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Original Article

Association between the telomerase reverse Transcriptase (TERT) rs2736098 polymorphism and cancer risks: a meta-analysis

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Abstract: Previous studies showed that genetic polymorphisms of TERT (telomere reverse transcriptase) were considered to be closely correlated with the susceptibility of cancers. However, in case of TERT rs2736098, two meta-analyses published in 2012 have shown that it has no correlation with cancer risks, whereas a case-control study and a meta-analysis published in 2013 have shown it can be used as a predictor for cancer risks, especially for Chinese population with lung adenocarcinoma. To further study its role in cancer prediction, here, we carried out an updated analysis in a more comprehensive way. Firstly, a systematic search through PubMed, Embase and Chinese National Knowledge Infrastructure (CNKI) was conducted and 21 case-control studies containing 13151 cases and 15857 controls were enrolled in our study. The results of random-model depending on the heterogeneity in studies demonstrated that TERT rs2736098 was closely related with the susceptibility of cancers (OR=1.11, 95% CI=1.02-1.21, P=0.020). Furthermore, the subgroup analysis indicated that a certain relevance was found in Asian group (OR=1.15 95% CI=1.07-1.25, P<0.001). The results of heterogeneity test were in the acceptable range. The Egger's tests and Begg's funnel plot did not show any publication biases. Moreover, the sensitivity analysis confirmed the reliability of our data. Taken together, our meta-analysis suggests that TERT rs2736098 can be used to predict the cancer susceptibility.

Keywords: TERT, polymorphism, rs2736098, cancer risk

Introduction

GLOBOCAN has previously reported that there are estimated 14.1 million new cases of cancers diagnosed worldwide and 8.2 million estimated deaths from cancers in 2012, as a result, cancer is one of the major causes of morbidity and mortality. It can be expected that cancer will become a more leading cause of premature deaths when compared with other noncommunicable diseases. Cancer is a kind of polygenetic disease, which is a result of inherited and/or environmental influences [1]. Therefore, genetic polymorphism susceptibility may play an important role in cancer risks.

Recent independent genome-wide association study (GWAS) has demonstrated that single nucleotide polymorphism (SNP), a single base-pair variation in the DNA-sequence, is significantly associated with cancer risks among three separated chromosome regions (5p15 [2], 6p21 [3], and 15q25 [4]). The chromosome 5p15.33 is a region with multi-cancer susceptibility that encodes TERT and cleft lip palate transmembrane 1-like (CLPTM1L). TERT is the catalytic subunit of telomerase that processes the elongation of telomeres by regulating its activities [5].

Telomerases usually add a small nucleotide sequence (TTAGGG) to the end of chromosomes in a repeatable way [6], leading to the prevention of chromosomal ends degradation and ensuring the elongation of chromosome termini in most eukaryotes [1]. Previous studies of telomerases have suggested that telomerase activity is associated with the number of cell division and it plays an important role in the immortal cell lines, such as cancer cells [7]. In

fact, 90% cancers are characterized by the elevation of telomerase activities. Consequently, the TERT, one of the telomerase catalytic subunits, is an important gene to be investigated. As a result, its SNP mutations and associations with cancer risks have been intensely studied. Massive of studies, including GWAS, case-control studies, the studies relying on fine-scale mapping technique and meta-analyses, have revealed associations of TERT gene polymorphisms mapping at 5p15.33 locus with variable cancers, such as non-small lung cancer [8, 9], breast cancer [10], prostate cancer [11], glioma [12], pancreatic cancer [13] and bladder cancer [14]. Many studies on the relationship between rs2736098, a synonymous SNP in the second exon of it mapping at chromosome 5p15.3, and the susceptibility of cancers have been increasingly designed. However, the results are still not in agreement. Results from two meta-analyses published in 2012 have suggested that rs2736098 has no association with cancer risks [15]. On the contrary, recent studies have indicated that the it is obviously involved in the susceptibility of different types of cancers, for instance, the bladder cancer and the renal cell carcinoma. Consequently, based on the new case-control studies, we conducted this updated meta-analysis in accordance with the criteria of PRISMA statement to clarify the relevance between TERT polymorphism rs2736098 and the susceptibility of cancers.

Materials and methods

Screening of related studies

Two independent investigators (XY. Zhu and Y. Wang) without language restrictions carefully conducted a comprehensive search through PubMed, Embase and CNKI database with an aim to cover all papers published prior to December 1st, 2015. The searching strategy used was shown as follows: ('telomere reverse transcriptase' or 'TERT'), ("variant" or "variation" or "polymorphism") and ("tumor" or "cancer" or "carcinoma"). In addition, references of retrieved researches on this issue were manually reviewed to identify extra eligible studies. Alternatively, only the latest or the most complete study was recruited when there are several publications focusing on the same population.

Inclusive and exclusive criteria

In our systematic review, the selected studies should meet the following standards: (1) case-control studies or cohort studies; (2) evaluation of TERT polymorphism rs2736098 and risks of cancers; (3) available and useable data of genotype frequencies or allele frequencies; (4) adequate information for OR and 95% CI; (5) original researches written in English or Chinese; (6) studies focusing on human being. Articles were excluded according to the following criteria: (1) abstract, case report, comment, review, and editorial; (2) lack of normal population as controls; (3) duplicated publications; (4) lack of sufficient data to obtain the genotype frequencies.

Data extraction

Information extraction from above eligible studies was conducted individually by two investigators (XY. Zhu and Y. Wang). Any different views were discussed until consensus was finally reached. The following data were collected: first author's name, years of publication, ethnicity of subjects (Caucasian or Asian), cancer types, sources of control groups (population or hospital-based controls), genotyping methods, genotype frequencies of haplotypesin groups and Hardy-Weinberg equilibrium (HWE) among controls. According to the criteria modified from previous publications [15, 16], the quality of studies was further evaluated.

