	0.877	1.169	0.166	0.868
	1.019	0.886	1.172	0.258	0.796

Odds ratio (95% CI) with study removed

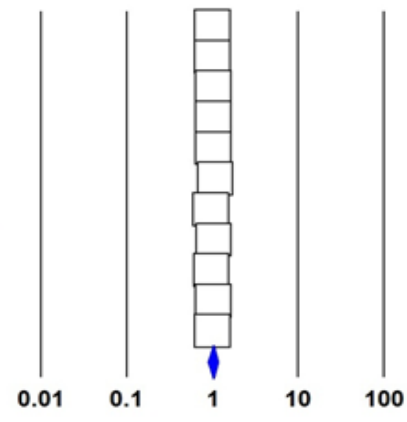


Figure 4. Sensitivity Analysis of Each Study Included in This Meta-Analysis for MTHFR rs1801133 (677C>T) Polymorphism by Omitting each Data Set in the Meta-Analysis. A: allele model (T vs. C); B: homozygote model (TT vs. CC); C: heterozygote model (TC vs. CC); D: dominant model (TT+TC vs. CC); E: recessive model (TT vs. TC+CC).

Table 3. Summary Risk Estimates for Association between MTHFR rs180113 (1298A>C) Polymorphism and Bladder Cancer Risk

Subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio				Publication Bias	
			I^2 (%)	P_H	OR	95% CI	Z_{test}	P_{OR}	P_{Beggs}	P_{Eggers}
Overall	A vs. C	Random	73.99	≤0.001	1.132	0.960-1.335	1.479	0.139	0.251	0.368
	AA vs. CC	Random	60.9	0.009	1.107	0.778-1.576	0.566	0.571	0.348	0.467
	AC vs. CC	Random	66.55	0.002	1.158	0.955-1.399	1.486	0.137	0.602	0.456
	AA+AC vs. CC	Random	73.59	≤0.001	1.203	0.978-1.481	1.746	0.081	0.175	0.353
	AA vs. AC+CC	Fixed	28.46	0.192	0.986	0.816-1.191	-0.147	0.883	0.754	0.457
Ethnicity										
Caucasian	A vs. C	Fixed	0	0.426	1.015	0.926-1.112	0.31	0.757	0.734	0.757
	AA vs. CC	Fixed	0	0.904	0.861	0.689-1.074	-1.326	0.185	0.734	0.484
	AC vs. CC	Fixed	0	0.757	1.038	0.918-1.175	0.598	0.55	0.308	0.033
	AA+AC vs. CC	Fixed	19.49	0.293	1.066	0.946-1.202	1.053	0.292	1	0.959
	AA vs. AC+CC	Fixed	0	0.684	0.879	0.709-1.089	-1.179	0.238	0.308	0.289
Asian	A vs. C	Random	89.76	≤0.001	1.267	0.727-2.207	0.836	0.403	0.296	0.49
	AA vs. CC	Random	73.95	0.021	1.58	0.479-5.210	0.751	0.452	1	0.2
	AC vs. CC	Random	88.07	≤0.001	1.361	0.714-2.595	0.935	0.35	1	0.496
	AA+AC vs. CC	Random	90.54	≤0.001	1.39	0.688-2.811	0.918	0.359	1	0.561
	AA vs. AC+CC	Fixed	44.14	0.167	1.73	1.051-2.848	2.154	0.031	1	0.176
Source of Controls										
HB	A vs. C	Random	82.12	≤0.001	1.102	0.879-1.383	0.842	0.4	0.452	0.533
	AA vs. CC	Random	74.69	0.001	1.146	0.681-1.927	0.513	0.608	0.452	0.626
	AC vs. CC	Random	75.73	0.001	1.145	0.886-1.478	1.036	0.3	1	0.636
	AA+AC vs. CC	Random	80.8	≤0.001	1.153	0.874-1.521	1.007	0.314	0.452	0.512
	AA vs. AC+CC	Fixed	54.68	0.051	0.971	0.781-1.207	-0.264	0.792	0.452	0.594
PB	A vs. C	Fixed	0	0.689	1.209	1.025-1.425	2.254	0.024	1	0.98
	AA vs. CC	Fixed	0	0.726	0.973	0.657-1.441	-0.136	0.892	1	0.139
	AC vs. CC	Fixed	37.1	0.204	1.158	0.932-1.441	1.322	0.186	1	0.717
	AA+AC vs. CC	Fixed	0	0.582	1.358	1.097-1.682	2.808	0.005	1	0.783
	AA vs. AC+CC	Fixed	0	0.963	1.032	0.705-1.511	0.164	0.869	0.296	0.384

