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Rs401681 polymorphism in *TERT-CLPTM1L* was associated with bladder cancer risk: A meta-analysis

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ABSTRACT

Objective(s): Genome-wide association studies have identified a number of genetic variants of telomerase reverse transcriptase (TERT), cleft lip and palate transmembrane1-like (CLPTM1L) associated with the risk of bladder cancer. Rs401681 polymorphism in *TERT-CLPTM1L* was of special interest for bladder cancer risk, whereas the results were inconclusive.

Materials and Methods: Publications illustrating the association between rs401681 polymorphism and bladder cancer risk were collected from the Embase, PubMed and Google scholar. Three independent reviewers worked on the data extraction. The meta-analysis was performed by STATA 12.0. The odds ratio (OR) with 95% confidence interval (CI) was calculated for these data.

Results: Six case-control studies were retrieved reporting a total of 9196 bladder cancer patients and 42570 controls. The strength of the relevance between rs401681 polymorphism and bladder cancer risk was evaluated by Stata 12.0 software. Rs401681[C] allele was identified marginally associated with increased bladder cancer risk, with per allele OR of 1.132 (95% CI=1.080-1.187, $P_{heterogeneity}$=0.701); in the stratified analysis by ethnicity, the increased cancer risk was revealed in Asian and Caucasian groups. Moreover, we also revealed that rs401681 polymorphism was associated with an increased risk of bladder cancer in Asian population with three publications under allele model (OR=3.722, 95% CI=1.311-10.568, *P*=0.014), whereas a decreased risk was identified in homozygote model (OR=0.692, 95 % CI=0.513-0.934, *P*= 0.016) and recessive model (OR=0.728, 95% CI=0.541-0.980, *P*=0.036).

Conclusion: In summary, our study provided evidence that rs401681 polymorphism is associated with the risk of bladder cancer.

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Introduction

Bladder cancer is one of the most frequent malignancies around the world (1). In the United States, the estimated number of new cases for 2014 is 69,000 while the estimated number of deaths is approximately 15,000 (2). Evidence suggests that the activation of the telomerase enzyme is a pivotal step in the development of bladder cancer; furthermore, somatic mutations in *TERT* promoters were identified in 55.6% of the bladder cancers (3). The addition of hexameric TTAGGG repeats to telomeres, located at the ends of chromosomal DNA (4), plays a critical role in counteracting the end-replication loss and consequent DNA damage repair, leading to genome instability, chromosomal fusions and rearrangements (5).

TERT and *CLPTM1L* were located in chromosome 5p15.33, which was regularly suggested to mediate the telomerase function. Moreover, rs401681 (located in 27 kb from the *TERT* and the intron 13 of *CLPTM1L*) is one of the most widely studied SNPs, which has been reported to be associated with an increased risk of many cancer types (e.g. prostate and lung cancers) via GWAS (6-9). Nevertheless, a decreased risk of colorectal cancer and melanoma was identified by some studies on the major (C) allele of rs401681 (10, 11).

Although several studies have paid attention to the relationship between rs401681 polymorphism and bladder cancer susceptibility (1, 9, 12), the reported data is inconclusive. Thus, we conducted a meta-analysis on all eligible studies to derive a more authentic estimation of the relevance and a better understanding of its possible influence on bladder cancer risk.

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Materials and Methods

Search strategy

We performed an *in silico* search of the Embase, PubMed and Google scholar databases to retrieve articles linking rs401681 polymorphism in *TERT-CLPTM1L* gene and susceptibility to bladder cancer available up to December 2014 using the combinations of "*TERT*", OR "telomerase reverse transcriptase," OR "*CLPTM1L*," OR "*CLPTM1*-like" AND "polymorphism," OR "gene," OR "variant", OR "mutation", OR "locus" OR "SNP" AND" association" OR "risk" AND "tumor" OR "cancer" OR "malignancy" OR "neoplasm" OR "carcinoma". All the searched publications were retrieved, and we also used a hand search of references of reviewed articles or original studies on this point to uncover additional studies. The search was limited to English language literatures, and all relevant studies were reviewed. Only the first published study was selected when overlapping studies existed. For republished studies, only the one with the largest sample size was enrolled. Finally, five eligible case–control studies of four publications were included in our meta-analysis.

