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ORIGINAL RESEARCH

Association of telomerase reverse transcriptase promoter mutations with clinicopathological features and prognosis of thyroid cancer: a meta-analysis

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Abstract: The clinicopathological and prognostic significance of telomerase reverse transcriptase (*TERT*) promoter mutations have been widely investigated in thyroid cancer; however, the results are still discrepant. Systematic searches were performed in PubMed, Web of Science, Scopus, Ovid, and the Cochran Library databases for relevant articles prior to April 2016. Mutation rates were synthesized by R statistical software. The odds ratio or standardized mean difference with 95% confidence interval was pooled by Stata. A total of 22 studies with 4,907 cases were included in this meta-analysis. *TERT* promoter mutations tended to present in aggressive histological types including poorly differentiated thyroid cancer (33.37%), anaplastic thyroid cancer (38.69%), and tall-cell variant papillary thyroid cancer (30.23%). These promoter mutations were likely to exist in older patients and males and were well associated with larger tumor size, extrathyroidal extension, vascular invasion, lymph node metastasis, distant metastasis, advanced tumor stage, disease recurrence/persistence, and mortality. In addition, *TERT* promoter mutations (especially C228T) tended to coexist with $BRAF^{V600E}$ mutation, which indicated more aggressive tumor behavior. Therefore, *TERT* promoter mutations may be promising biomarkers for early diagnosis, risk stratification, prognostic prediction, and management of thyroid cancer.

Keywords: *TERT* promoter mutations, thyroid cancer, clinicopathological features, prognosis, $BRAF^{V600E}$ mutation

Introduction

Telomerase, a RNA-dependent DNA polymerase, adds repeat segments to the end of linear chromosomes stabilizing the length of telomere and enabling the immortalization of malignant cells.[1] Telomerase reverse transcriptase (TERT) is a rate-limiting catalytic subunit of telomerase complex taking part in telomerase reactivation and telomere elongation.[2,3] Overexpression of TERT and activation of telomerase are found in various malignancies, which are linked to cancer hallmarks including proliferation, antiapoptosis, angiogenesis, invasion, and metastasis.[4,5] Two mutations in −124 bp (chr5: 1,295,228; termed C228T) and −146 bp (chr5: 1,295,250; termed C250T) upstream from the translation start site of *TERT* gene have been identified in melanomas[6,7] and have further been found in glioma,[8,9] liposarcoma,[9] urothelial carcinoma,[8,10] hepatocellular carcinoma,[8,11] and thyroid cancers.[12–17] Functional research studies showed that *TERT* promoter mutations enhanced the transcriptional activity of the *TERT* promoter, which highly upregulated the mRNA level and increased telomerase activity.[8]

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This may be because both mutations generate novel binding sites (GGAA/T) for E-twenty-six (ETS) transcription factors and enhance the transcriptional level, which provides an alternative mechanism of TERT activation.[6–8]

Thyroid cancer is the most common endocrine malignancy, with an increasing incidence in the last few decades.[18,19] Among the follicular-cell-derived thyroid cancer (FCDTC), papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC) are well-differentiated and classified as differentiated thyroid cancer (DTC),[20] while anaplastic thyroid cancer (ATC) is undifferentiated with limited survival of <6 months.[21] Another rare histological type is medullary thyroid cancer (MTC) originating from parafollicular or C cells. Development and progression of thyroid cancer are accompanied by accumulation of genetic and epigenetic alterations which vary from different histological types of thyroid cancer. The aberrant activation of *RET* signaling is the primary mechanism of MTC, while MAPK pathway (mainly triggered by $BRAF^{V600E}$ mutations) and PI3K pathway (which can be caused by mutations in *RAS*, *PTEN*, and *PIK3CA*), respectively, lead to PTC and FTC.[20] *TERT* promoter mutations also participate in the carcinogenesis of thyroid cancer, and the frequency ranges from 9% to 37% in different studies.[12–14,16,17,22] Although the clinicopathological and prognostic significance of *TERT* promoter mutations have been investigated in various research studies,[14–17,22,23] no consistent conclusion has been achieved.[15,24–26] Besides, some researchers have reported that the coexistence of *BRAF* and *TERT* promoter mutations contributes to more aggressive tumor and worse outcome;[15,23,27] however, other researchers have reported contrary results.[24,28]