HB, Hospital Based; PB, Population Based

CI 0.879-1.241, p=0.623, Fig 2D), and recessive (TT vs. TC+CC: OR = 1.019, 95% CI 0.886-1.172, p=0.796, Fig 2E). Moreover, we have performed subgroup analysis by ethnicity, country of origin and source of controls. Stratified analysis by ethnicity showed that MTHFR rs1801133 (677C>T) polymorphism was associated with an increased risk of bladder cancer in Asians under the recessive genetic model (TT vs. TC+CC: OR = 1.357, 95% CI 1.041-1.770, p=0.024, Fig 2F), but not in Caucasians. There was no significant association between MTHFR rs1801133 (677C>T) polymorphism and risk of bladder cancer by source of controls and in Chinese population.

MTHFR rs180113 (1298A>C) Polymorphism

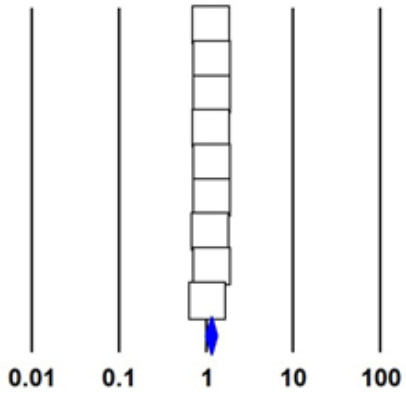
The summary of association between the MTHFR rs180113 (1298A>C) polymorphism and risk of bladder cancer are presented in Table 3. Pooled ORs demonstrated that MTHFR rs180113 (1298A>C) polymorphism was not significantly associated with bladder cancer risk globally under all five genetic models, i.e., allele (C vs. A: OR = 1.132, 95% CI 0.960-1.335, p=0.139, Fig 3A), homozygote (CC vs. AA: OR = 1.107, 95% CI 0.778-1.576, p=0.571, Fig 3B), heterozygote (CA vs. AA: OR = 1.158, 95% CI 0.955-1.399, p=0.137, Fig 3C), dominant (CC+CA vs. AA: OR = 1.203, 95% CI 0.978-1.481, p=0.081, Fig 3D), and recessive (CC vs. CA+AA: OR = 0.986, 95% CI 0.816-1.191, p=0.883, Fig 3E). Moreover, we carried out subgroup analyses by ethnicity and source of controls. Stratified analysis by ethnicity revealed that there was a significant association between the MTHFR rs180113 (1298A>C) polymorphism and increased risk of bladder cancer in Asians under the recessive genetic model (CC vs. CA+AA: OR = 1.730, 95% CI 1.051-2.848, p=0.031, Fig 3F), but not in Caucasians. Moreover, subgroup analysis by source of controls showed a significant association between MTHFR rs180113 (1298A>C) polymorphism and risk of bladder cancer in population based (PB) group of studies under two genetic models, i.e., allele (C vs. A: OR = 1.209, 95% CI 1.025-1.425, p=0.024) and dominant (CC+CA vs. AA: OR = 1.358, 95% CI 1.097-1.682, p=0.005), but not in hospital based (HB) studies (Table 3).

