Association between functional TERT promoter polymorphism rs2853669 and cervical cancer risk in South Indian women

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Abstract. A single nucleotide polymorphism (SNP) rs2853669 (A>G) in the telomerase reverse transcriptase (TERT) promoter has recently been reported in chr5:1,295,349 T>C (T349C), and was shown to be associated with increased cancer risk and poor survival in a specific population. However, at present, the role of this particular SNP with TERT promoter driver mutations and its genetic association with human papilloma virus (HPV) in patients with cervical cancer has not been determined. In the present study, the genetic association of the functional SNP rs2853669 in the presence/absence of TERT promoter hotspot mutations and HPV in patients with cervical cancer of South Indian origin was evaluated. To understand and compare the frequency of the variant allele and its risk association in different cancer types of various populations, the SNP was genotyped in 257 cervical cancer samples and 295 controls, and its associations with TERT promoter hotspot mutations and HPV were analyzed. Furthermore, an extensive search of previously published articles in PubMed, Embase and Web of Science was conducted; a meta-analysis was carried out to elucidate the association of the SNP with different cancer types in global populations. The SNP analysis showed significantly high frequency (41%) of homozygous variant allele rs2853669 (GG) in patients with cervical cancer compared with control samples [Recessive allele model odds ratio (OR)=1.71; 95% CI=1.20-2.43; P=0.003]. No significant interaction was observed between the TERT SNP rs2853669 and HPV status as well as other hotspot TERT promoter (C228T and C250T) mutations determined in our previous study. In addition, the overall meta-analysis revealed a significant association of the SNP rs2853669 with other cancer types in different ethnic populations (OR=1.09; 95% CI=1.03-1.16; P=0.004). The present results suggested that the TERT SNP rs2853669 could play an important role in the risk of cervical cancer in a South Indian population.

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Abbreviations: HPV, human papilloma virus; TERT, telomerase reverse transcriptase; ETS, E-twenty six

Key words: human papilloma virus, telomerase reverse transcriptase, cervical cancer, promoter single nucleotide polymorphism, rs2853669, polymorphism

Introduction

Telomeres are special structures at the ends of the chromosomes consisting of 'TTAGGG' tandem repeat sequences along with its associated protein complex called 'shelterin' (1). As a result of the inability of DNA polymerases to duplicate the ends of the linear DNA molecules, the lengths of the human telomeres get shortened to ~50 bp with each cell division and the attrition of telomere length is counteracted by telomerase reverse transcriptase (TERT), which is the catalytic component of the enzyme telomerase (2). It has been reported that TERT is reactivated in cancer cells and was found to be overexpressed in tumors (3). Several mechanisms are associated with TERT reactivation, including TERT promoter mutations or rearrangements, and copy number amplification and methylation (4). Cancer cells survive by exploiting the telomere maintenance mechanism. The details of cellular immortality through the telomere length maintenance are poorly understood in many human cancer types, including cervical cancer.

The prevalence of cervical cancer has increased rapidly in rural areas and overall it ranks second in both incidence and mortality rates in India, regardless of its incidental difference between rural and urban areas (5). In total, 95% of cases are caused by persistent infection with high-risk human papilloma

virus (HPV) (6). Persistent HPV infection and viral oncogene expression results in the inactivation of tumor suppressor genes, including tumor protein 53 and pRb, that consequently leads to increased genomic instability and accumulation of mutations, which often results in tumorigenesis (7). Cervical cancer remains a serious problem among women, especially in developing nations like India even after several decades of cervical cancer research that has identified various therapeutic regiments.

Recent advancement in next generation sequencing has enabled the whole genome sequencing of tumors with their paired normal controls. The Cancer Genome Atlas and International Cancer Genome Consortium studies revealed significant non-coding mutations and single nucleotide polymorphism (SNPs) in the regulatory regions of genes associated with tumorigenesis. The pan-cancer analysis of the whole genome aimed to analyze whole genomes of ~2,500 tumors and matched normal controls, mainly to identify driver mutations and to differentiate from passenger mutations (8). The previously identified driver mutations (C228T and C250T) in the TERT promoter were found to create a new binding site for the E-twenty six (ETS) family of transcription factors, resulting in the increased expression of TERT, which was initially observed in melanomas (9,10) and later in other cancer types (11). A previous study also observed the same functional TERT promoter mutations with high frequency (21.4%) in South Indian cervical cancer (12).