Therefore, this meta-analysis was conducted to clarify the distribution of *TERT* promoter mutations in different histological types of thyroid cancer and then analyze their association with high-risk clinicopathological features, adverse outcomes, and $BRAF^{V600E}$ mutation. Furthermore, the practical values of *TERT* promoter mutations in preoperative diagnosis, risk stratification, prognostic prediction, and therapeutic option were evaluated.

Materials and methods

Search strategy and selection criteria

Systematic searches were performed in PubMed, Web of Science, Scopus, Ovid, and the Cochran Library databases for relevant studies before April 2016. The search terms were: ((thyroid cancer) or (thyroid neoplasm) or (thyroid tumor)) and ((TERT) or (telomerase reverse transcriptase)). Relevant articles and reviews were also inspected for additional studies. Studies were included according to the following criteria: 1) detecting *TERT* promoter mutations in thyroid cancer; 2) data availability of mutation rate, clinicopathological features, prognosis, or $BRAF^{V600E}$ mutation; and 3) evaluation of the summary odds ratio (OR) or standardized mean difference (SMD) with 95% confidence interval (CI). Studies were excluded based on these criteria: 1) review, case report, editorial, or comments; and 2) research studies with repeated or unusable data.

Data extraction and quality assessment

Details including first author, year of publication, country, number of centers, study design, number of participants, histological type of thyroid cancer, mean age, gender, sample source, sequencing method, cases and duration of follow-up, $BRAF^{V600E}$ mutation, clinicopathological features (mean diameter of tumor, extrathyroidal extension, vascular invasion, distant metastasis, lymph node metastasis, and tumor stage), and adverse outcome (persistence/recurrence and disease specific mortality) were obtained from the studies. Tumor stage was standardized by the tumor-node-metastasis (TNM) system of the American Joint Committee on Cancers.[25] Persistence/recurrence was defined as the presence of abnormality confirmed by pathology. The quality of studies was assessed by two investigators according to the Newcastle–Ottawa scale (NOS) comprising three dimensions: four scores for subject selection, two scores for subject comparability, and three scores for prognostic assessment.[26] Studies with >7 scores were regarded as high quality, 4–6 scores were mid-range, and ≤3 were low quality.

Statistical analysis

Mutation frequencies were synthesized by R statistical software (version 3.2.1; R Foundation for Statistical Computing, Vienna, Austria). OR and SMD, respectively, quantify the association between *TERT* promoter mutations and dichotomous variables (gender, extrathyroidal extension, vascular invasion, lymph node metastasis, distant metastasis, stage, recurrence/persistence, mortality, and $BRAF^{V600E}$ mutation) and continuous variables (age and tumor size). Pooled OR and SMD with 95% CI were achieved by STATA (version 12.0; Stata Corporation, College Station, TX, USA). The potential heterogeneity was evaluated by Cochrane Q test and inconsistency index (I^2). I^2>50% suggested significant heterogeneity and so random effects model (DerSimonian–Laird method) was chosen; otherwise, fixed effects model (Mantel–Haenszel method) would be considered.[29] Continuous data were pooled by Cohen method for SMD when the

number of studies >10 (both fixed effects model and random effects model). For all analyses, $P<0.05$ was regarded as statistically significant.

Results

Search results and quality assessment

A flowchart of the literature research is shown in Figure 1. A total of 1,106 articles were initially included. After removal of the duplicates, 894 studies remained. Then, 854 studies were excluded after reviewing the titles and abstracts. Full-text of the remaining 40 studies were further evaluated, and 22 studies with 4,907 patients were ultimately included in this meta-analysis.[12–17,22,23,27,30–42] All the 22 studies reported the frequency of *TERT* promoter mutations,[12–17,22,23,27,30–42] 18 studies were available for analyzing the clinicopathological features and prognostic significance,[14–17,22,23,27,31–33,35–42] 15 studies investigated the relationship of *TERT* promoter and $BRAF^{V600E}$ mutations,[13,15,16,22,23,27,33–37,39–42] and six of them evaluated the synergetic effect of both mutations.[15,24,25,35,37,41] According to the NOS system, 11 studies were classified as high-quality and the other 11 were mid-range. Main characteristics and methodological quality of all the 22 studies are listed in Table 1 according to the publication year. The structures of *TERT* core promoter and BRAF protein kinase are shown in Figure 2.