A

Study name	Point	Lower limit	Upper limit	Z-Value	p-Value
Moore 2004	1.137	0.952	1.358	1.417	0.157
Lin 2004	1.165	0.968	1.402	1.613	0.107
Sanyal 2004	1.153	0.958	1.387	1.505	0.132
Karagas 2005	1.126	0.932	1.361	1.232	0.218
Moore 2007	1.159	0.950	1.412	1.457	0.145
Cai 2009	1.148	0.956	1.378	1.482	0.138
Rouissi 2009	1.111	0.932	1.324	1.171	0.242
Wang 2009	1.160	0.971	1.385	1.639	0.101
Safarinejad 2011	1.028	0.948	1.115	0.670	0.503
	1.132	0.960	1.335	1.479	0.139

Statistics with study removed

Odds ratio (95% CI) with study removed

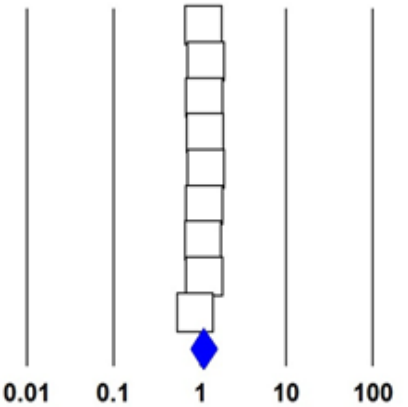


B

Study name	Point	Lower limit	Upper limit	Z-Value	p-Value
Moore 2004	1.103	0.753	1.616	0.503	0.615
Lin 2004	1.175	0.783	1.764	0.779	0.436
Sanyal 2004	1.122	0.745	1.690	0.551	0.581
Karagas 2005	1.159	0.763	1.760	0.694	0.488
Moore 2007	1.177	0.768	1.802	0.749	0.454
Cai 2009	1.127	0.771	1.648	0.617	0.537
Rouissi 2009	1.097	0.746	1.612	0.471	0.638
Wang 2009	1.129	0.779	1.637	0.640	0.522
Safarinejad 2011	0.888	0.723	1.090	1.138-	0.255
	1.107	0.778	1.576	0.566	0.571

Statistics with study removed

Odds ratio (95% CI) with study removed

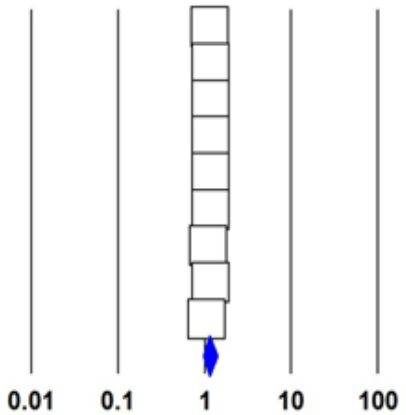


C

Study name	Point	Lower limit	Upper limit	Z-Value	p-Value
Moore 2004	1.166	0.949	1.432	1.462	0.144
Lin 2004	1.188	0.954	1.478	1.540	0.123
Sanyal 2004	1.193	0.968	1.470	1.654	0.098
Karagas 2005	1.180	0.947	1.471	1.475	0.140
Moore 2007	1.172	0.922	1.489	1.299	0.194
Cai 2009	1.175	0.947	1.457	1.467	0.142
Rouissi 2009	1.115	0.916	1.358	1.086	0.277
Wang 2009	1.190	0.967	1.465	1.647	0.100
Safarinejad 2011	1.057	0.951	1.175	1.025	0.305
	1.156	0.955	1.399	1.486	0.137

