

RESEARCH ARTICLE

Association of TERT rs2736098 Polymorphism with Cancer Risk: a Meta-analysis

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Abstract

Studies have reported an association between the TERT rs2736098 single nucleotide polymorphism (SNP) and cancer susceptibility, but the results remain inconclusive. To provide a more precise estimation of the relationship, a meta-analysis of 8 published studies including 8,070 cases and 10,239 controls was performed. Stratification by sample size, genotyping method, source of controls and ethnicity were used to explore the source of heterogeneity. In the overall analysis, no significant association was found between the TERT rs2736098 polymorphism and cancer risk. However, the result showed the rs2736098 was significantly associated with an increased cancer risk and the heterogeneity was effectively decreased for homozygote comparison by removal of two studies: OR = 1.337 (95% CI = 1.183-1.511; P heterogeneity = 0.087). In the subgroup analysis by ethnicity, a significantly increased risk of cancers was found among Asians (OR = 1.413, 95% CI = 1.187-1.683 for AA versus GG). Our meta-analysis did not show that the TERT rs2736098 plays an important role in cancer risk. More studies with larger sample size and well-matched controls are needed to confirm the findings.

Keywords: TERT - GWAS - cancer susceptibility - meta-analysis

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Introduction

Cancer is a complex and multifactor disease that is thought to result from an interaction between genetic background and environmental factors (Pharoah et al., 2004). It has been suggested that low-penetrance susceptibility genes combined with environmental factors may be important in the development of cancer and are likely to modulate the effect of environmental risk factors.

Telomeres are specialized nucleic acid-protein complexes that protect chromosomes from degradation, end-to-end fusion, and atypical recombination; thus, telomeres play a key role in the maintenance of chromosomal stability (Blackburn, 1984). Telomerase, a ribonucleoprotein, consists of a telomere reverse transcriptase (TERT) and a telomere RNA component. Telomerase adds the telomeric repeat sequence directly to the single-strand 3' overhang to maintain telomere ends that have been incrementally shortened by each cell division. Enzymatic activity is absent in somatic cells but can be found in most cancer cells (Shay et al., 1997).

TERT gene is located on the short (p) arm of chromosome 5 at position 15.33 (5p15.33) which also containing another well-known gene, cleft lip and palate transmembrane 1 like (CLPTM1L). The sequence variants in the TERT and CLPTM1L gene regions have been implicated in carcinogenesis (McKay et al., 2008; Rafnar et al., 2009; Wang et al., 2010). TERT rs2736098, a synonymous coding single-nucleotide polymorphism (SNP) in exon 2 of TERT located on chromosome 5p15 (Gago-Dominguez et al., 2011), was found to be associated

with risk of cancers.

Recently, several genome-wide association studies (GWAS) have investigated the role of the TERT rs2736098 polymorphism in the etiology of cancers, but with inconclusive results. In this report, the aim was to estimate the effect of this polymorphism on cancer susceptibility as well as to explore sources of heterogeneity among the studies.

Materials and Methods

Search strategy and Study selection

PubMed and Embase were searched using following search terms: 'TERT' or telomere reverse transcriptase, 'polymorphism or variant', and 'cancer'. Related reference articles were searched to identify other relevant publications. Moreover, references of all included articles were screened. When more than one study of the same population was included in several publications, only the most recent or complete study was used in this meta-analysis (Liu et al., 2011). The following inclusion criteria were used for selecting studies: (i) evaluation of the rs2736098 polymorphism and cancer susceptibility; (ii) use a case-control design; and (iii) data was presented on genotype counts of cases and controls for TERT rs2736098 polymorphism. We excluded only case population studies, duplicates of earlier publication and no usable genotype frequency data studies.

Data extraction

From each eligible report, we recorded last name

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Table 1. Characteristics of the Literature Included in the Meta-analysis