Statistical analysis

ORs and 95% CIs were defined as measurements of the association between TERT polymorphism rs2736098 and cancer risks. Each study was estimated for HWE using the Chi-Square test with significance set that p value <0.05. The pooled OR and its 95% CI were calculated based on five genetic comparisons: a heterozygote model (AG vs. GG), a homozygote model (AA vs. GG), an allelic comparison (2*AA+AG vs. 2*GG+AG), a dominant comparison (AA+AG vs. GG) and a recessive comparison (AA vs. AG+GG). In addition to comparing the pooled effects to assess the variance among all groups, the stratification was also carried out on the basis of ethnicity and different phenotypes of cancers. Z-tests were used to identify the significance of the summarized OR and p-value less than 0.05 was considered

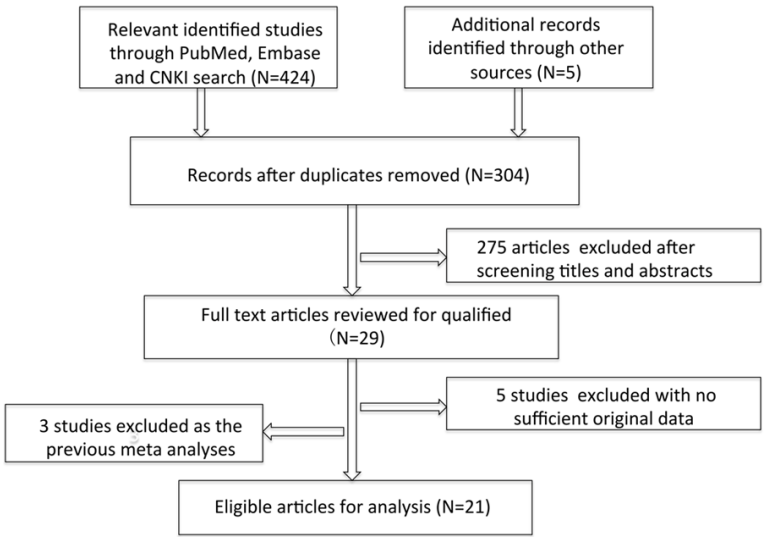

Figure 1. The graphically depicted steps of the process for selecting eligible studies.

as statistically significant. The heterogeneity among each study was measured by Higgins's (I^2) tests and Q tests [17]. Besides, the sensitivity analyses were conducted by omitting one single study each time to estimate the stability of the pooled results and to confirm the reliability of our data. Potential publication bias was accessed using Egger's linear regression tests and Begg's tests through visual inspections of asymmetric plot and funnel plot [18]. All statistical analyses and figures were handled using Stata software 13.0 (Stata Corporation, College Station, TX, USA).

Results

Features of selected studies

On the basis of our initial searching strategies, 424 articles were found. When we manually observed the references of above original articles, additional 5 articles were recruited in our study. After screening the titles, abstracts and full-texts of these articles 400 articles were excluded due to duplicate ones (n=125) and no association with the TERT rs2736098 polymorphism (n=275). Finally, 29 articles were left for further evaluation. Five studies were further excluded because of the unavailability of original data or the shortage of the frequencies of exact genotypes to calculate the OR and 95% CI. Additionally, 3 more were excluded for the reason that the previous meta-analyses have reported. Finally, 21 studies with 15857 controls and 13151 cases were appropriately recognized. The detail of selecting progress was shown in **Figure 1**. The characteristic baseline of selected studies were summarized in **Table 1**, including cancer phenotypes, first author, publication year, country of study population, ethnicity, gene typing techniques, source of control and the conformity to HWE. Most of the studies were carried out in Asians and 6 studies were conducted in Caucasians. Among the 21 studies, five gave evidence for the correlation between TERT-CLPM1L genomic polymorphism and digestive tract tumors, including one colorectal cancer studies [19], two esophageal cancer studies [20, 21] and two gastric cancer studies [22, 23]; four were referred to lung cancer [16, 24-26], three were referred to the hepatocellular carcinoma [27-29] and the bladder cancer [30, 31], respectively, two were referred to the breast cancer [32, 33], one was referred to head and neck cancer [34], glioma [35], Acute Lymphoblastic Leukemia[36] and renal cancer [37], respectively.

Meta-analysis results

Overall, 21 prospective studies enrolling 13151 cases and 15857 controls were included in our meta-analysis. Q test and I^2 test were used to measure the heterogeneity among studies. We selected the random model to calculate the pooled OR depending on the characteristics of our data and the heterogeneity test (I^2=80.4%, P<0.001). A statistically significant association between TERT polymorphism rs2736098 and cancer risks was found in four models, the dominant comparison (OR=1.134, 95% CI 1.009-1.274, P=0.034) (Figure S1), the recessive comparison (OR=1.188, 95% CI 1.038-1.361, P=0.013) (Figure S2), the homozygous model (OR=1.258, 95% CI 1.047-1.512, P=0.014) (Figure S4), and the allelic model (OR=1.11, 95% CI 1.02-1.21, P=0.020) (**Figure 2**). Unfortunately, there was no statistical association under the heterozygous model (OR=1.099, 95% CI 0.985-1.226 P=0.092) (Figure S3). In addition, in our subgroup analy-