Distribution of *TERT* promoter mutations in thyroid cancer

Table 2 summarized the distribution of *TERT* promoter mutations in different histological types of thyroid cancer. Random effects model was used in the analysis with obvious heterogeneity ($I^2>50\%$); otherwise, the fixed effects model was chosen. The *TERT* promoter mutations only existed in FCDTC, but were absent in MTC and benign lesions (data not shown). Two types of *TERT* promoter mutation (C228T and C250T) were mutually exclusive. Besides, C228T (0.1126; 95% CI 0.0820–0.1433) was more common than C250T (0.0271; 95% CI 0.0174–0.0368). Their frequencies in poorly differentiated thyroid cancer (PDTC) (0.3337; 95% CI 0.2068–0.4606) and ATC (0.3869; 95% CI 0.2866–0.4872) were three times higher than that in DTC (0.1091; 95% CI 0.0819–0.1363). And the rate in FTC (0.1703; 95% CI 0.1277–0.2128) was nearly twice in PTC (0.0941; 95% CI 0.0716–0.1165). Among the subcategories of PTC, tall-cell PTC (TCPTC, 0.3023; 95% CI 0.1650–0.4396) harbored especially higher rate than conventional (0.0342; 95% CI 0.0362–0.1490) and follicular variant (0.0809; 95% CI 0.0207–0.1824) PTCs.

Clinicopathological and prognostic significance of *TERT* promoter mutations

Age, gender, tumor size, extrathyroidal extension, vascular invasion, lymph node metastasis, distant metastasis, tumor stage, persistence/recurrence, and mortality were obtained from the studies 11, 16, 7, 8, 4, 14, 8, 12, 8, and 5, respectively. Fixed effects model was used in the analysis of gender, vascular invasion, persistence/recurrence, and mortality, while random effects model was chosen for the other analyses.

As shown in Figure 3, *TERT* promoter mutations tended to present in older patients (SMD 0.79; 95% CI 0.61–0.96)