First author	Year	Country	Cancer types	Source of controls	Ethnicity	Genotyping method	Cases			Controls		
							GG	GA	AA	GG	GA	AA
Hofer	2012	Austria	Colorectal cancer	Population	European	TaqMan	86	45	6	963	623	119
Wang	2012	China	Cervical cancer	Population	Asian	TaqMan	375	444	174	397	480	138
CY	2011	China	Hepatocellular carcinoma	Hospital	Asian	TaqMan	500	563	210	526	604	198
Chen	2010	China	Glioma	Hospital	Asian	PCR	351	461	141	430	486	117
Gago-	2011	USA	Bladder cancer	Population	European	TaqMan	217	189	43	278	210	43
Dominguze		China	Bladder cancer	Population	Asian	TaqMan	178	236	85	203	270	54
Liu	2010	USA	Head and neck cancer	Population	European	TaqMan	588	419	72	576	461	78
Choi	2009	Korea	Lung cancer	Population	Asian	PCR-RELP	311	322	87	345	320	55
Savage	2007	poland	Breast cancer	Population	European	TaqMan	1171	699	97	1313	811	141

PCR, polymerase chain reaction

of first author, year of publication, country of the study and ethnicity, source of control groups (population- or hospital-based controls), genotyping method and numbers of genotyped cases and controls. Different ethnic descents were categorized as European, Asian or mixed that included subjects of more than one ethnicity.

Statistical analysis

The strength of the association between the TERT rs2736098 polymorphism and cancer susceptibility was assessed by odds ratios (ORs) and 95% confidence intervals (CIs). The OR and the 95% CI in each comparison were assessed in a codominant model (AA versus GG; AG versus GG), a dominant model (AA + AG versus GG), a recessive model (AA versus AG + GG). The statistical significance of the pooled OR was determined using the Z-test. Pooled estimates of the OR were obtained by calculating a weighted average of OR from each study (DerSimonian, 2011; Liu et al., 2011). To assess the source of heterogeneity, stratified analyses were also performed based on by ethnicity of study population, the source of controls, sample size (subjects >500 in both cases and controls) and genotyping method.

Heterogeneity assumption was checked by the Q-test (Parmar et al., 1998). If the result of heterogeneity test was $P > 0.05$, we used a fixed-effects model with the Mantel-Haenszel method. If heterogeneity was present, a random effect model with the DerSimonian and Laird method was then used to account for inter-study heterogeneity instead (Moher et al., 2009). Potential publication bias was analyzed by Funnel plots and Egger's linear regression test. The statistical analysis was conducted by with Stata software (version 10.0; Stata Corp LP, College Station, TX), using two-sided P values.

Results

Summary of enrolled studies

According to our inclusion criteria, a total of 8 eligible studies involving 8070 cases and 10239 controls were included in the pooled analysis (Savage et al., 2007; Choi et al., 2009; Liu et al., 2010; Gago-Dominguez et al., 2011; Ding et al., 2011; Chen et al., 2011; Wang et al., 2012; Hofer et al., 2012). The characteristics of selected studies are summarized in Table 1. Genotyping was conducted using TaqMan assay for all studies except Chen et al. and Choi et al. There were 5 studies of Asians and 4 studies of Europeans. In addition, all controls matched for sex

Table 2. Stratified Analyses of the TERT rs2736098 Polymorphism on Cancer Risk, All Eligible Studies

Variables	n ^a	Odds Ratio	95% CI	P ^b
Overall	9	1.196	0.972-1.471	0.001
Sample size				
<500	3	1.221	0.714-2.086	0.048
≥500	6	1.17	0.928-1.476	0.001
Genotyping method				
TaqMan	7	1.092	0.869-1.374	0.005
PCR	2	1.573	1.256-1.970	0.468
Source of controls				
Population based	7	1.163	0.876-1.544	<0.001
Hospital based	2	1.267	0.964-1.666	0.132
Ethnicities				
Asian	5	1.413	1.187-1.683	0.143
European	4	0.881	0.686-1.131	0.208

^aNumber of comparisons; ^bP value of Q-test for heterogeneity

and age were population based with the exception of two studies by CY et al. and Chen et al. The distribution of genotypes in the controls of all studies was consistent with Hardy-Weinberg equilibrium but two studies (Gago-Dominguez et al., 2011; Ding et al., 2011).