Table 1. General features of studies included in the meta-analysis

Study	Year	Country	Ethnicity	Cancer types	Cases	Controls	Source	Genetyping methods	HWE
de Martino [37]	2015	Austria	Caucasian	Renal cancer	239	366	HP	PCR	——
Yin Wang [20]	2014	China	Asian	Esophageal Cancer	600	651	HP	PCR (LDR)	YES
MM Zhao [43]	2014	China	Asian	Lung Cancer	952	955	HP	PCR	YES
Lingyan Su [28]	2014	China	Asian	Hepatocellular Carcinoma	201	210	HP	TaqMan	YES
Vibha Singh [30]	2014	India	Asian	Bladder cancer	225	240	HP	TaqMan	YES
Hashemi M [33]	2014	Iran	Caucasian	breast cancer	252	222	HP	PCR-RFLP	YES
Chao Zhang [27]	2013	China	Asian	Hepatocellular carcinoma	400	400	HP	PCR-RFLP	NO
Haijian Wu [16]	2013	China	Asian	Non-Small-Cell Lung Cancer	539	627	HP	TaqMan	YES
Cheng Li [24]	2013	China	Asian	Lung Cancer	468	544	HP	TaqMan	YES
Xiaojing Sheng [36]	2013	China	Asian	Acute Lymphoblastic Leukemia	567	670	HP	TaqMan	YES
Philipp Hofer [19]	2013	Austria	Caucasian	Colorectal cancer	137	1705	PB	TaqMan	YES
Baohua XU [23]	2012	China	Asian	Gastric cancer	297	306	HP	PCR-RFLP	YES
Sumin Wang [44]	2012	China	Asian	Cervical Cancer	993	1015	PB	TaqMan	YES
Ding [29]	2011	China	Asian	Hepatocellular carcinoma	1273	1328	HP	TaqMan	YES
Hongyan Chen [35]	2011	China	Asian	Glioma	953	1033	HP	PCR	YES
Zhensheng Liu [34]	2010	USA	Caucasian	Head and Neck cancer	1079	1115	PB	TaqMan	YES
Gago-Dominguez [31]	2010	USA	Caucasian	Bladder cancer	449	531	PB	TaqMan	NO
		China	Asian		499	467			
Choi [45]	2009	Korea	Asian	Lung Cancer	720	720	PB	PCR-RFLP	YES
Guolei Wang [21]	2009	China	Asian	Esophageal Cancer	181	200	PB	PCR	YES
Shunmei Wang [22]	2008	China	Asian	Gastric cancer	160	152	PB	PCR-RFLP	YES
Savage [32]	2007	Poland	Caucasian	Breast cancer	1967	2265	PB	TaqMan	YES

HB, hospital-based; PB, population-based; HWE, Hardy-Weinberg equilibrium; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

sis, a significant association was found in the Asian group under the dominant comparison (OR=1.14, 95% CI 1.02-1.27 P=0.007), the recessive comparison (OR=1.30, 95% CI 115-1.46, P<0.001), the homozygous comparison (OR=1.36, 95% CI 1.16-1.58, P<0.001) and the allelic comparison (OR=1.15, 95% CI 1.07-1.25, P<0.001) (**Figure 3**). The Table S1 represents the results of overall subgroup analyses.

Susceptibility analysis

In the sensitivity analysis, the influence of each study on the pooled OR was examined. Even though the genotype distributions of the control groups in the researches of Zhang et al. [27] and Gago Dominguez et al. [31] did not satisfy HWE, there is no substantial alteration of the corresponding pooled OR and the inter-study heterogeneity in the absence or presence of the above two studies, suggesting that our meta-analysis results were trustable and reliable.

Publication bias

The publication bias between included studies was evaluated through Begg's funnel plots and Egger's tests. As shown in **Table 2** and **Figure 4**, no obvious asymmetry was observed in funnel plots. Afterwards, the Egger's tests based on linear regression methods where the normal deviation is plotted against precision were used to provide evidence for the symmetry of the plots. All the *P* values of Egger's tests were more than 0.05 (P =0.344 for rs2736098 dominant, P = 0.229 for recessive variant, P = 0.745 for homozygous, P = 0.313 for heterozygous variant and 0.873 for a allelic variant). **Table 2** presented the results of Begg's funnel plots and Egger's tests under the five genetic models.

Discussion

Telomeres are protective caps that contain unique hexanucleotide sequencerepeats $(TTAGGG)_n$ at the end of chromosomes, which are thought to be essential for maintaining the cell life span and chromosome integrity. Telomerase, playing an important role in cellular immortality and tumorigenesis [38], participates in the synthesis of telomeric DNA and in the maintenance of functional telomeres via its reverse transcriptase activities [39]. There are