Inclusion and exclusion criteria

Articles which met the following criteria were included: (1) Parameters about the rs401681 polymorphism and bladder cancer risk are evaluated; (3) Race and numbers of affected and unaffected subjects are reported; (5) Sufficient data for calculating an odds ratio (OR) with 95 percent confidence interval (95%CI) in additive model is available; (6) Sufficient data for detail genotype frequencies in Asian population is available. Exclusion criteria were: (1) Studies on the subjects of family cancer risks or cancer-prone disposition; (2) The study which has no usable data reported or contains duplicated data; (3) Abstract, comment, review and editorial; (4) When multiple publications reported the same or to overlapping patients, we retained only the largest study to avoid duplication of information.

Data extraction

Three investigators (Meng Zhang, Xun Wu and Wei Lu) independently extracted data in a standardized form and have reached a consensus of all publications. For each eligible study, the following information was recorded: the name of the first author, the publication year, ethnicity, source of controls, minor allele frequency (MAF), genotype frequency and/or additive OR and 95% CI and the number of the cases and controls. The detail information about the genotype frequency in Asian population was provided by three case-control studies. The association between rs401681 polymorphism and bladder cancer risk was evaluated under four genetic models.

Statistical analysis

We evaluated the association between rs401681 polymorphism and bladder cancer risk by using crude OR with 95% CI in overall population. The heterogeneity of the individual studies was evaluated by Q test (for the association between rs401681 polymorphism and bladder cancer risk in Asian). If the *P* value of Q test was ≥0.05, the fixed effects model was used to pool the data; otherwise, random effects model will be selected. However, the test for heterogeneity does not have enough power for selecting the effects model for the pooling analysis of the association between rs401681 polymorphism and overall bladder cancer risk. Thus, random effects model was selected for all the analyses. Both funnel plot and Egger's test were applied to evaluate the publication bias (*P*<0.10 was considered representative of statistical significance). We used STATA Software (version 12.0, Stata Corp) to perform all statistical tests and for any test or model, *P*<0.05 was considered to be statistically significant. Further, the four genetic models: allele contrast (T vs. C), homozygote (TT vs. CC), recessive (TT vs. TC/CC), and dominant (TT/TC vs. CC) models were used to evaluate the association between polymorphism and bladder cancer risk in Asian population group.

Results

Eligible studies

In total, five eligible case–control studies of four publications involving 9,196 cases and 42570 controls were selected in this meta-analysis (1, 6, 9, 12). And three case-control studies including 1044 cases and 1869 controls were selected to evaluate the association between the genetic models of the polymorphism and bladder cancer risk in Asian. The main characteristics of these studies are demonstrated in Table 1 (1, 9, 12). The ethnicity origins of these eligible publications are Asia and Caucasian. Besides, a study was excluded for an overlap (13). The distribution of rs401681[C] allele and the genotype frequencies of Asian publications among bladder cancer cases and controls are shown in Table 1 and methodological quality of the included studies according to the Newcastle-Ottawa Scale was shown in Table 2.

Meta-analysis

The main results of this meta-analysis and the heterogeneity tests are shown in Table 3. Rs401681[C] allele was proved to be associated with bladder cancer risk in overall population (per allele, OR=1.132, 95% CI: 1.080–1.187; *P*<0.001, Figure 1a). In the stratified analysis by ethnicity, the rs401681[C] locus conferred susceptibility to bladder cancer in Asian group (per allele, OR=1.172, 95 % CI 1.039–1.322; *P*=0.010) and Caucasian group (per allele, OR=1.125, 95%CI=1.068-1.184; *P*<0.001).

Furthermore, our work also showed that rs401681 polymorphism is associated with bladder cancer risk in Asian population under four models: the rs401681 polymorphism was associated with increased risk of

Table 1. Study characteristics in an analysis of the association between rs401681 polymorphism and bladder cancer risk