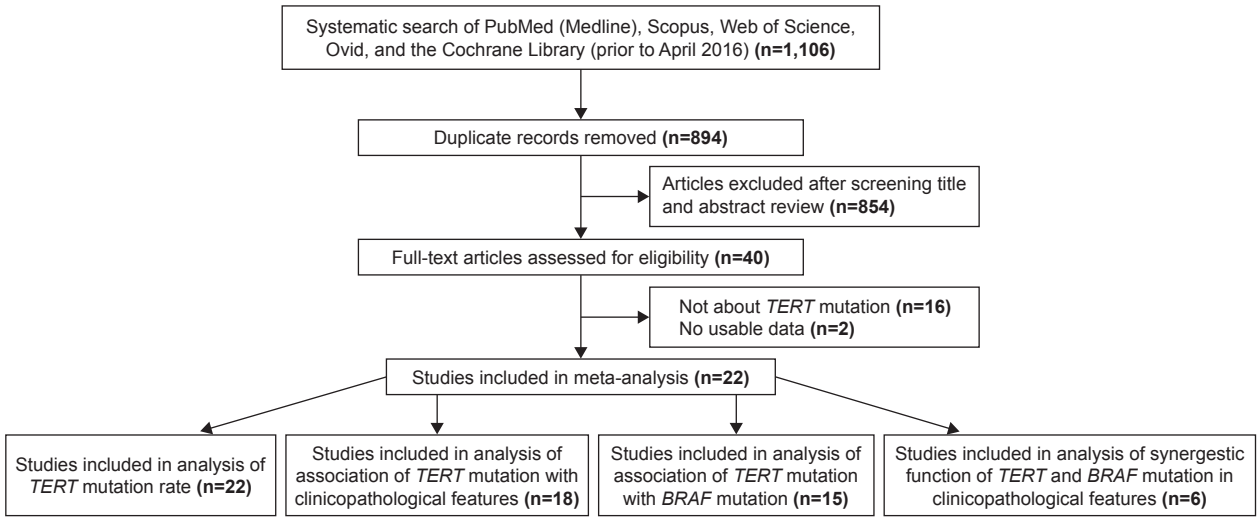


Figure 1 Flowchart of study selection process.
Abbreviation: TERT, telomerase reverse transcriptase.

Table 1 Characteristics and methodological quality of 22 studies included in the meta-analysis

Study	Year	Country	Study design	Number of centers	Number of cases	Histotype	Mean age (years)	Sex (F/M)	*TERT* mutation			Follow-up		Quality (NOS)
									Sample source	Sequencing method	Mutation type	Cases	Duration (months)	
Landa et al[12]	2013	USA	Retro	Multiple	183	TC	NA	NA	Surgical specimen	Direct sequencing	C228T + C250T	NA	NA	4
Liu et al[13]	2013	USA	NA	NA	414	TC	NA	NA	Surgical specimen	Direct sequencing	C228T + C250T	NA	NA	6
Vinagre et al[17]	2013	Portugal	Retro	Multiple	263	TC	48	189/61	FFPE	Direct sequencing	C228T + C250T	NA	NA	6
Liu and Xing[30]	2014	USA	Pro	Single	129	DTC	NA	NA	FNAB	Direct sequencing	C228T + C250T	NA	NA	5
Liu et al[14]	2014	Sweden	Retro	Single	144	TC	NA	NA	Surgical specimen	Direct sequencing	C228T + C250T	51	NA	9
Liu et al[15]	2014	USA	Retro	Multiple	430	DTC	NA	NA	FFPE	Direct sequencing	C228T + C250T	NA	NA	5
Melo et al[16]	2014	Portugal	Retro	Multiple	469	FCDTC	48.2	342/117	FFPE	Direct sequencing	C228T + C250T	469	93.6	8
Wang et al[31]	2014	Sweden	Retro	Single	52	FTC	53.19	33/19	Surgical specimen	Direct sequencing	C228T + C250T	52	111	8
Xing et al[23]	2014	USA	Retro	Single	507	PTC	45.9	365/142	FFPE	Direct sequencing	C228T	507	24	8
Crescenzi et al[32]	2015	Italy	Retro	Multiple	30	PTC	NA	NA	CNB	Direct sequencing	C228T + C250T	NA	NA	5
De Biase et al[33]	2015	Italy	Retro	Multiple	404	PTC	NA	294/82	FFPE	NGS	C228T + C250T	306	58	8
Dettmer et al[34]	2015	Switzerland	Retro	Multiple	125	PTC	NA	91/34	Surgical specimen	Direct sequencing	C228T + C250T	NA	NA	5
Gandolfi et al[27]	2015	Italy	Retro	Single	121	PTC	NA	83/38	FFPE	Direct sequencing	C228T + C250T	121	NA	8
Muzza et al[35]	2015	Italy	Retro	NA	182	DTC	NA	171/69	Surgical specimen	Direct sequencing	C228T + C250T	240	78.9	9
Qasem et al[22]	2015	Saudi Arabia	Pro	NA	265	DTC	34	201/64	FFPE	Direct sequencing	C228T + C250T	244	6–12	9
Shi et al[36]	2015	USA	Retro	Multiple	106	ATC	63.7	65/41	FFPE	Direct sequencing	C228T + C250T	NA	NA	6
Bullock et al[37]	2016	Australia	Pro	Single	80	PTC	47.3	66/14	FFPE	Direct sequencing	C228T + C250T	80	106	8
Myung et al[38]	2016	Korea	Retro	Single	74	PTC	48	57/17	FFPE	Direct sequencing	C228T + C250T	74	NA	8
Bae et al[39]	2016	Korea	Retro	Single	222	DTC	NA	173/49	FFPE	Direct sequencing	C228T + C250T	222	NA	5
Jeon et al[40]	2016	Korea	Pro	Single	35	PTC	45	28/7	Surgical specimen	Direct sequencing	C228T	35	66	8
Jin et al[41]	2016	China	Retro	Single	653	PTC	46.5	503/150	FFPE	Direct sequencing	C228T + C250T	NA	NA	5
Sohn et al[42]	2016	Korea	Retro	Single	19	DTC	59.79	13/6	Surgical specimen	NGS	C228T + C250T	19	65.95	5