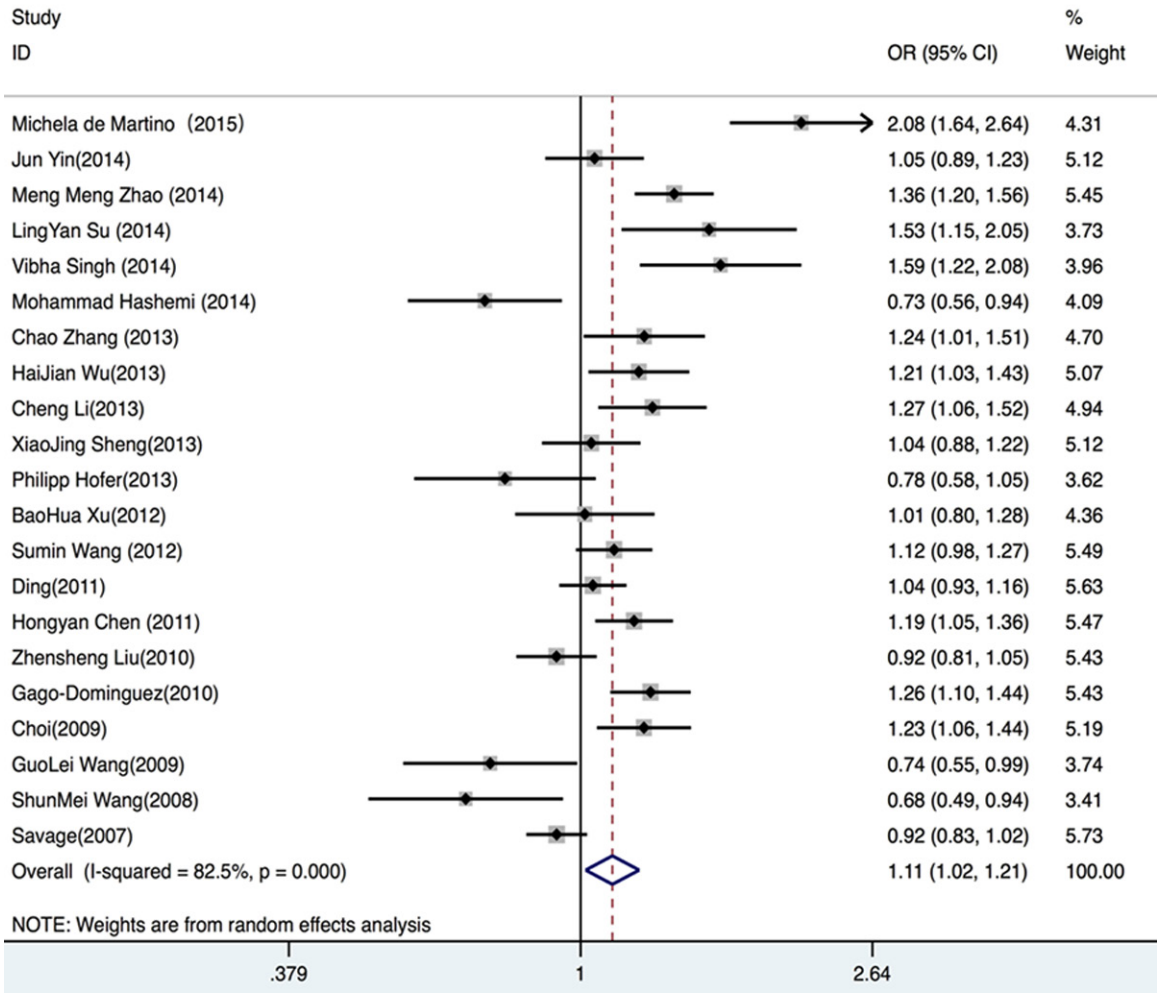

Figure 2. Forest plot of TERT polymorphism rs2736098 and cancer risks in the allelic model. CI, confidence interval; OR, odds ratio.

two components of telomerase, one is the RNA subunit, serving as a template for the synthesis of telomeric repeats, the other is the human TERT protein, similar to viral reverse transcriptase in both structure and functions [40]. Solid evidence by screening of most types of human cancers have shown a tight correlation of telomerase activity with malignancy, suggesting that this enzyme can be used as a novel and useful tumor marker.

SNPs, the most common type of sequence variations in human genome, have been suggested to cause human phenotypic differences and possibly contributing to the individual cancer risks. In case of the polymorphism in tumorigenesis, although the conclusions are not always in agreement, some TERT polymorphisms have been implicated in predicting outcomes for cancer patients [41]. Among these genetic polymorphisms, rs2736098, a synonymous polymorphism (G>A; Ala305Ala) located in the second exon of TERT, has shown to be related with the length of telomere [42]. An increasing number of studies have focused on exploring its relationship with cancer susceptibility. However, the results are still in debate. A meta-analysis, published in 2012 [15], has shown that there is no association between rs2736098 and cancer risks in any of genetic models, whereas previous data from GWAS studies on the same issue demonstrated a contrary conclusion [30]. Maybe due to the fact that there was only allele frequency available in some studies or the data accessed from studies did not meet the inclusion criteria of the meta-analysis, some searching data were not included in previous reviews on this issue, which may limit the reliability of the conclusions. After that, several new case-control studies of this issue based on different study popu-