Previously, an SNP rs2853669 (A>G) in the TERT promoter was identified in chr5:1,295,349 T>C (T349C) and was shown to be associated with cancer risk in a different population (13-15). The presence of the SNP rs2853669 along with reactivating promoter mutations has been reported to increase the risk and shown to be associated with a poor survival rate in hepatocellular carcinoma (16). At present, the role of this particular SNP with TERT promoter driver mutations and its genetic association with HPV in patients with cervical cancer has not been determined. To understand the genetic association of the TERT SNP with cervical cancer risk, the present study focused on analyzing the SNP rs2853669 in cervical cancer samples from South Indian women and healthy controls. Furthermore, a meta-analysis of the SNP rs2853669 in various cancer types of world populations was conducted to elucidate the distribution and risk association of variant alleles with different types of cancer.

Materials and methods

Study design and subjects. The present study was conducted after obtaining approval from the Institutional Ethics Committee (approval no. 04092010) of Madras Medical College and Hospital (Chennai, India). The present study was conducted between January 2011 and June 2015. The tissue/blood samples were collected at The Institute of Social Obstetrics and Government Kasturba Gandhi Hospital for Women and Children (Chennai, India) and Government Royapettah Hospital (Chennai, India) after obtaining written informed consent from each patient. The present study included tissue samples from 257 patients with cervical cancer confirmed by histologic examination of biopsies and curettage specimens in pathology laboratory. For histologic classification, a two-tiered cervical intraepithelial neoplasia system was employed (17). The samples with no reported malignancy in the pathology were excluded. The biopsy samples were collected during the diagnosis of the patients and transported to the laboratory in RNAlater solution (Thermo Fisher Scientific, Inc.). The transported samples were homogenized and stored in RNAlater solution at 4˚C overnight. On the subsequent day, RNAlater solution was discarded and DNA was isolated from the tissues after PBS washes following a standard phenol-chloroform extraction method. In total, 295 healthy women were recruited as controls. About 2 ml of blood was collected in EDTA-coated vacutainers by venipuncture of the dorsal hand veins. Blood samples were also collected from patients with cervical cancer to sequence them to confirm the germline nature of the TERT SNP. The mean age of the patients and controls were 51.2±11.3 and 39.41±10.6 years, respectively. DNA was isolated from all the blood samples using a standard phenol-chloroform extraction and ethanol precipitation method (18).

SNP genotyping. To analyze the frequency of the SNP rs2853669 A>G in patients with cervical cancer and the controls, a hydrolysis probe-based allelic discrimination assay was performed (Thermo Fisher Scientific, Inc.). The PCR reaction was carried out in a total of 5 μl comprised of 10 ng DNA and 2.5 μl 2X TaqMan Universal PCR master mix (No UNG) and 0.125 μl 40X TaqMan SNP genotyping assay mix (Assay ID: C-8773290_10; Thermo Fisher Scientific, Inc.). The reaction was performed in QuantStudio 6 Flex Real-Time PCR (Thermo Fisher Scientific, Inc.) using a standard protocol (2 min at 50˚C, 10 min at 95˚C followed by 15 sec at 92˚C and 60 sec at 60˚C for 40 cycles) and the allelic discriminations were conducted by detecting the fluorescence in the PCR reactions. A control with no template was included in each plate. Genotype calls of 95% quality were scored using Sequence Detection Software v2.4.1 (Thermo Fisher Scientific, Inc.).

DNA sequencing. The germline nature of the identified TERT SNP was confirmed by sequencing 2% parallel blood DNA available from the patients with cervical cancer and the controls. The human TERT promoter region was amplified from cervical cancer tissue DNA samples and sequencing was performed, as previously described (12). The promoter-specific primers TERT forward: 5'-TGTAAAACGACGGCCAGT GGCCGATTCGACCTCTCTC-3' and reverse: 5'-CAGCGC TGCCTGAAACTCG-3' (the underlined sequence in the TERT F is the M13 universal sequencing primer) were used to amplify the TERT promoter. Briefly, the thermocycling conditions for PCR were 5 min at 95˚C once followed by 30 sec at 95˚C, 45 sec at 60˚C, and 45 sec at 72˚C for 10 cycles and 30 cycles of 30 sec at 95˚C, 45 sec at 60˚C and 30 sec (with 5 sec increases in each cycle) at 72˚C and final extension for 7 min at 72˚C. The PCR products were purified using a commercial kit (Qiagen, Inc.) and sequenced by the Sanger sequencing method (Macrogen, Inc.). The representative sequence for all three genotypes is presented in Fig. S1.