Statistics with study removed

Odds ratio (95% CI) with study removed



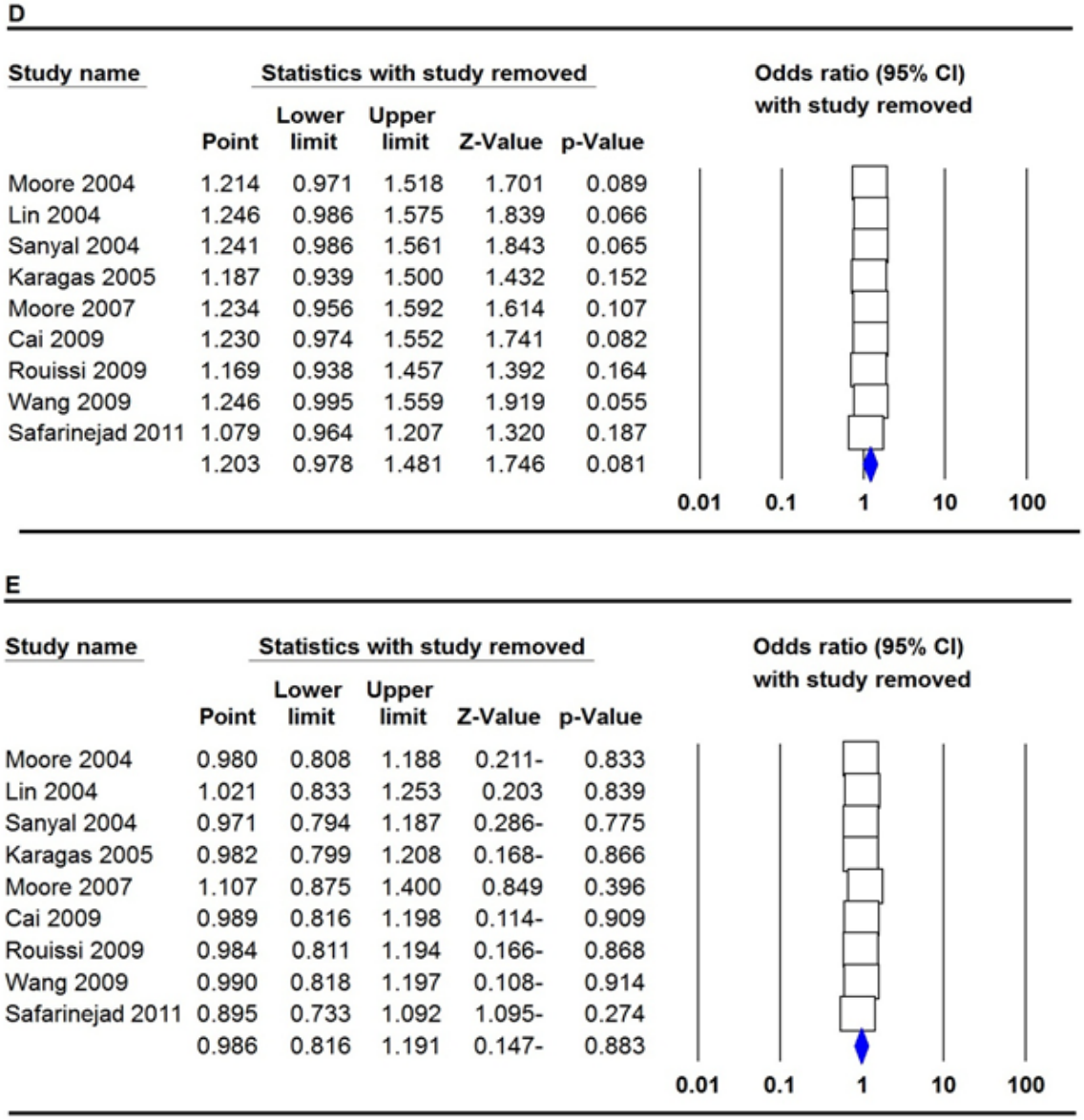


Figure 5. Sensitivity Analysis of each Study Included in This Meta-Analysis for MTHFR rs180113 (1298A>C) by Omitting each Data Set in the Meta-Analysis. A: allele model (A vs. C); B: homozygote model (AA vs. CC); C: heterozygote model (AC vs. CC); D: dominant model (AA+AC vs. CC); E: recessive model (AA vs. AC+CC).

Minor Allele Frequencies (MAFs)

The minor allele frequencies (MAFs) for MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms in healthy controls is shown in Table 1. There were ethnic variations in the allele and genotype distributions for these polymorphisms. MAFs in controls for MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms were 36.6% and 26.6%, respectively. Moreover, the mutant allele frequency for MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms were 39.0% and 27.4%, respectively. Thus, the mutant and wild allele frequency of MTHFR rs180113 (1298A>C) polymorphism were less than MTHFR rs1801133 (677C>T) polymorphism.