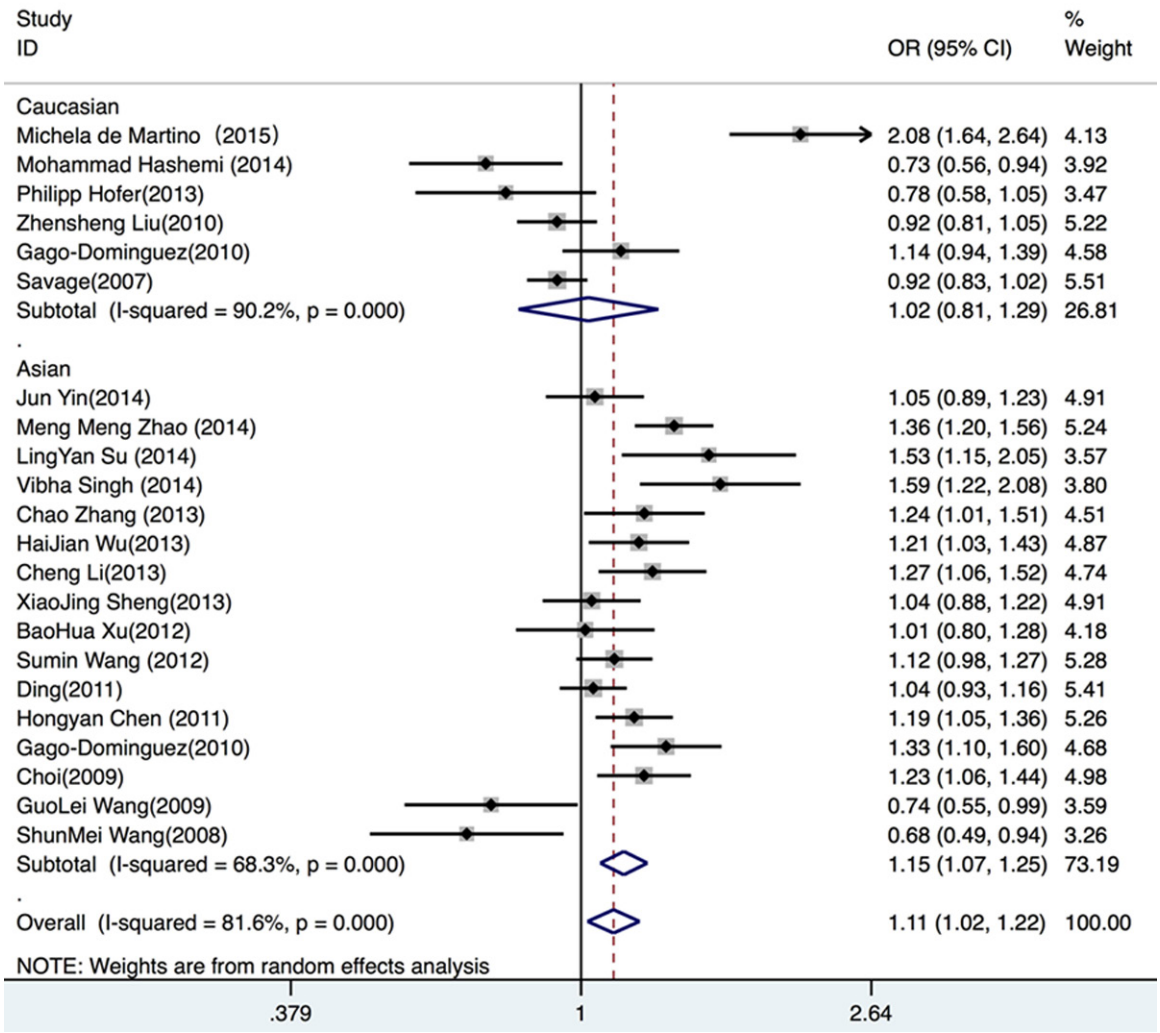

Figure 3. Stratified subgroup analysis of races in the allelic model.

Table 2. A summary of *P*-values for Begg's funnel plots and egger's tests in five genetic model

	Begg's funnel plot	Egger's test
Dominant model	0.566	0.344
Recessive model	0.216	0.229
Homozygote comparison	0.833	0.745
Heterozygote comparison	0.651	0.313
Allelic model	1	0.873

lation have been published these years. Therefore, updating previous systematic reviews is necessary to make a clarified conclusion as new evidence have been produced.

21 studies were enrolled in our meta-analysis and the corresponding data containing 13151 cases and 15857 controls merged for analysis can reach a valid conclusion concerning the potential association between TERT polymorphisms rs2736098 and cancer risks. Notably, the data of these samples are all accessed from the original publications. Our analysis revealed that there was a significant correlation with pooled OR in 4 genetic models except for the heterozygous model (AG vs. GG). However, with a large amount of heterogeneity, the stratified analysis based on ethnicity was applied to find potential cause of heterogeneity between-study. We found that Asians had higher risks of cancers than Caucasians under all the four models apart from the heterozygous model. This discrepancy can possibly be explained by the different life styles and environments between the two ethnicities. Additionally, the

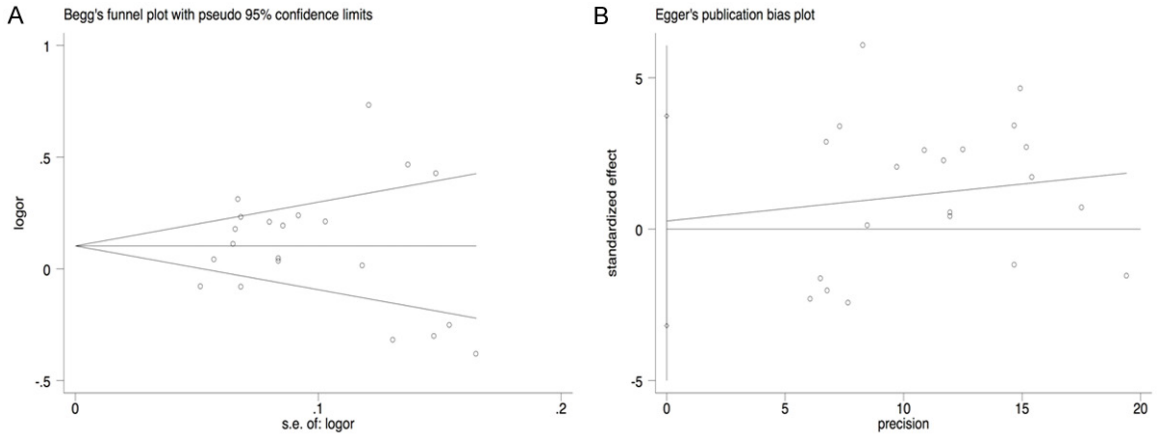

Figure 4. Publication bias analysis. A. Begg's funnel plots of TERT polymorphism rs2736098 in the allelic model. B. Egger's tests of the TERT polymorphism rs2736098 in the allelic model.

heterogeneity was significantly decreased when removing the Caucasian group. Compared with previous meta-analyses [15, 16], our study updated the recent data of the correlation between this polymorphism and cancer risks. For the reason that all published studies satisfying with our inclusion criteria were enrolled in this updated meta-analysis, our data should be recognized more powerfully. Furthermore, the methodological issues for the meta-analysis, from heterogeneity, publication bias to the stability of results, were all well investigated.