Statistical analysis. The genotype and allele frequency of the cervical cancer and control groups were estimated using the gene count method. Hardy-Weinberg Equilibrium (HWE)

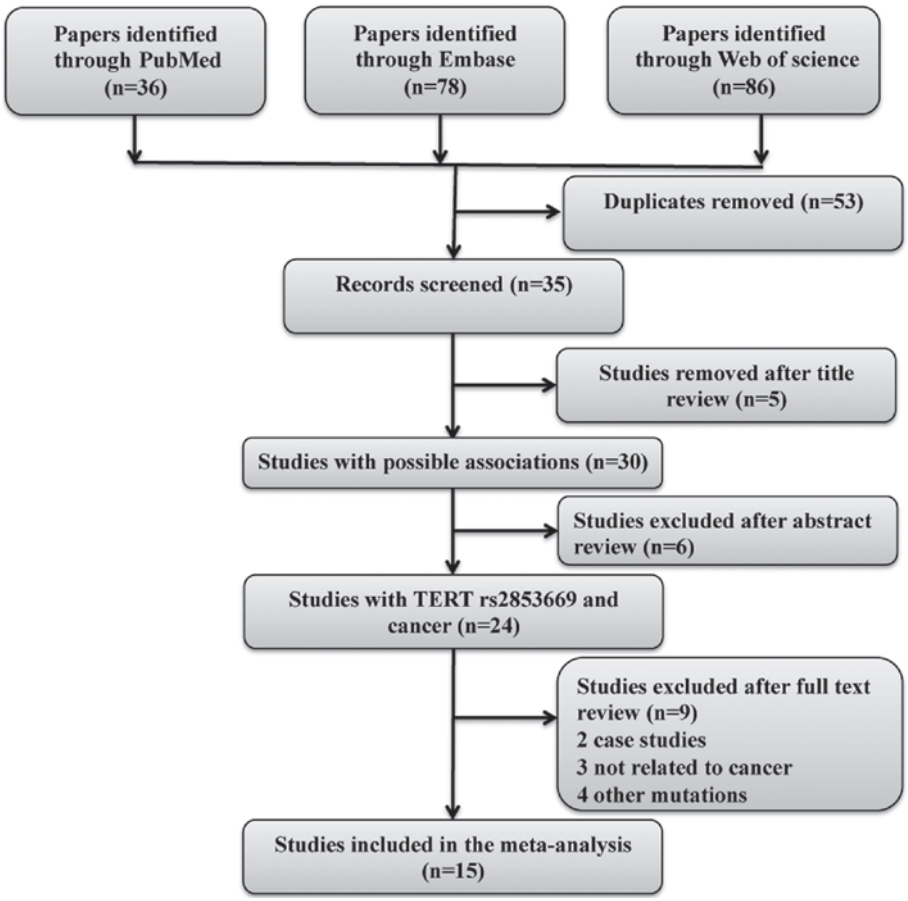

Figure 1. Flow chart shows the selection of eligible studies included in the meta-analysis. TERT, telomerase reverse transcriptase.

was assessed by the goodness-of-fit χ^2 test (19). The association between the polymorphism and cancer was analyzed using a χ^2 test. The odds ratios (ORs) with 95% CIs were calculated to evaluate the association between the rs2853669 polymorphism and cervical cancer risk, including variant heterozygous vs. wild-type homozygous model, and variant homozygous vs. wild-type homozygous model and dominant model. All tests were two-tailed and $P<0.05$ was considered to indicate a statistically significant difference. All the analyses were performed using SPSS, version 20.0. (IBM, Corp.).

Meta-analysis. Meta-analysis was performed to identify the distribution of rs2583669 variant allele across various types of cancers among different populations. An extensive literature search was conducted using PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), Embase (https://www.embase.com/) and Web of Science (https://clarivate.com/products/web-of-science/) databases (last search date January 10, 2019). The key words used in the search included 'TERT or telomerase reverse transcriptase', 'cancer or carcinoma' and 'rs2583669 polymorphism'. After the initial screening, the full text of all relevant papers was obtained and further filtered in order to fit into the inclusion criteria: i) Studies examining the association of TERT rs2583669 polymorphisms with cancer; ii) prospective case-control studies; and iii) studies with information to calculate ORs. Then, two authors independently extracted the genotype frequencies of the TERT rs2583669 polymorphism. This meta-analysis evaluated the association of TERT rs2583669 polymorphism with cancer risk according to the guidelines issued in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (20). A flow-chart describing the present study and the selection criteria for the meta-analysis is presented in Fig. 1. All meta-analyses were conducted using OpenMetaAnalyst software (21). All the previous studies included in the meta-analysis were evaluated for the HWE using a χ^2 test in the control groups and $P>0.05$ was considered as the sample neutrality. The carrier risk associations for rs2853669 were calculated in the allelic, dominant and recessive models using R (version 3.2.0) statistical software (https://cran.r-project.org/bin/windows/base/old/3.2.0/). The OR and 95% CIs were illustrated using Forest plot graphical representation. Furthermore, a sensitivity analysis was conducted by excluding one study in each analysis to examine the robustness of the method used for the meta-analysis. The potential publication bias was assessed by Begg's funnel plot and Egger's test (22).