Between-Study Heterogeneity

As shown in Tables 2 and 3, there was a significant between-study heterogeneity in overall population under most genetic models for both MTHFR rs1801133 (677C>T) and rs180113 (1298A>C). Thus, we utilized a random-effects model (DerSimonian and Laird method) for those genetic models. To explore the potential sources of between-study heterogeneity, we conducted subgroup analyses by ethnicity, country of origin and source of controls. Results revealed a significant heterogeneity in Asians studies for both MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms. However, the heterogeneity was reduced or disappeared in Caucasian studies. Subgroup analysis revealed that ethnicity and source of controls (for rs180113) might be source of heterogeneity in the current meta-analysis.

Sensitivity Analysis

We conducted a sensitivity analysis to evaluate the stability of the results by sequentially removing each study from our meta-analysis. As shown in Figures 4 and 5, the

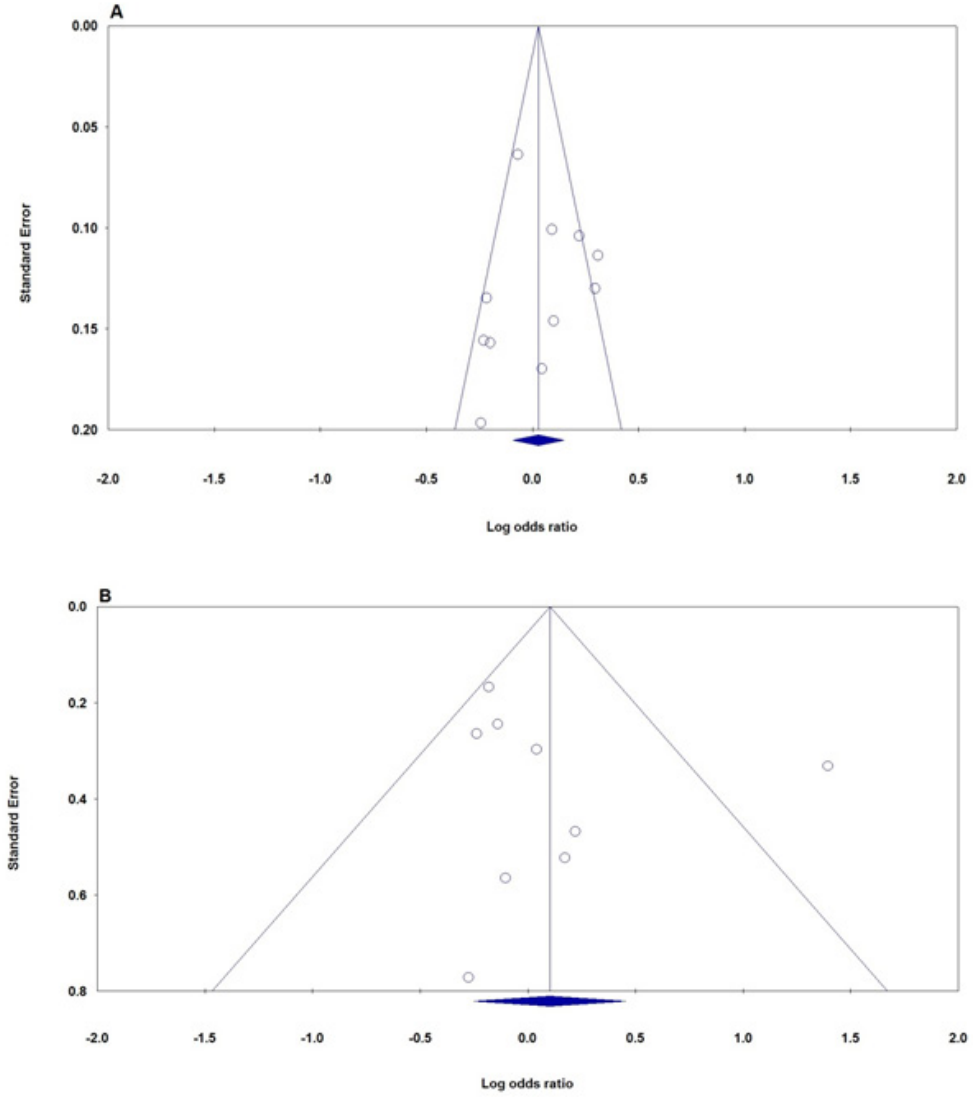


Figure 6. The Funnel Plots of Publication Bias for Association between MTHFR Polymorphisms and Bladder Cancer Risk. A: MTHFR rs1801133 (677C>T) (allele model: T vs. C); B: MTHFR rs180113 (1298A>C) (homozygote model: AA vs. CC).

data from sensitivity analysis revealed that none of the studies changed the pooled OR for MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) under all five genetic models, and it shows that the meta-analysis is stable.