Author	Year	Country	Ethnicity	Source	No. of (case/control)	MAF	OR(95%CI)	Case	Control					
								CC	CT	TT	CC	CT	TT	HWE
Rafnar *et al* (6)	2009	Iceland	Caucasian	Population	780/ 28,890	45.5	1.16 1.05–1.29	-	-	-	-	-	-	-
	2009	Iceland	Caucasian	Population	578/ 28,890	45.5	1.17 1.03–1.32	-	-	-	-	-	-	-
	2009	The Netherlands	Caucasian	Population	1,277/ 1,832	43.0	1.06 0.96–1.17	-	-	-	-	-	-	-
	2009	UK	Caucasian	Hospital	707/ 506	48.6	1.23 1.04–1.44	-	-	-	-	-	-	-
	2009	Italy-Torino	Caucasian	Hospital	329/ 379	45.5	1.02 0.84–1.24	-	-	-	-	-	-	-
	2009	Italy-Brescia	Caucasian	Hospital	122/ 156	43.6	1.04 0.74–1.46	-	-	-	-	-	-	-
	2009	Belgium	Caucasian	Population	199/ 378	44.6	1.22 0.95–1.56	-	-	-	-	-	-	-
	2009	Eastern Europe	Caucasian	Hospital	214/ 515	42.5	1.20 0.96–1.51	-	-	-	-	-	-	-
	2009	Sweden	Caucasian	Population	346/ 905	47.9	1.10 0.92–1.31	-	-	-	-	-	-	-
	2009	Spain	Caucasian	Hosptial	173/ 1,427	46.2	1.03 0.83–1.29	-	-	-	-	-	-	-
	2009	Combined	Caucasian	-	4147/34988	46.5	1.12(1.06-1.18)	-	-	-	-	-	-	-
Gago-Dominguez *et al* (9)	2011	America	Caucasian	Population	472/554	44.2	1.18(0.98-1.41)	-	-	-	-	-	-	-
	2011	China	Asian	Population	500/529	33.8	1.20(1.00-1.45)	248	207	45	237	226	66	0.29
Ma *et al* (12)	2012	China	Asian	Community	184/962	33.0	1.04(0.83-1.32)	85	70	22	424	381	115	0.04
Zhang *et al* (1)	2014	China	Asian	Hospital	367/420	65.1	1.26(1.02-1.57)	173	166	28	180	187	53	0.68

MAF: Minor Allele Frequency; **"-":** not mentioned; Population: population-based; Hospital: hospital-based.

Table 2. Methodological quality of the included studies according to the Newcastle-Ottawa Scale

Author (number)	Country	Adequacy of Case Definition	Representativeness of the Cases	Selection of Controls	Definition of Controls	Comparability Cases/Controls	Ascertainment of Exposure	Same Method of Ascertainment
Rafnar *et al* (6)	Iceland	*	*	NA	NA	*	*	*
	Iceland	*	*	NA	NA	*	*	*
	The Netherlands	*	*	*	NA	*	*	*
	UK	*	*	NA	NA	*	*	*
	Italy-Torino	*	*	NA	NA	*	*	*
	Italy-Brescia	*	*	NA	NA	*	*	*
	Belgium	*	*	NA	NA	*	*	*
	Eastern Europe	*	*	NA	*	*	*	*
	Sweden	*	*	NA	NA	*	*	*
	Spain	*	*	NA	*	*	*	*
Gago-Dominguez *et al* (9)	China	*	*	*	NA	*	*	*
	USA	*	*	*	NA	*	*	*
Ma *et al* (12)	China	*	*	NA	NA	*	*	*
Zhang *et al* (1)	China	*	*	NA	NA	*	*	*

*, Yes; NA, not applicable; The last item "non-response rate" was eliminated from this study