Results

Frequency of rs2853669 in South Indian cervical cancer. In the present study, the promoter SNP rs2853669 (-245A>G) was genotyped in 552 samples, consisting of 257 cervical cancer samples and 295 controls from South India. The distribution of alleles along with various clinical variables is presented in Table I. The proportions of genotypes were 23.3% AA, 35.4% AG and 41.3% GG in the cervical cancer cases, and

Table I. Clinicopathological characteristics among the rs2853669 genotypes in patients with cervical cancer.

Clinicopathological characteristics	Total (%)	SNP rs2853669	
		AA (%)	AG+GG (%)
No. of cases	257	60 (23.3)	197 (76.7)
Age in years (mean ± SD)	51.2 ±11.3		
<51	138 (53.7)	29 (21.1)	109 (78.9)
≥51	116 (45.1)	31 (26.7)	85 (73.3)
Unknown	3 (1.2)	-	3 (100)
Tumor cell differentiation			
Well differentiated	54 (21)	11 (20.4)	43 (79.6)
Moderately differentiated	116 (45.1)	34 (29.4)	82 (70.6)
Poorly differentiated	52 (20.2)	10 (19.3)	42 (80.7)
Unknown	35 (13.6)	5 (14.7)	30 (85.7)
CIN grade			
IB1 or IB2	17 (6.6)	6 (35.3)	11 (64.7)
II or IIA2 and IIB	86 (33.5)	18 (20.9)	68 (79.1)
III or IIIB	58 (22.6)	17 (29.3)	41 (70.7)
IV or IVB	3 (1.2)	-	3 (100)
Unknown	93 (36.2)	19 (20.4)	74 (79.6)
Infiltration			
Infiltr\nfiltrated	208 (80.9)	50 (24.1)	158 (75.9)

SNP, single nucleotide polymorphism; AA, wild-type; GG+AG, carrier allele; CIN, cervical intraepithelial neoplasia.

21.4% AA, 49.5% AG and 29.1% GG in the controls (Table II). A higher incidence [41.25% (106/257)] of homozygous variant 'GG' was identified in the cervical cancer samples compared with the controls [29.15% (86/295)] in the study population, although without any statistical significance (P=0.265). The TERT rs2853669 polymorphism showed significant association with cervical cancer in the recessive model (GG vs. AA+AG; OR=1.71; 95% CI=1.20-2.43; P=0.003; Table II). The distribution of TERT rs2853669 polymorphism among TERT activating promoter mutations in our previous study (C228T and C250T) is documented in Table III (12). The individuals carrying rs2853669 variant allele (CT+CC) was found in 66.7% (20/30) of TERT promoter mutation-positive patients and this interaction did not increase the risk (OR=1.54; 95% CI=0.62-3.67; P=0.33). Furthermore, the distribution of the TERT rs2853669 polymorphism among patients with high-risk HPV also did not increase the risk of cervical cancer (OR=1.54; 95% CI=0.79-3.01; P=0.203; Table IV).

Meta-analysis results. Based on the inclusion criteria, a total of 17 studies from 14 previously published articles (15,23-35) along with the present study results were analyzed for the association between the TERT rs2583669 polymorphism and cancer susceptibility; this included 9,537 cancer cases and 12,370 controls (Table V). By pooling all the previous studies, a statistically significant association between the TERT rs2583669 polymorphism and cancer risk was identified (dominant model: Pooled OR=1.09; 95% CI, 1.03-1.16; P=0.004; Fig. 2). Further, stratification analyses were performed to assess the risk by type of cancer and ethnicity (Table SI). TERT rs2583669 polymorphism and cancer risk was not statistically significant in different ethnicities. Stratification analyses by type of cancer showed significant association of TERT rs2583669 polymorphism with acute myeloid leukemia, hepatocellular carcinoma and lung cancer (Table SI). Significant heterogeneity was observed in all genetic models tested (Table SI). Sensitivity analysis performed by omitting individual studies revealed that there was no change in the pooled ORs (Fig. 3). The shape of the funnel plot (Fig. 4) and Egger's tests did not reveal any evidence for asymmetry in all three genetic models (Table SI).