Publication Bias

We performed potential publication bias with the Begg's test and the Egger's test. As Figure 6 indicated, the symmetrical funnel plot indicated that there is no significant publication bias for both for MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms under all five genetic models. Moreover, the Egger's test was performed to provide the statistical evidence of funnel plot (Tables 2 and 3).

Discussion

MTHFR is a key enzyme in folate metabolism, which converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate that is required for the remethylation of homocysteine to methionine (Gohari et al., 2019; Karimi-Zarchi et al., 2019; Sadeghiyeh et al., 2020; Bahrami et al., 2021). The rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms are responsible for the synthesis of a thermolabile form of MTHFR enzyme with decreased enzymatic activity (Kiseljaković et al., 2008). It is suggested that the homozygote mutant genotypes of MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms are linked with higher plasma homocysteine level (Azarpira et al., 2018; Niktabar et al., 2021). Some published molecular epidemiological studies have demonstrated an association between MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms and an increased risk of bladder cancer. However, Safarinejad et al., reported that MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms did not risk factor for development of bladder cancer among Iranian population (Safarinejad et al., 2011). This trend inconsistent results between the rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms at MTHFR gene and risk of bladder cancer may be caused for limited number of related studies

and small sample sizes. In the current meta-analysis, we evaluated the associations between rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms at MTHFR gene and susceptibility to bladder cancer based on 20 eligible studies. Our pooled OR indicated that both rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms at MTHFR gene were not associated with susceptibility to bladder cancer globally.

In the current meta-analysis, pooled ORs showed that MTHFR rs1801133 (677C>T) polymorphism was not associated with an increased risk of bladder cancer in overall population. Stratified analysis by showed that both polymorphisms were associated with bladder cancer in Asians, but not in Caucasian. Similarly, Shi et al., (2014) in a meta-analysis of 3,463 cases and 3,927 controls revealed that the MTHFR rs1801133 (677C>T) polymorphism was not associated with risk of bladder cancer in overall population. However, their stratified analysis by ethnicity showed that the MTHFR rs1801133 (677C>T) polymorphism was significantly associated with susceptibility to bladder cancer in Middle Eastern populations. Li et al., (2013) in a meta-analysis based on 15 case-control studies with 3,570 cases and 3,926 healthy subjects demonstrated that the MTHFR rs1801133 (677C>T) polymorphism did not associate with risk of bladder cancer. Their subgroup analysis still revealed that the rs1801133 (677C>T) polymorphism was not risk factor for bladder cancer by ethnicity and sources of controls. The previous meta-analyses were not performed subgroup analyses by country of origin for MTHFR rs1801133 (677C>T) polymorphism. Thus, this polymorphism association with bladder cancer needs to be evaluated. Moreover, their conclusions reliability is considerably smaller than that needed to achieve the robust conclusions.

Our pooled data failed to show a significant association between the MTHFR rs180113 (1298A>C) and an increased risk of bladder risk in the global population. Our subgroup analysis by ethnicity showed a significant association between MTHFR rs180113 (1298A>C) and bladder cancer risk in Asians, but not in Caucasians. Moreover, some epidemiological studies revealed that the MTHFR rs180113 (1298A>C) polymorphism was associated with susceptibility to bladder risk in Chinese (Lin et al., 2004; Cai et al., 2009). However, our subgroup analysis by country of origin did not show an association between the MTHFR rs180113 (1298A>C) polymorphism and susceptibility to bladder cancer in Chinese patients. Similarly, Safarinejad et al., in case-control study revealed that the MTHFR rs180113 (1298A>C) was not risk factor for development of bladder cancer in Iranian population (Ghaemmaghami et al., 2008; Safarinejad et al., 2011; Binesh et al., 2012). According to our findings, the association of the MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms with bladder cancer may be due to differences in ethnicity, genetic background, life style, smoking habits, and etc. in a meta-analysis, Zhang et al., (2018) verified the relationship between TP53 codon 72 and bladder cancer risk in Asians, but not Caucasians. These results indicated that genetic variants might be an ethnicity related factor of susceptibility to bladder cancer. In another way, it seemed that different populations with multiple genetic backgrounds have different genetic variants risk in development of bladder cancer (McConkey et al., 2010; Meng et al., 2017). Still, further studies are needed to explore this difference between Asians and Caucasians.