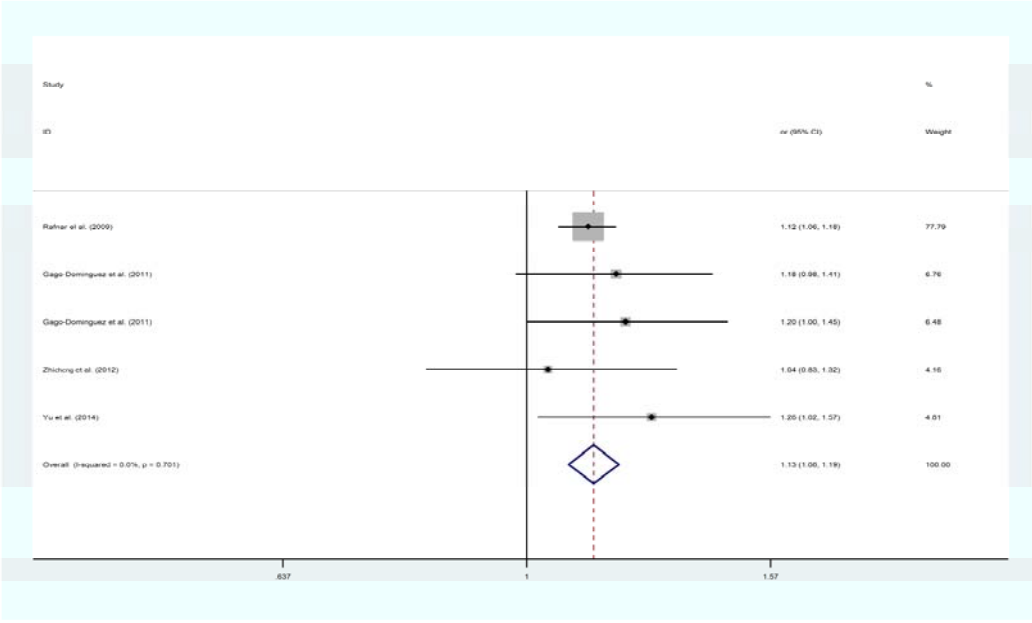

Figure 1a. Odds ratio of bladder cancer risk associated with rs401681 under the additive model by fixed effects

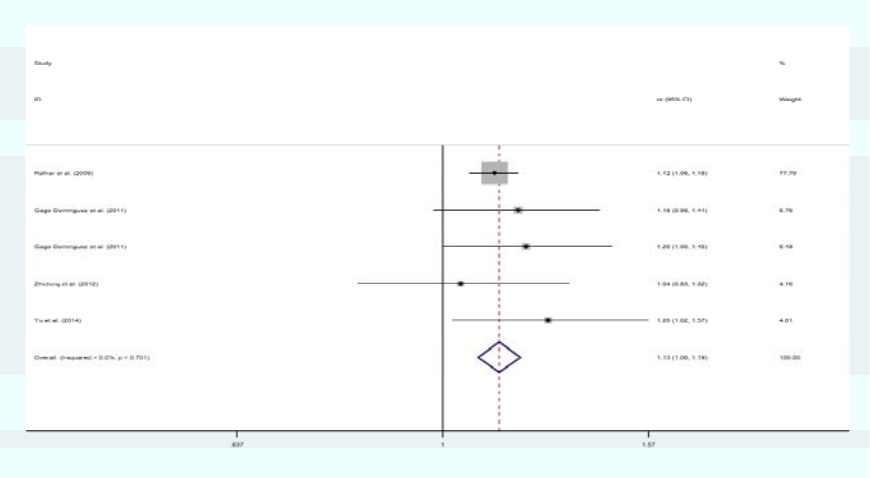

Figure 1b. Odds ratio of bladder cancer risk associated with rs401681 under the homozygote model in Asian by fixed effects

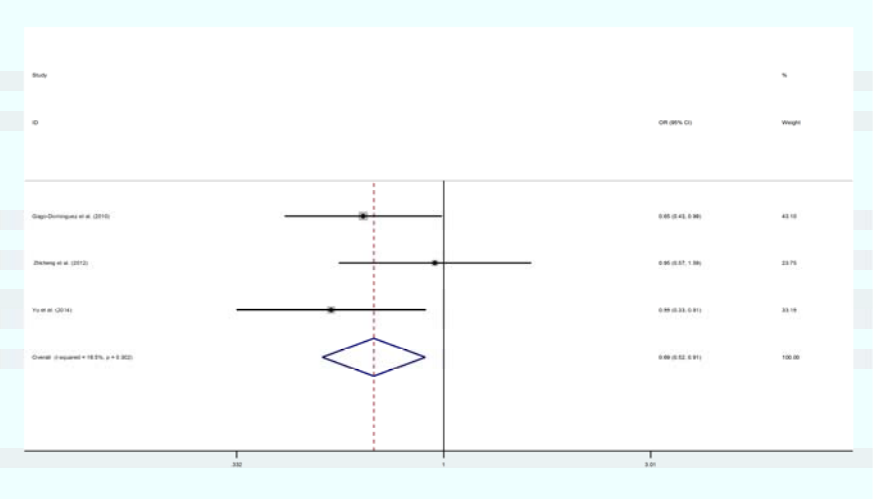

Figure 1c. Odds ratio of bladder cancer risk associated with rs401681 under the recessive model in Asian by fixed effects

bladder cancer in allele model (OR=3.722, 95% CI=1.311-10.568, *P*=0.014), and decreased risk in homozygote model (OR=0.692, 95 % CI=0.513-0.934, *P*= 0.016, Figure 1b) and recessive model (OR=0.728, 95% CI=0.541-0.980, *P*=0.036, Figure 1c) in Asian (Table 3).