Similar with other meta-analyses [15, 16], there are still limitations in our study, which are required to be addressed in the future. Firstly, we have combined the computational and manual strategies to identify the eligible studies, there are still related reports in other language or are still unpublished, which are likely to lead to distorted results. Secondly, some studies were excluded for not mentioning the original genotype distribution and allele frequencies. Although we had tried our best to communicate with first authors and corresponding authors, the original data of certain studies were still unable to get, thus making the sample size in the study was not large enough to obtain a more comprehensive analysis. In addition, studies containing different ethnicities were conducive to estimate the effects of this functional polymorphism on cancer risks, while only six studies originating from Caucasian group in our analysis, so the size of sample was not large enough to attain statistical significance in each term. When the meta-analysis was stratified by ethnicity, the heterogeneity of Asian groups dropped obviously from more than 80% to 60%, suggesting that ethnicity maybe a factor partially contributing to the heterogeneity. Thirdly, cancer is caused by a complex interplay between genetic and environmental factors, but our data were not stratified by some specifically environmental factors and lifestyles such as diet, daily alcohol intake, drug use and smoking status in developing cancers due to the unavailability of original data. Consequently, further well-designed studies of this issue are required to draw definitive conclusions. Finally, only TERT polymorphism rs2736098 was included in this study. According to previous researches, the 5p15.33 locus has been characterized by a 62-kb region block including the 5'-end of TERT, the rate-limiting component for telomerase activity and the entire gene CLPTM1L, these two genes participated in promoting cancer process were epidemiologically related to the polymorphisms [9]. The TERT gene alone has more than 500 known SNPs, but only minorities of the polymorphisms have been investigated in different tumor types. Meanwhile, the evidence of association between the polymorphism and certain tumor types may not be compatible with the susceptibility of other tumor types [42]. Only one comprehensive synopsis of this topic has been published, which indicates that much work remains to be done to find out more information and to entirely understand the relevant role of TERT locus in carcinogenesis [41]. Furthermore, not only the effects of genetic polymorphisms, but also the interactions or networks among these genetic loci or the telomere length should be studied in the future.

Above all, this updated meta-analysis study demonstrates that the TERT polymorphism rs2736098 is closely correlated with the susceptibility of cancer risks. However, our renewed findings need to be verified by more extensive and well-designed epidemiological studies.

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Disclosure of conflict of interest

None.

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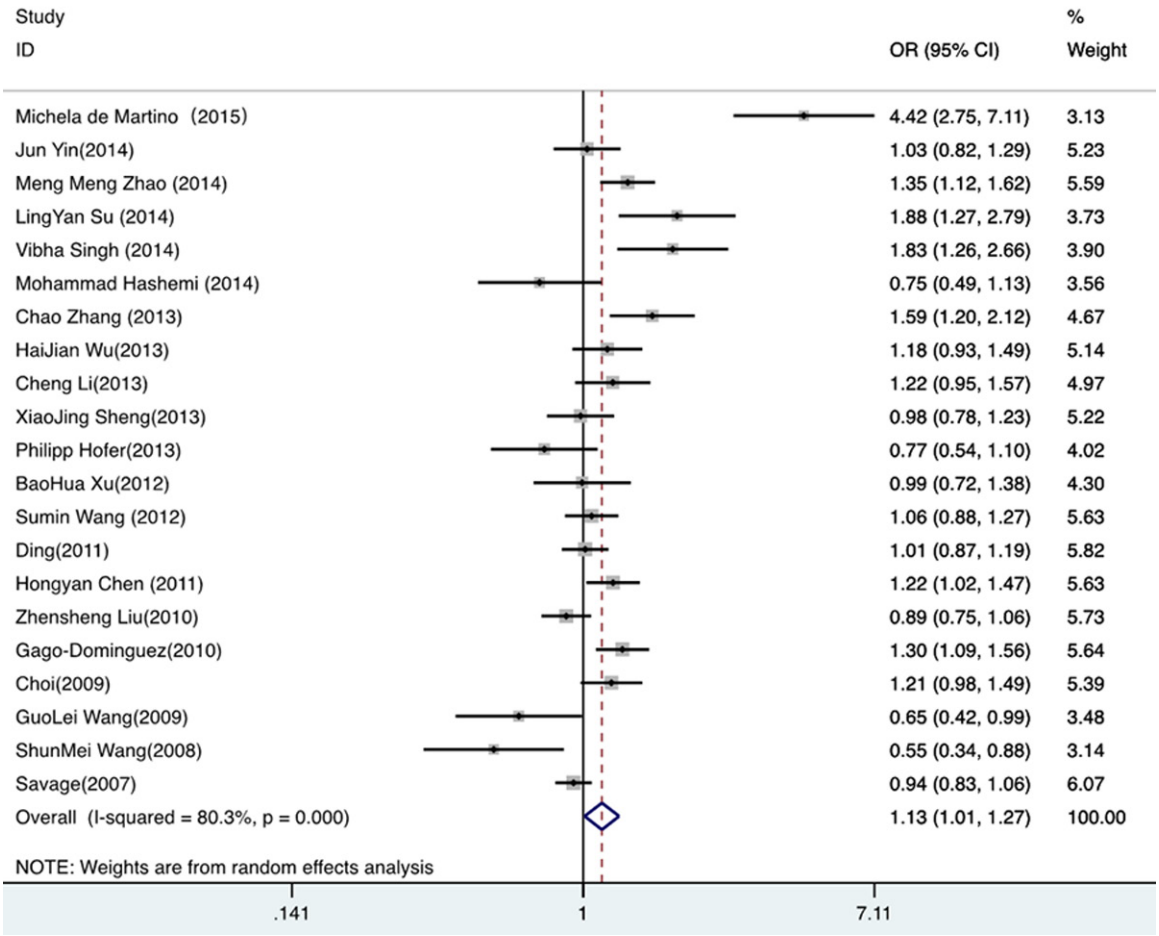

Figure S1. Forest plots of TERT polymorphism rs2736098 and cancer risks in the dominant model (Abbreviation: CI, confidence interval OR, odds ratio).