Abbreviations: Retro, retrospective; Pro, prospective; TC, all the histological types of thyroid cancer; DTC, differentiated thyroid cancer; FCDTC, follicular-cell-derived thyroid cancer; FTC, follicular thyroid cancer; PTC, papillary thyroid cancer; ATC, anaplastic thyroid cancer; FFPE, formalin-fixed, paraffin-embedded tissues; FNAB, fine-needle aspiration biopsy; CNB, core-needle biopsy; NA, not available; NGS, next-generation sequencing; NOS, Newcastle–Ottawa scale.

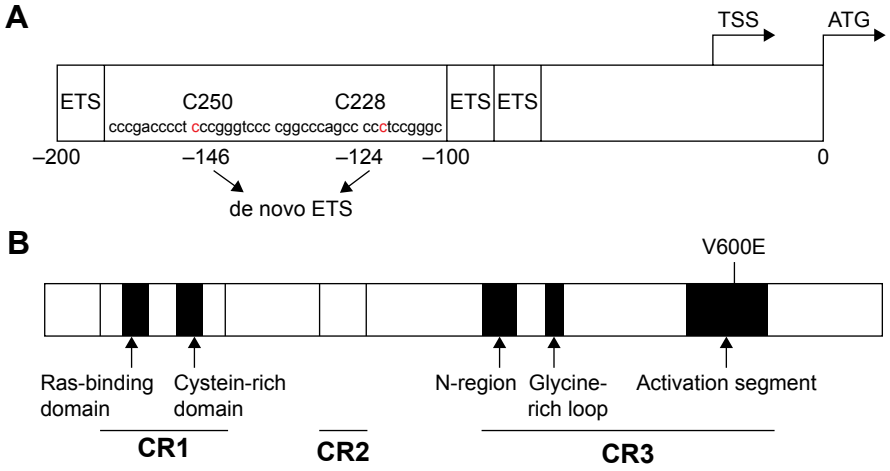


Figure 2 Schematic representation of TERT core promoter and BRAF protein kinase.
Notes: (**A**) The C228T and C250T mutations are located in −124 bp and −146 bp upstream from the TSS, which induce novel ETS binding sites. (**B**) There were three conserved regions (CR1, 2, 3) in the BRAF protein kinase. CR1 and CR2 are regulatory domains and CR3 is the catalytic domain. RBD and CRD are located in CR1. The N-region, glycine-rich loop, and activation segment are located in CR3. $BRAF^{V600E}$ is located in the activation segment.
Abbreviations: TERT, telomerase reverse transcriptase; TSS, translation start site; ETS, E-twenty-six; RBD, Ras-binding domain; CRD, cystein-rich domain.

and males (OR 1.64; 95% CI 1.31–2.05). Besides, they were relevant to larger tumor size (SMD 0.67; 95% CI 0.31–1.04), extrathyroidal extension (OR 2.86; 95% CI 1.68–4.86), vascular invasion (OR 1.81; 95% CI 1.22–2.68), lymph node metastasis (OR 1.80; 95% CI 1.11–2.91), distant metastasis (OR 8.19; 95% CI 4.11–16.32), and advanced tumor stage (OR 5.39; 95% CI 2.90–10.00). They also indicated adverse outcomes including tumor persistence/recurrence (OR 3.75; 95% CI 2.58–5.45) and disease-related mortality (OR 8.39; 95% CI 4.13–17.03).