Discussion

TERT has been found to be overexpressed in 90% of human cancer (36). Furthermore, genetic alterations in the proximal promoter of TERT were shown to be significantly associated with a range of clinical stages of different cancer types (37). Recently, one of the mechanisms of TERT regulation through the non-coding driver mutations (C228T and C250T) in the TERT promoter has been reported in several cancer types with different frequencies (38-40). The mutations created a new binding site for the ETS family of transcription factors, resulting in the overexpression of TERT (9,10). However, the mechanism by which it overexpresses and the length of the telomere were reported to be different in different types of tumor. TERT has been shown to co-operate with activated oncogenes and inactivated tumor suppressor genes in

Table II. Association between TERT rs2853669 polymorphism and cervical cancer.

TERT SNP rs2853669 A>G	Cervical cancer (n=257) (Percentage frequency)	Control (n=295) (Percentage frequency)	OR	95% CI	P-value
Genotype					
AA	60 (23.3)	63 (21.4)		Reference	
AG	91 (35.4)	146 (49.5)	0.66	0.42-1.02	0.058
GG	106 (41.3)	86 (29.1)	1.29	0.82-2.04	0.265
Dominant model AG+GG vs. AA	197 (76.7)	232 (78.6)	0.89	0.60-1.33	0.575
Recessive model GG vs. AA+AG	106 (41.3)	86 (29.1)	1.71	1.20-2.43	0.003
Allelic model					
A	211 (41.1)	272 (46.1)		Reference	
G	303 (58.9)	318 (53.9)	1.23	0.97-1.56	0.091
HWE χ^2	18.51	0.005			
HWE P-value	<0.001	0.944			

ORs with the 95% CIs were calculated using SPSS. TERT, telomerase reverse transcriptase; SNP, single nucleotide polymorphism; OR, odds ratio; HWE, Hardy Weinberg Equilibrium; CI, confidence interval.

Table III. Association of TERT promoter mutations (C228T and C250T) with rs2853669 (-245T/C) in cervical cancer from our previous study (12).

	TERT mutation status			
SNP rs2853669T>C (n=140)	Wild-type	Mutated	OR (95% CI)	P-value
TT (37)	27	10	Reference	
CT+CC (103)	83	20	1.54 (0.62-3.67)	0.333

ORs with 95% CIs were calculated using SPSS. TERT, telomerase reverse transcriptase; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Table IV. Association of HPV and telomerase reverse transcriptase rs2853669 (-245T/C) in cervical cancer.

	HPV status			
SNP rs2853669T>C (n=257)	Positive (%)	Negative (%)	OR (95% CI)	P-value
TT (60)	46 (76.7)	14 (23.3)	Reference	
CT+CC (197)	134 (68.0)	63 (32.0)	1.54 (0.79-3.01)	0.203

ORs with 95% CIs were calculated using SPSS. HPV, human papilloma virus; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

tumorigenesis (41). In addition, viral oncogene expression and other genetic alterations, including mutations and SNPs in both coding and non-coding regions, were shown to play a major role in carcinogenesis (42). Advances in next generation sequencing resulted in the identification of several SNPs and mutations in non-coding regulatory regions, highlighting the role of genetic variants in the regulatory region of genes in tumorigenesis (8).

The TERT promoter SNP rs2853669 and its association with cancer risk has been reported in various cancer types in different populations (13-15). However, the role of this particular SNP with the TERT promoter driver mutations and its genetic association with HPV in patients with cervical cancer had not yet been studied, to the best of our knowledge. In the present study, to understand the genetic association of the TERT SNP with cervical cancer risk, the SNP rs2853669 was genotyped in 257 cervical cancer samples of South Indian origin and 295 control samples, and a higher frequency of the rs2853669 variant allele (G) was observed in the cervical cancer samples compared with the control samples. The SNP rs2853669 in the TERT promoter has been shown to have a functional impact on TERT regulation and telomere length

Table V. Telomerase reverse transcriptase rs2853669 and cancer association studies included in the meta-analysis.