Between-study heterogeneity may have affected a meta-analysis result when interpreting of the pooled ORs (Edraki et al., 2019; Sayad, Ahmadi, Nekouian, et al., 2020; Sayad, Ahmadi, Moradi, et al., 2020). In the current meta-analysis, significant between study heterogeneity existed for both MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms under most genetic models. Subgroup analysis revealed that ethnicity might be the potential source of heterogeneity in the meta-analysis (Karimi-Zarchi et al., 2013; Mojtaba Sohrevardi et al., 2016). However, heterogeneity may be due to many factors, such as differences in the characteristics of controls, life style, diverse genotyping methods, small sample size, and a mixed population from different geographic regions (Yang et al., 2014). Moreover, certain HWE deviations were revealed in the distributions of controls in some included studies, which may be due to the small sample size or other experimental technique errors in the study. However, we have selected all eligible studies even HWE-violating studies.

There are potential limitations in our meta-analysis should be considered. First, the numbers of studies as well as sample sizes for each ethnicity and some subgroup analyses were relatively limited, which Type-II error might not be dismissed. Second, the research subjects of the included studies were mostly from Asian and Caucasian origins. Thus, the bias of racial diversity could not be avoided and the results are not applicable to all populations. Third, the reliability and authenticity of our results may be influenced by the limited number of studies and small sample sizes. Fourth, moderate heterogeneity existed in some genetic models for both MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms. And the subsequent meta-regression could not identify any interfering factors contributing to heterogeneity. Selection bias, although no publication bias was observed, might be a possible major source of between-study heterogeneity in this meta-analysis. Fifth, the detailed individual data in some studies was not available; leading to failure to adjust risk of MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms with bladder cancer based on potential risk factors, such as age, gender, smoking, occupation, environmental factors, lifestyle habits, and other covariates of this disease. Finally, bladder cancer as other malignancies is a multi-factorial disease that results from complex interactions between various genetic and environmental factors. However, due to the lack of the individual original data in the selected studies, we were unable to evaluate the effect of gene-environment interactions, gene-gene interactions and also different polymorphisms within MTHFR gene on development of bladder cancer.

In conclusion, our pooled data revealed that MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms might be not risk factor for development of bladder cancer. However, stratified analysis by ethnicity

revealed that both MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms were significantly associated with an increased risk of bladder cancer in Asians. Further studies with larger sample size, well-designed, and population-based studies among different ethnicities required to validate our findings.

Abbreviations

MTHFR: Methylenetetrahydrofolate Reductase
CBD: Biomedical Database
CNKI: Chinese National Knowledge Infrastructure
NOS: Newcastle-Ottawa Score
OR: Odds Ratio
CI: Confidence Interval
HWE: Hardy-Weinberg Equilibrium
CMA: Comprehensive Meta-Analysis
HB: Hospital-Based
PB: Population-Based
SOC: source of controls
MAFs: Minor Allele Frequencies
PCR-RFLP: Polymerase Chain Reaction Restriction Fragment Length Polymorphism

Author Contribution Statement

Conceived and designed the study and experiments: SAD and FA. Performed the experiments: HM, JSY and SHS. Analyzed the data: SAD and HN. Contributed reagents/materials/analysis tools: FA, SK and MZS. Wrote the paper: HN, JSY and EA. All authors reviewed the manuscript.

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Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate

Not applicable for this manuscript.

Vailability of data and material

The datasets generated during and/or analyzed during this study are the corresponding author on reasonable request.

Conflicts of interest

The authors declare that they have no conflict of interest.

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