Association between TERT rs2736098 and cancer risk

We found no significant association between rs2736098 polymorphism and cancer risk in any of genetic models. The results were as followed: AA versus GG (OR = 1.196, 95% CI = 0.972-1.471, $P_{\text{heterogeneity}} = 0.001$), AG versus GG (OR = 0.999, 95% CI = 0.937-1.066, $P_{\text{heterogeneity}} = 0.455$), AA/AG versus GG (OR = 1.026, 95% CI = 0.966-1.091, $P_{\text{heterogeneity}} = 0.058$), AA versus AG/GG (OR = 1.192, 95% CI = 0.989-1.435, $P_{\text{heterogeneity}} = 0.002$). There was significant heterogeneity for homozygote comparison (AA versus GG: $P_{\text{heterogeneity}} = 0.001$) and recessive model comparison (AA versus AG /GG: $P_{\text{heterogeneity}} = 0.002$). As shown in Table 2, we explored the source of high among-study heterogeneity for homozygote comparison by sample size, genotyping method, source of controls and ethnicity. In the subgroup analysis by ethnicity, statistically significantly increased risk was found among Asians for AA versus GG genotype (OR = 1.413, 95% CI = 1.187-1.683, $P_{\text{heterogeneity}} = 0.143$), but not among Europeans (OR = 0.881, 95% CI = 0.686-1.131, $P_{\text{heterogeneity}} = 0.208$). A forest plot of homozygote comparison on the bias of studies was shown in Figure 1. There was significant heterogeneity for studies with sample size ≥ 500 (OR = 1.17, 95% CI = 0.928-1.476, $P_{\text{heterogeneity}} = 0.001$), studies using TaqMan assay genotyping method (OR = 1.092,

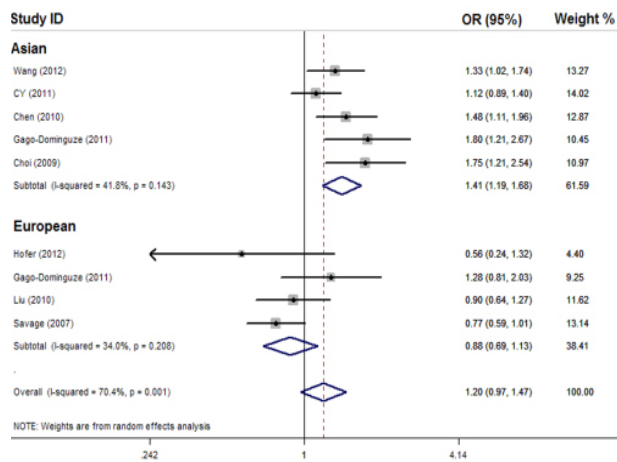


Figure 1. Forest Plot of TERT rs2736098 Polymorphism and Cancer Risk for Homozygote Comparison (AA versus GG)

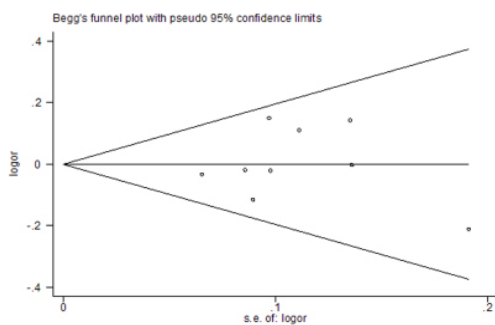


Figure 2. Funnel Plot of TERT rs2736098 Polymorphism and Cancer Risk for Heterozygote Comparison (AG versus GG) on the Basis of all Studies ($p = 0.772$)

95% CI = 0.869–1.374, $P_{\text{heterogeneity}} = 0.005$) and studies with population based controls (OR = 1.163, 95% CI = 0.876–1.544, $P_{\text{heterogeneity}} < 0.001$). Sensitivity analyses indicated that two independent studies by Savage et al. and Liu et al. were the main origin of the heterogeneity in homozygote comparison. Heterogeneity was effectively decreased and there was a significant association between rs2736098 polymorphism and cancer risk by exclusion of those studies: OR = 1.196 (95% CI = 0.972–1.471, $P_{\text{heterogeneity}} = 0.001$) and OR = 1.337 (95% CI = 1.183–1.511; $P_{\text{heterogeneity}} = 0.087$) before and after removal, respectively. No significant associations were detected upon repeating previous stratification analyses (Table 3). Furthermore, no other single study influenced the pooled OR qualitatively as indicated by sensitivity analyses, suggesting that the results of this meta-analysis are stable.

Publication bias

We performed Begg's funnel plot and Egger's tests to assess publication bias. The shape of the funnel plot seemed symmetrical in all comparison models. The Egger's test results also did not show any evidence of publication bias, indicating our results to be statistically robust ($P = 0.772$ for AG versus GG; Figure 2).