Relationship of *TERT* promoter and $BRAF^{V600E}$ mutations

As shown in Figure 4, *TERT* promoter mutations were likely to occur in $BRAF^{V600E}$-positive thyroid cancer (OR 1.88; 95% CI 1.41–2.51), which was especially obvious in C228T (OR 2.53; 95% CI 1.77–3.62) rather than C250T mutation (OR 0.64; 95% CI 0.13–3.06). Random effects model was used to evaluate the association between $BRAF^{V600E}$ and C250T mutations.

Patients harboring both *TERT* promoter and $BRAF^{V600E}$ mutations tended to be male (OR 3.71; 95% CI 1.66–8.29) and have larger tumor size (SMD 0.80; 95% CI 0.24–1.35), extrathyroidal extension (OR 5.85; 95% CI 2.14–16.01), and advanced tumor stage (OR 7.90; 95% CI 3.22–19.37) in comparison with patients with *TERT* promoter mutations only (Table 3). When compared with patients having $BRAF^{V600E}$ mutation only, patients harboring both *TERT* and $BRAF^{V600E}$ mutations seemed to be older patients (SMD 0.77; 95% CI 0.40–1.15) and males (OR 2.38; 95% CI 1.59–3.56) and tended to suffer from extrathyroidal extension (OR 5.76; 95% CI 3.45–9.63), lymph node metastasis (OR 1.58; 95% CI 1.01–2.47), distant metastasis (OR 13.07; 95% CI 2.57–66.59), advanced tumor stage (OR 4.22; 95% CI 2.71–6.58), recurrence/persistence (OR 8.50; 95% CI 4.20–17.19), and mortality (OR 8.14; 95% CI 2.38–27.89) (Table 3).