					Cases			Control			HWE	
Author, year	Serial no.	Disease	Country	Ethnicity	CC	CT	TT	CC	CT	TT	P-value	(Refs.)
Present Study	1	Cervical cancer	India	Asian	106	91	60	86	146	63	0.944	-
Zhang *et al*, 2017	2	Gastric cancer	China	Asian	44	136	180	36	219	473	0.109	(23)
Xing *et al*, 2016	3	Lung cancer	China	Asian	41	162	215	30	145	235	0.249	(24)
Bayram *et al*, 2016	4	Gastric cancer	Turkey	Caucasian	13	47	44	35	99	75	0.810	(25)
Oztas *et al*, 2016	5	Breast cancer	Turkey	Caucasian	24	47	36	25	52	31	0.723	(26)
Jannuzzi *et al*, 2015	6	Colorectal cancer	Turkey	Caucasian	15	50	31	17	58	40	0.587	(27)
Mosrati *et al*, 2015	7	Glioblastoma	Sweden	Caucasian	11	48	69	65	341	373	0.293	(15)
Mosrati *et al*, 2015	8	AML	Sweden	Caucasian	38	99	89	65	341	373	0.293	(15)
Yoo *et al*, 2015	9	Lung cancer	Korea	Asian	137	477	478	105	490	485	0.242	(28)
Shadrina *et al*, 2015	10	NHL	Russia	Caucasian	35	213	272	71	322	491	0.079	(29)
Zhong *et al*, 2013	11	Lung cancer	China	Asian	108	242	148	72	224	206	0.381	(30)
Liu *et al*, 2011	12	SCCHN	USA	Caucasian	79	381	428	85	375	425	0.863	(31)
Shen *et al*, 2010	13	Breast cancer	USA	Caucasian	86	445	503	128	432	522	0.009	(32)
Park *et al*, 2010	14	HCC	Korea	Asian	35	121	134	68	110	99	0.001	(33)
Varadi *et al*, 2009	15	Breast cancer	Poland	Caucasian	58	299	411	38	154	244	0.059	(34)
Varadi *et al*, 2009	16	Breast cancer	Sweden	Caucasian	47	310	409	143	558	818	0.001	(34)
Savage *et al*, 2007	17	Breast cancer	Poland	Caucasian	1,095	766	124	1,224	900	158	0.669	(35)

AML, acute myeloid leukemia; NHL, non-Hodgkin lymphoma; SCCHN, squamous cell carcinoma of the head and neck; HCC, hepatocellular carcinoma; HWE, Hardy-Weinberg equilibrium.

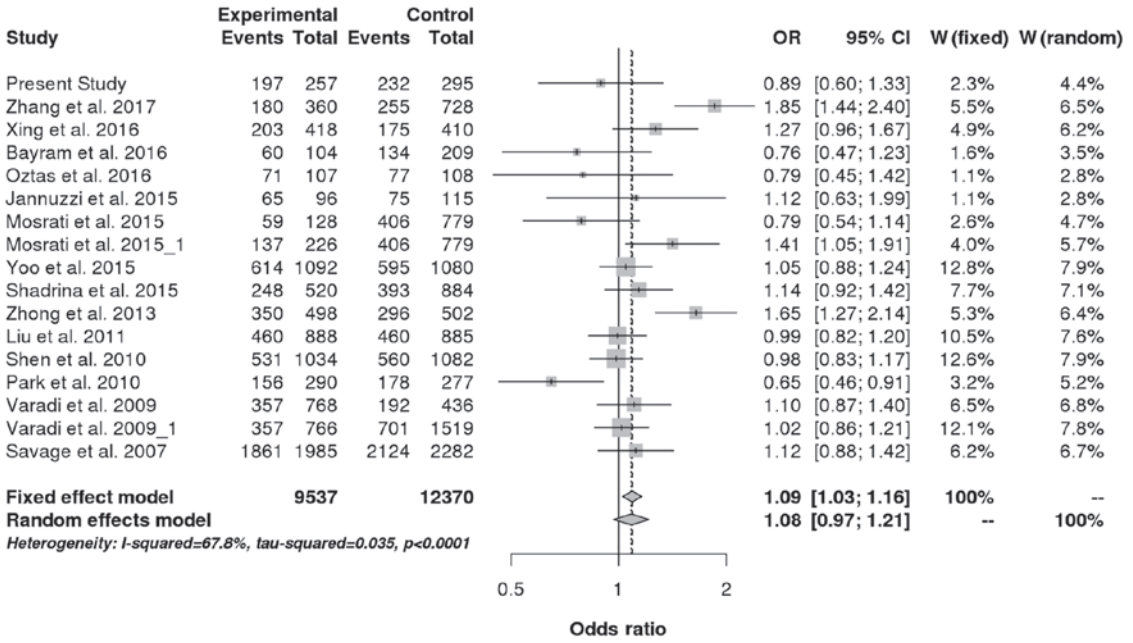

Figure 2. Meta-analysis for the association between various cancer types and telomerase reverse transcriptase rs2853669 polymorphism. Reference numbers of the respective studies are provided in Table V. The squares indicate the ORs in each study, with square sizes inversely proportional to the standard error of the OR. The diamond shape indicates the pooled ORs. Horizontal lines represent 95% CI. OR, odds ratio.