Discussion

TERT is only expressed in embryonic stem cells and germ cells as reported in previous studies (Pettigrew et

Table 3. Stratified Analyses of the TERT rs2736098 Polymorphism on Cancer Risk, All Eligible Studies

Variables	n ^a	Odds Ratio	95% CI	P ^b
Overall	7	1.337	1.183-1.511	0.087
Sample size				
<500	3	1.332	1.161-1.527	0.048
≥500	4	1.356	1.031-1.784	0.178
Genotyping method				
TaqMan	5	1.246	1.076-1.443	0.1
PCR	2	1.574	1.258-1.971	0.468
Source of controls				
Population based	5	1.423	1.202-1.685	0.111
Hospital based	2	1.247	1.044-1.490	0.132
Ethnicities				
Asian	5	1.376	1.209-1.565	0.143
European	2	1.029	0.697-1.520	0.094

^aNumber of comparisons; ^bP value of Q-test for heterogeneity test

al., 2012). As the catalytic subunit of telomerase, TERT is the most important determinant in the regulation of telomerase activity, and the telomerase activity is crucial in the elongation of telomere length in tumor cells. Activation of telomerase induced by TERT is a pivotal step during cellular immortalization and malignant transformation of human cells (Collins et al., 2002). Shorter telomeres have been associated with an increased risk of cancers including lung cancer, bladder cancer and other tumors (McGrath et al., 2007; Jang et al., 2008). This was consistent with a study by Baird et al. that suggested telomerase and the control of telomere length are intimately linked to the process of tumorigenesis in humans (Baird et al., 2010). TERT rs2736098 has been shown to be associated with telomere length but not with TERT expression (Rafnar et al., 2009). The biology of TERT makes it a compelling candidate gene for factors that influence cancer risk (Kyo et al., 2002) and the TERT gene has been recognized as one of the most common tumor markers.

It is well known that single nucleotide polymorphisms (SNPs) are the most common sources of human genetic variation, which may contribute to an individual's susceptibility to cancer. Previous conclusions of several GWAS on the association between TERT rs2736098 and cancer risk remain conflicting. To better examine the association between TERT rs2736098 and cancer susceptibility, we performed a comprehensive meta-analysis which includes 8070 cases and 10239 controls from 8 case-control studies

The results of our meta-analysis did not show significant association between the TERT rs2736098 polymorphism and cancer risk. According to the value of OR, rs2736098 genotype seems to increase cancer risk although not statistically significant. When stratifying the ethnicity, our results indicated that an increased risk was observed among Asians but not Europeans. There are several reasons for the inconsistent results. First, carcinogenesis is a multistep process involving multifactorial interplay between genetic and environmental factors. Environmental and lifestyles are very different among individuals of different races. Second, the difference in the linkage disequilibrium (LD) structure in the TERT region among different populations

was a more likely reason. Third, that maybe because the polymorphism site varied in different ethnicities (Deng et al., 2001) and certain effects of genetic polymorphisms are population specific.

To identify source of heterogeneity, an important goal of the meta-analysis, we stratified the studies according to sample size, genotyping method, source of controls and ethnicity. Two studies by Savage et al. and Liu et al. were disproportionately driving the apparent association and heterogeneity. Rs2736098 polymorphism was found to be associated with an increased risk of cancers and the heterogeneity was decreased in homozygote comparison after removing those studies from the meta-analysis.

Some limitations should be considered when we interpret the results. First, the numbers of published studies were not sufficiently large for a comprehensive analysis, publication bias might have occurred. Second, some other important SNPs that scan in the TERT-CLPTM1L region especially in high LD with rs2736098 or those in the similar biological pathways involved in the cancer risk are neglected. Third, lacking the original data of the reviewed studies limited our further evaluation of potential interactions, because the interactions among gene–gene, gene–environment and even different polymorphic loci of the same gene may modulate cancer risk. Our meta-analysis also had advantages. First, the quality of case-control studies included was satisfactory and met our inclusion criterion. Second, substantial numbers of cases and controls were pooled from different studies, which significantly increased the statistical power of the analysis.

In conclusion, our meta-analysis demonstrated that there was no significant association between rs2736098 polymorphism and cancer risk overall. However, the TERT rs2736098 was significantly associated with an increased cancer risk in Asians. Considering the limitations of the present meta-analysis, it is necessary to conduct further research with standardized unbiased methods, larger sample studies and well-matched controls.

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