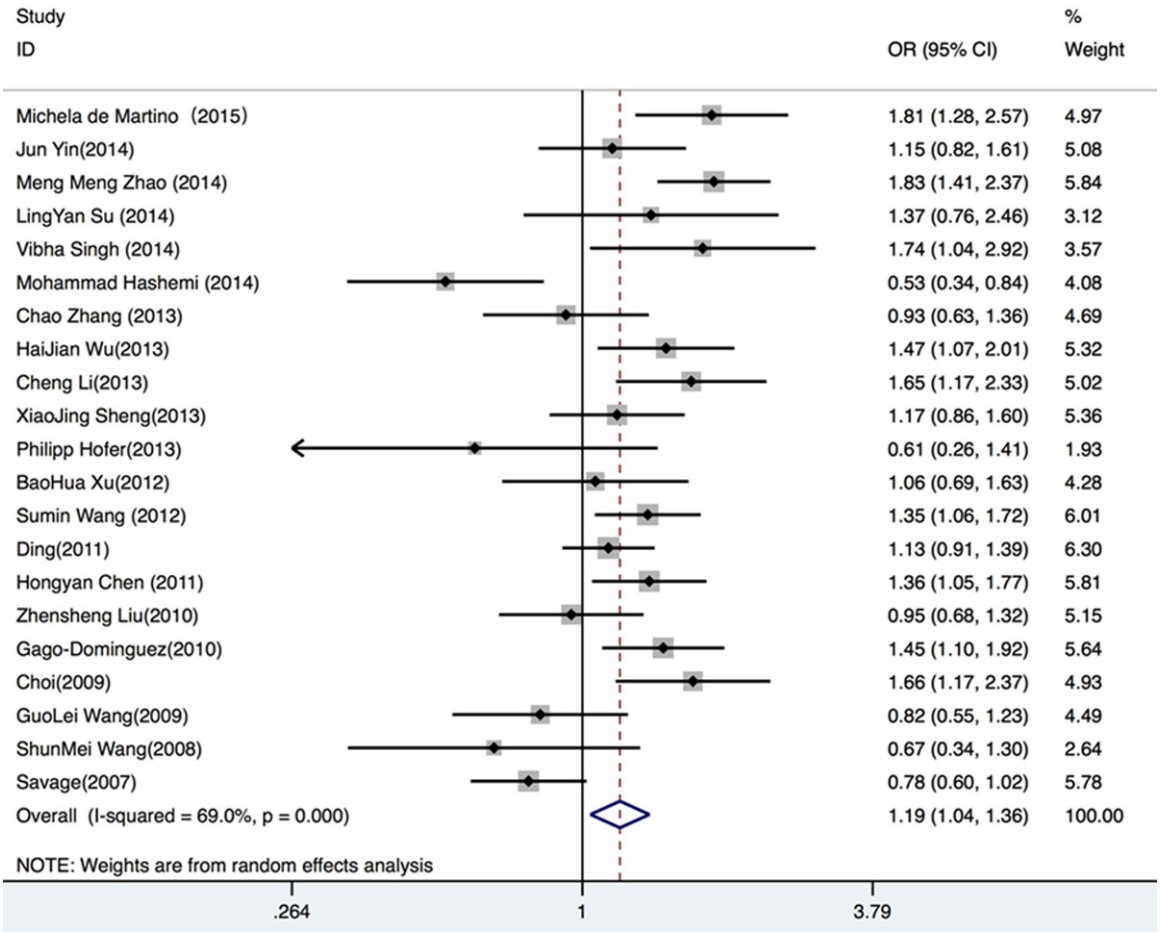

Figure S2. Forest plots of TERT polymorphism rs2736098 and cancer risks in the recessive model.