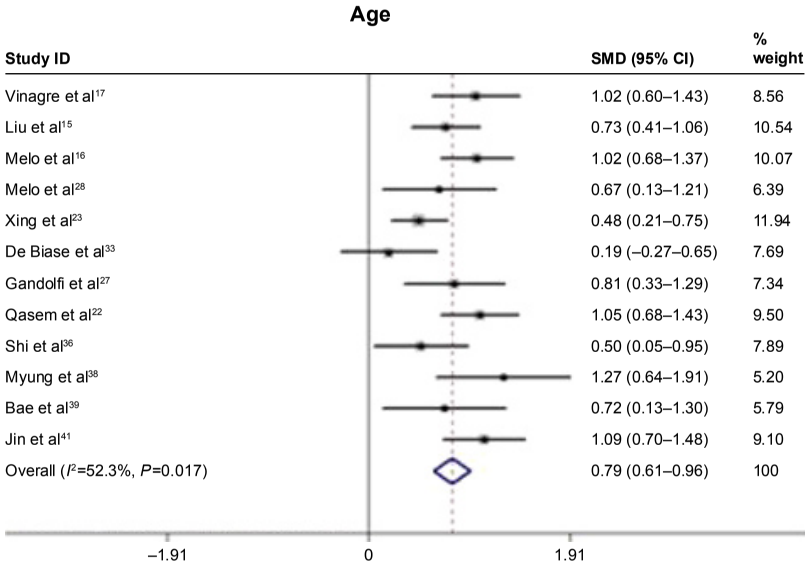
Discussion

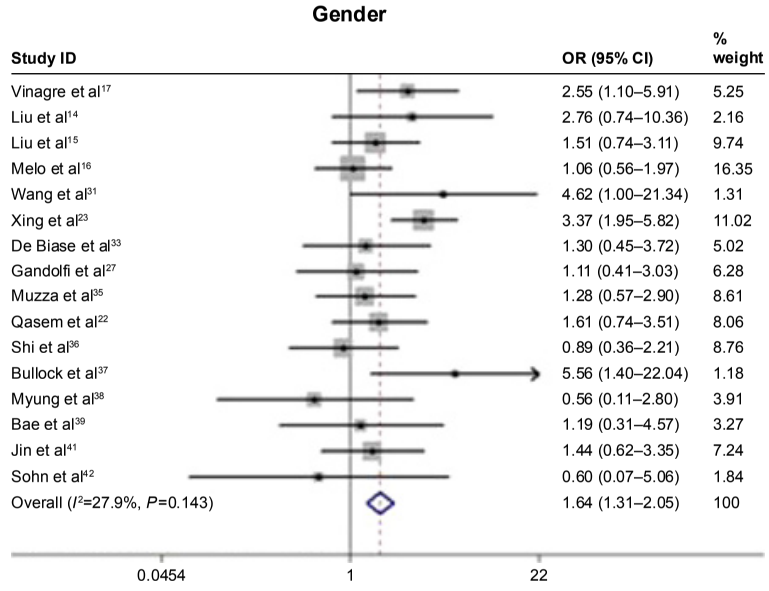
The majority of thyroid cancer has excellent prognosis after thyroidectomy with/without radioiodine ablation.[43] However, a small group of patients suffer from unfavorable outcome.[44,45] During the pathogenesis and progression of thyroid cancer, a number of genetic and epigenetic alterations are accumulated. These alterations provide potential biomarkers to discriminate aggressive cases from those with indolent behavior. In recent years, the clinicopathological and prognostic significance of *TERT* promoter mutations have been widely evaluated in thyroid cancer, and the discrepancies among studies are probably caused by small sample size of individual studies.[14–17,22,23] This meta-analysis demonstrated that *TERT* promoter mutations were likely to aggregate in aggressive histological types and associated with high-risk clinicopathological features and adverse outcome of thyroid cancer. The present study also confirmed the coexistence of *TERT* promoter (C228T) and $BRAF^{V600E}$ mutations, which contributed to more aggressive tumor behavior. De-Tao et al conducted a similar meta-analysis recently, but it only included 8 studies comprising 2,035 patients and excluded studies analyzing fine-needle aspiration biopsy (FNAB) which was an important and

Table 2 Frequencies of *TERT* promoter mutations in different histological types of thyroid cancer

	C228T					C250T					C228T or C250T				
	n	Events	Pooled	95% CI	Heterogeneity, I^2 (%)	n	Events	Pooled	95% CI	Heterogeneity, I^2 (%)	n	Events	Pooled	95% CI	Heterogeneity, I^2 (%)
DTC	2,828	258	0.0936	0.0634–0.1238	86.32	2,561	78	0.0284	0.0153–0.0415	75.98	3,587	339	0.1091	0.0819–0.1363	87.55
PTC	2,443	220	0.0878	0.0586–0.1170	84.98	2,118	63	0.0253	0.0124–0.0382	78.60	3,170	276	0.0941	0.0716–0.1165	79.67
CPTC	723	77	0.0961	0.0425–0.1497	84.02	340	3	0.0078	0.0075–0.0231	51.35	340	33	0.0342	0.0362–0.1490	70.62
FVPTC	216	16	0.0636	0.0313–0.0960	23.07	113	2	0.0141	0.0074–0.0357	0	113	10	0.0809	0.0207–0.1824	75.05
TCPTC	62	17	0.2736	0.1627–0.3845	0	43	1	0.0341	0.0195–0.0876	0	43	13	0.3023	0.1650–0.4396	0
FTC	160	27	0.1522	0.0973–0.2071	10.09	218	13	0.0393	0.0139–0.0647	13.95	294	53	0.1703	0.1277–0.2128	0
PDTC	86	27	0.3025	0.2065–0.3984	0	86	12	0.0955	0.0052–0.1857	51.99	131	51	0.3337	0.2068–0.4606	54.37
ATC	205	80	0.3885	0.3221–0.4549	0	205	9	0.0387	0.0125–0.0649	0	257	103	0.3869	0.2866–0.4872	59.16
Total	3,654	413	0.1126	0.0820–0.1433	90.65	3,387	109	0.0271	0.0174–0.0368	69.71	4,190	510	0.1428	0.1088–0.1768	92.76

Abbreviations: DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; CPTC, conventional papillary thyroid cancer; FVPTC, follicular-variant papillary thyroid cancer; TCPTC, tall-cell papillary thyroid cancer; FTC, follicular thyroid cancer; PDTC, poorly differentiated thyroid cancer; ATC, anaplastic thyroid cancer; TERT, telomerase reverse transcriptase; CI, confidence interval; I^2, inconsistency index.