in various cancer types (34,35,43,44). Furthermore, the genetic association of the TERT SNP rs2853669 has been reported in many cancer types of different populations where the disease association was different between the population and the cancer types. The variant rs2853669 is located in the binding site of ETS-2, another transcription factor of the ETS family, which regulates various genes involved in cellular senescence and tumorigenesis (45,46). The results of either the SNP of TERT (rs2853669) alone or the modifying effect of rs2853669 on TERT hotspot promoter mutations have been shown to

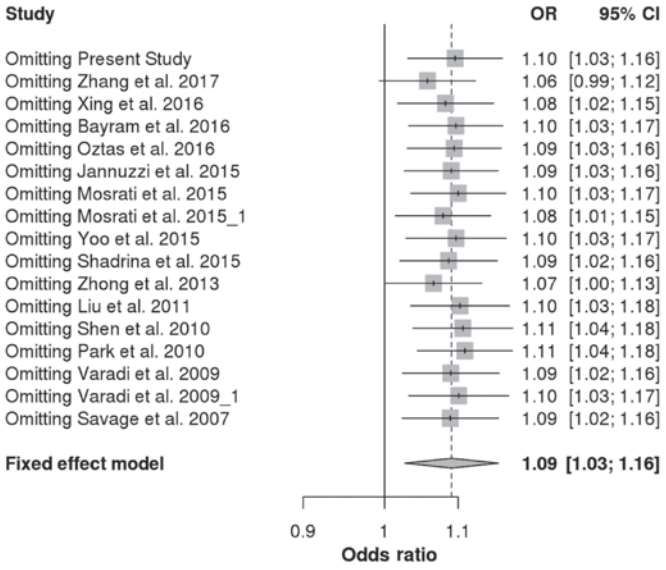

Figure 3. Sensitivity analysis of meta-analysis results of telomerase reverse transcriptase rs2853669 polymorphism. Sensitivity analysis was performed to generate a forest plot by excluding one of the studies in each time. Reference numbers of the respective studies are presented in Table V. The squares indicate the ORs after omitting each study and the diamond shape indicates the pooled ORs. Horizontal lines represent 95% CI. OR, odds ratio.

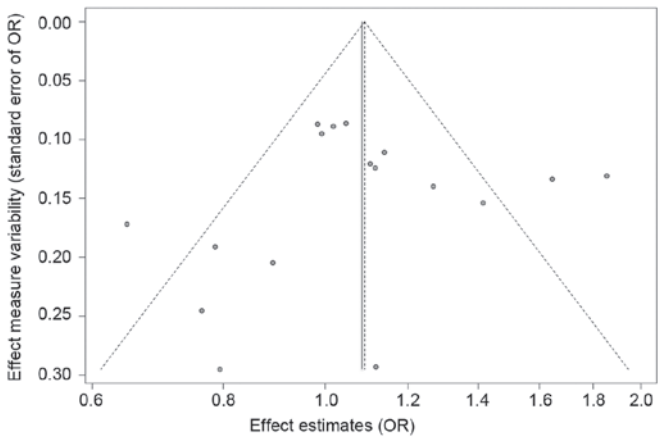

Figure 4. Funnel plot used in assessing publication bias in the meta-analysis of telomerase reverse transcriptase rs2853669. The dotted vertical line indicates the triangular region within which 95% of studies are expected to lie in the absence of bias and heterogeneity. The solid vertical line corresponds to no intervention effect. OR, odds ratio.

be highly controversial with varying results across different previous studies (15,31,34,35,44,47,48).