Publication bias

A sensitivity analysis was done to explore the influence of individual publications on the collected results by removing a single publication from the pooled analysis once at a time and no individual study influenced the pooled OR value. Then, we

Table 3. Results from stratified analysis of the rs401681 polymorphism and bladder cancer risk in Asian

Comparison	Test of association (OR)	95%CI	*P*	Test of heterogeneity	
				P	I^2
T vs. C	3.722	1.311-10.568	0.014	0.000	99.1
TT vs. CC	0.690	0.525-0.907	0.008	0.302	16.5
TT vs. TC/CC	0.724	0.558-0.941	0.016	0.275	22.6
TT/TC vs. CC	1.171	0.998-1.375	0.054	0.849	0.0

OR odds ratio, CI confidence interval

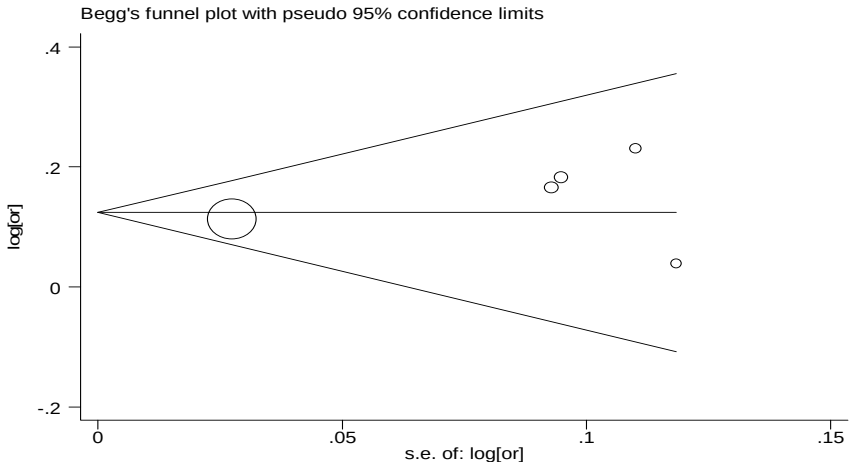

Figure 2. Funnel plot analysis to detect publication bias for the rs401681 in the involved four data sets

performed both Begg's funnel plot and Egger's test to assess the publication bias of the literature (per allele, Begg's test: t=0.97, *P*= 0.406, Figure 2). No obvious asymmetry was obtained from the shape of funnel plots in overall meta-analysis.

Discussion

Chromosome 5p15.33 region contains the *CLPTM1L* and *TERT* genes and genetic variations in this region have been associated with increased or decreased risk of multiple cancer types (14, 15). The rs401681 polymorphism was located in the intron 13 of *CLPTM1L* and 27 kb from the *TERT*, which has been widely reported to be associated with an increased risk of lung, prostate and bladder cancer. Rafnar *et al,* first conducted GWAS which composed of 3,945 bladder cancer patients and 34,988 controls, and showed that the rs401681[C] allele was associated with an increased cancer risk with a combined OR of 1.12 (95% CI, 1.03–1.11) (6). Recently, Yu *et al,* examined the association between SNP rs401681 and bladder cancer risk in a Chinese population of 367 cases and 420 controls (1). Moreover, in the present study, we confirmed that the rs401681 polymorphism was associated with bladder cancer risk that was consistent with a previous study (6). Heterogeneity and sensitivity analyses were conducted to promise the reliability of the data.

To sum up, we conducted a comprehensive research for all eligible studies and provided an overview of the association between rs401681[C] allele and bladder cancer risk, as well as the association between the four genetic models and bladder cancer risk. Still, there exist several limitations in our meta-analysis that should be noted. First, the non-English literatures were excluded, which may result in publications bias. Second, we have calculated the pooled ORs in Asian group under four genetic models; however, since another two studies provided insufficient genotype frequencies, we were unable to calculate the pooled ORs in addition to additive model. Besides, ORs with and without adjustment were pooled together, which might be a consideration source of heterogeneity.

Conclusion

Based on larger sample size, our meta-analysis provided a more precise estimation that rs401681[C] is a risk factor for bladder cancer in Asian and Caucasian groups and rs401681 polymorphism was a risk factor for bladder cancer under allele model and a protective factor in homozygote model and recessive model in Asian group. Future well-designed studies are warranted to refine the investigation on this issue of interest.

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Conflicts of interest statement

The authors declare no competing financial interests.

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