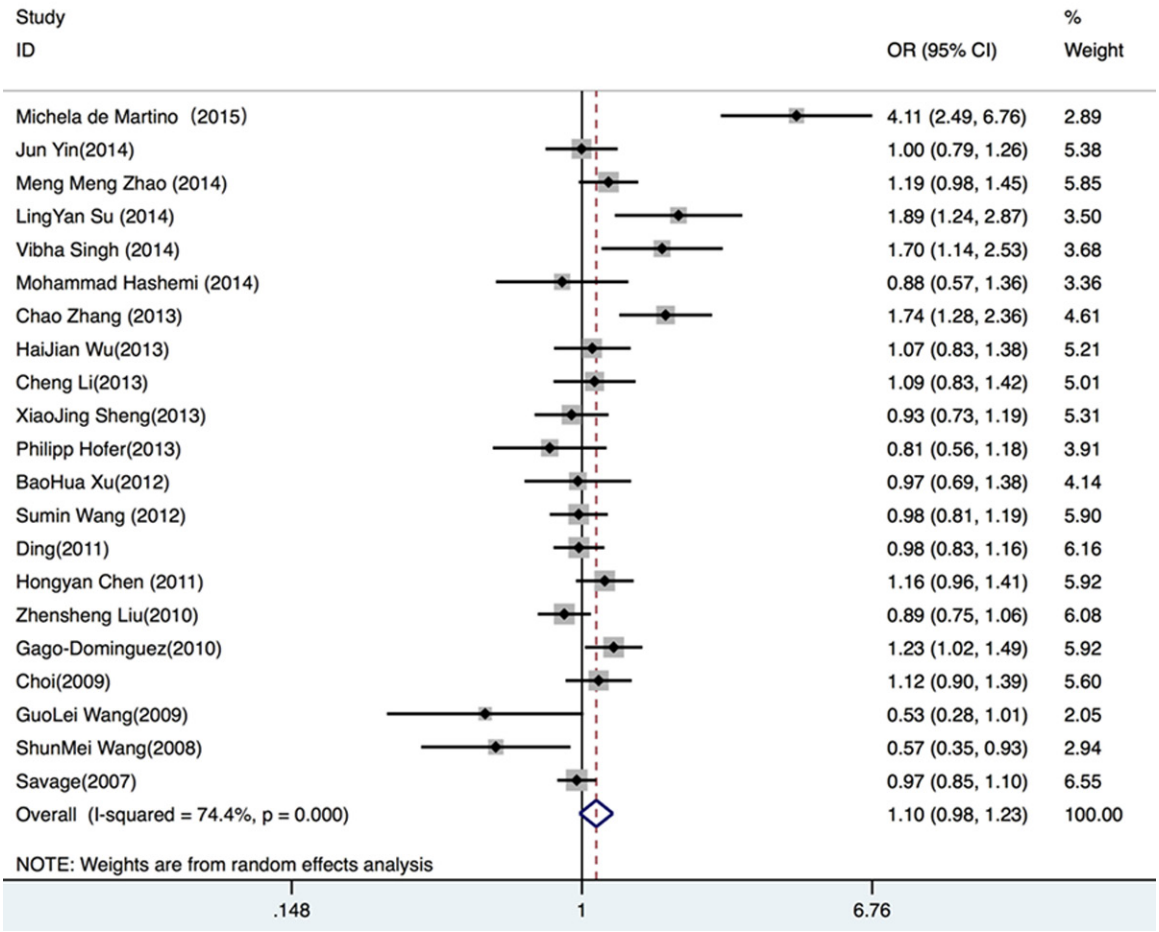

Figure S3. Forest plots of TERT polymorphism rs2736098 and cancer risks in the heterozygote comparison.

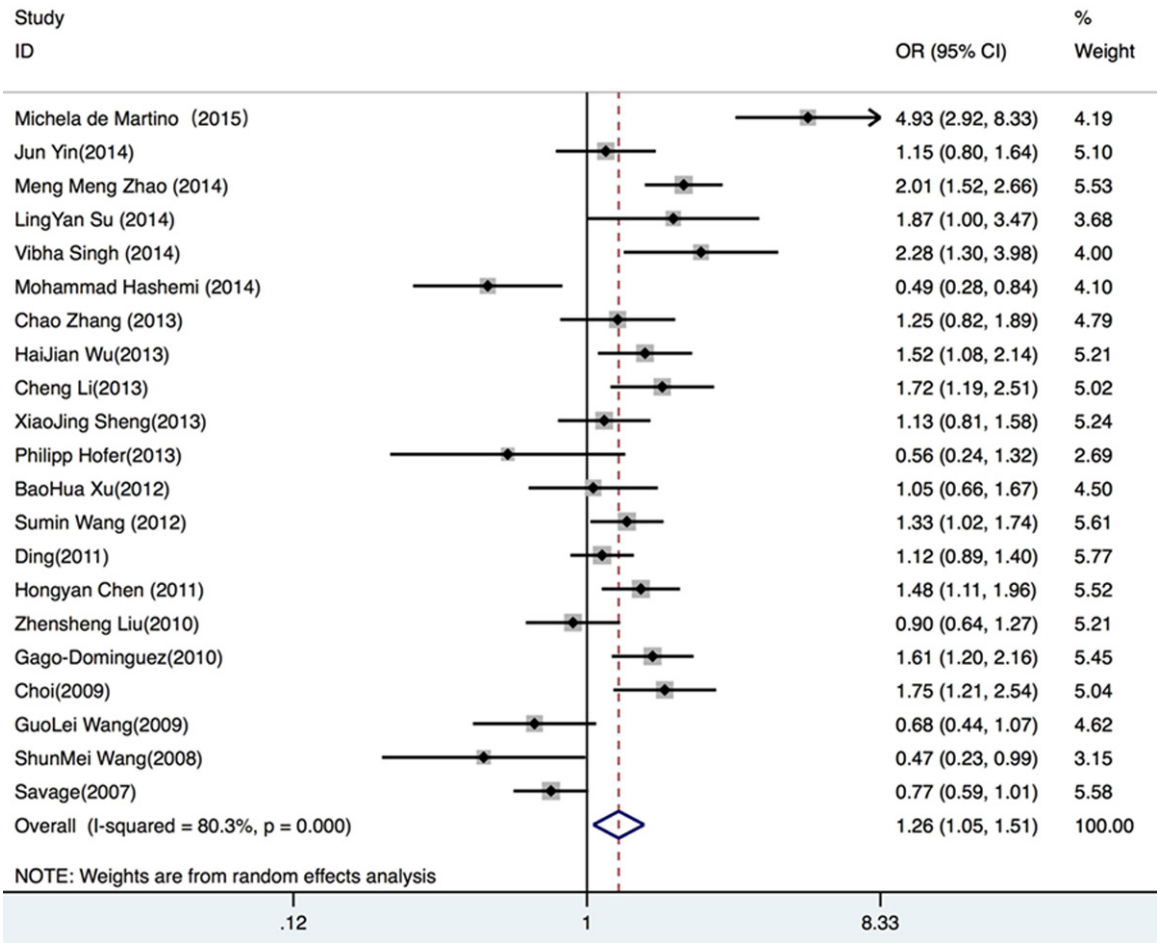

Figure S4. Forest plots of TERT polymorphism rs2736098 and cancer risks in the homozygote comparison.

Table S1. A summary of ORs for the overall and subgroup analyses of rs2736098 and cancer risk

Subgroup	Dominant model			Recessive model			Homozygote comparison		
	ORs	95% CI	*P*-value	ORs	95% CI	*P*-value	ORs	95% CI	*P*-value
Overall	1.134	1.009-1.274	0.034	1.188	1.038-1.361	0.013	1.258	1.047-1.512	0.014
Asian	1.16	1.040-1.293	0.473	1.298	1.154-1.461	<0.001	1.355	1.163-1.579	<0.001
Caucasian	1.117	0.826-1.509	0.007	0.932	0.655-1.326	0.693	1.047	0.596-1.841	0.011

Subgroup	Heterozygote comparison			Allelic model		
	ORs	95% CI	*P*-value	ORs	95% CI	*P*-value
Overall	1.099	0.985-1.226	0.092	1.111	1.016-1.215	0.02
Asian	1.105	0.992-1.231	0.071	1.154	1.066-1.248	<0.001
Caucasian	1.132	0.856-1.497	0.385	1.025	0.811-1.295	0.836

Abbreviations: ORs, odds ratios.