The combination of the rs2853669 variant allele (G) and TERT promoter hotspot mutations was also high compared with hotspot mutations and the wild-type allele. Almost all the patients with the rs2853669 and hotspot mutations were observed in poorly differentiated squamous cell carcinoma tumors except one patient where it was a moderately differentiated tumor (49). It has been reported that the TERT hotspot mutation-positive cases showed poor survival in the absence of the variant allele (47). On the contrary, previous studies on glioblastoma showed a shortest mean overall survival, which was mainly detected in patients harboring both an activating TERT promoter mutation and the rs2853669 variant homozygous allele (14,15). Moreover, the role of rs2853669 with or without TERT hotspot mutations on TERT regulation and activation has been demonstrated through functional experiments in hepatocellular carcinoma (HCC) cell lines. E2F1 binding to the TERT promoter can enhance interaction with other epigenetic modifiers like DNMT1 and HDAC1 to enable promoter methylation-mediated regulation of TERT transcription, which suggested that E2F1 could potentially function as a transcriptional repressor of TERT (16). The SNP (rs2853669) may interfere with the binding of E2F1 and influence promoter methylation associated with the TERT transcription as the SNP resides at 2 bp downstream of E2F1 binding site on the TERT promoter (16). Recently, the combination of -124 C>T (C228T) mutation and rs2853669 (-245T>C) variant was reported to be correlated with increased TERT transcription activity in HCC cases that frequently resulted in higher risk of recurrence (16). The increased mortality in HCC has been observed in cases with the co-existence of the two hotspot TERT promoter mutations and SNPs (16). The expression level of TERT is increased by the inhibition of the transcriptional repressor E2F1 in the presence of the variant allele -245T>C, together with the activation of the ETS2 transcription factor due to the -124C>T mutation. A recent study reported that the -245T region located on an ETS2 binding site is not a native ETS2 binding site, suggesting that the TERT promoter mutation (-124C>T and -146C>T) cooperates with its native ETS binding sites to form high-order structures such as G-quadruplexes, contributing to the recruitment rs2853669 with the non-coding driver hotspot mutation in the TERT promoter has been shown to be associated with poor prognosis in HCC (16). Furthermore, the present results suggested that the hotspot TERT promoter mutations and the SNP combinations might play an important role in TERT regulation. In addition, the expression of TERT was shown to be regulated by HPV E6 oncoprotein (50,51). The combination of the rs2853669 variant allele and TERT promoter hotspot mutation (C228T and C250T) along with HPV could be an additional risk to patients with cervical cancer. However, the present study did not identify any significant association between the TERT SNP (rs2853669) and the presence of HPV.

In addition, to understand and compare the genotype frequency and the risk associated with the SNP rs2853669 among various cancer types and different populations, a meta-analysis was conducted. Due to the lack of previous studies on the association of rs2853669 and cervical cancer risk in different populations, a meta-analysis using different cancer types of the world population was conducted. In the present study, the variant allele 'G' showed a significantly increased association with cancer risk in the dominant model. This result was consistent with previous studies reported from other human cancer types and this result was also obtained in the present meta-analysis (14,28,29,46). Overall, the present meta-analysis showed a significant risk association of the SNP rs2853669 with various cancer types of the different ethnic population. In contrast, Shen *et al* (52) reported that the SNP had no association with cancer risk and prognosis. The number and ethnic origin of the samples could influence the outcome of the results. However, when the SNP was combined with TERT promoter mutations, a modifying

effect of rs2853669 among patients with cancer with TERT promoter mutations was observed and only those patients carrying the TT genotype had a poor survival (52). Although the effect of both the SNP and the TERT promoter mutations were analyzed in the present study, it was not possible to identify an association between the TERT SNP with the clinicopathological features of the patients with cervical cancer, as the majority of the patient history showed poor follow up and/or drop out from the treatments. Moreover, all previous genome-wide association studies (GWAS) showed an association of this SNP with telomere length (53). To the best of our knowledge, no previous GWAS study has reported the association of SNP with cancer risk/clinical features, although many individual previous studies have reported the association of this SNP with cancer risk across human cancer but not in cervical cancer (14-16,33).

In conclusion, the present study suggests that the TERT rs2853669 variant 'GG' may play a role in the progression of cervical cancer in South India as well in different cancer types of world populations. The present study enrolled female patients from a relatively homogenous population of South India; however, the limitations of the present study should be considered when interpreting the results. A major limitation of the present study is the collection of tissue from cases and blood samples from controls for DNA extraction and genotyping. Furthermore, a number of demographic and clinicopathological characteristics limited the present study. Owing to inadequate data, the impact of demographic and clinicopathological characteristics was not considered to perform multivariate analysis. Furthermore, non-availability of the data on *Helicobacter pylori* infection, alcohol consumption and smoking, limited the evaluation of the potential interactions between these risk factors and TERT rs2853669 polymorphism. In conclusion, within the limitations, the present study provides an insight on the significance of genetic variants present in the non-coding regions of genes and their association with the hotspot mutations. However, functional studies are warranted to establish the role of rs2853669 in cervical carcinogenesis.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

AKMun conceived the study, designed the experiments and supervised the study. VV and SR contributed to the study design, performed the experiments, analyzed the data and drafted the manuscript. KA and GA helped conduct the experiments and drafted the manuscript. RR provided tumor samples and clinical data. AKMur, LVKSB and AKMun analyzed the data and critically reviewed the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted after obtaining approval from The Institutional Ethics Committee (approval no. 04092010) of Madras Medical College and Hospital (Chennai, China). The tissue/blood samples were collected from The Institute of Social Obstetrics and Government Kasturba Gandhi Hospital for Women and Children, Triplicane, Chennai and Government Royapettah Hospital after obtaining written informed consent from each patient.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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