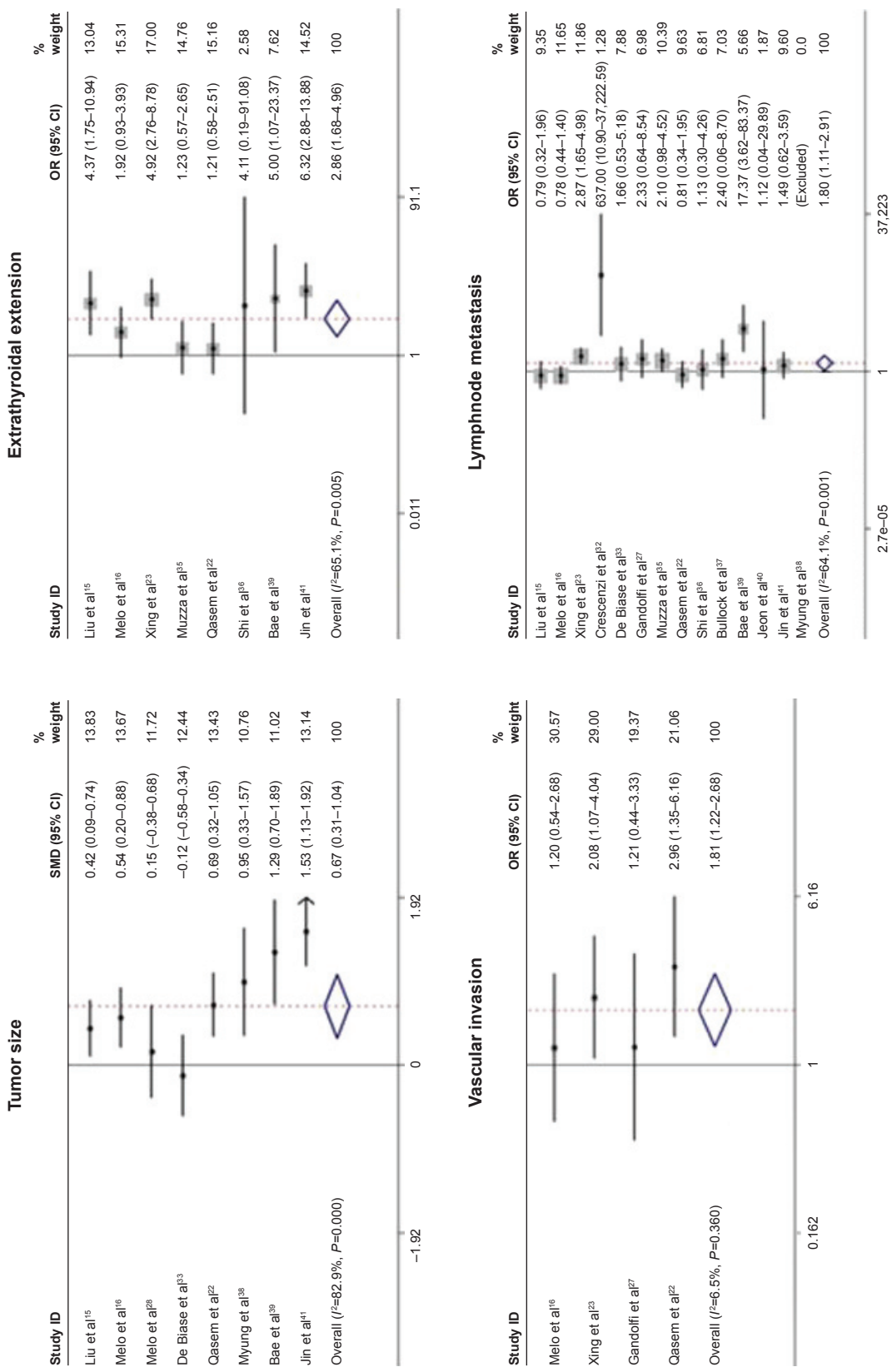


Figure 3 (*Continued*)

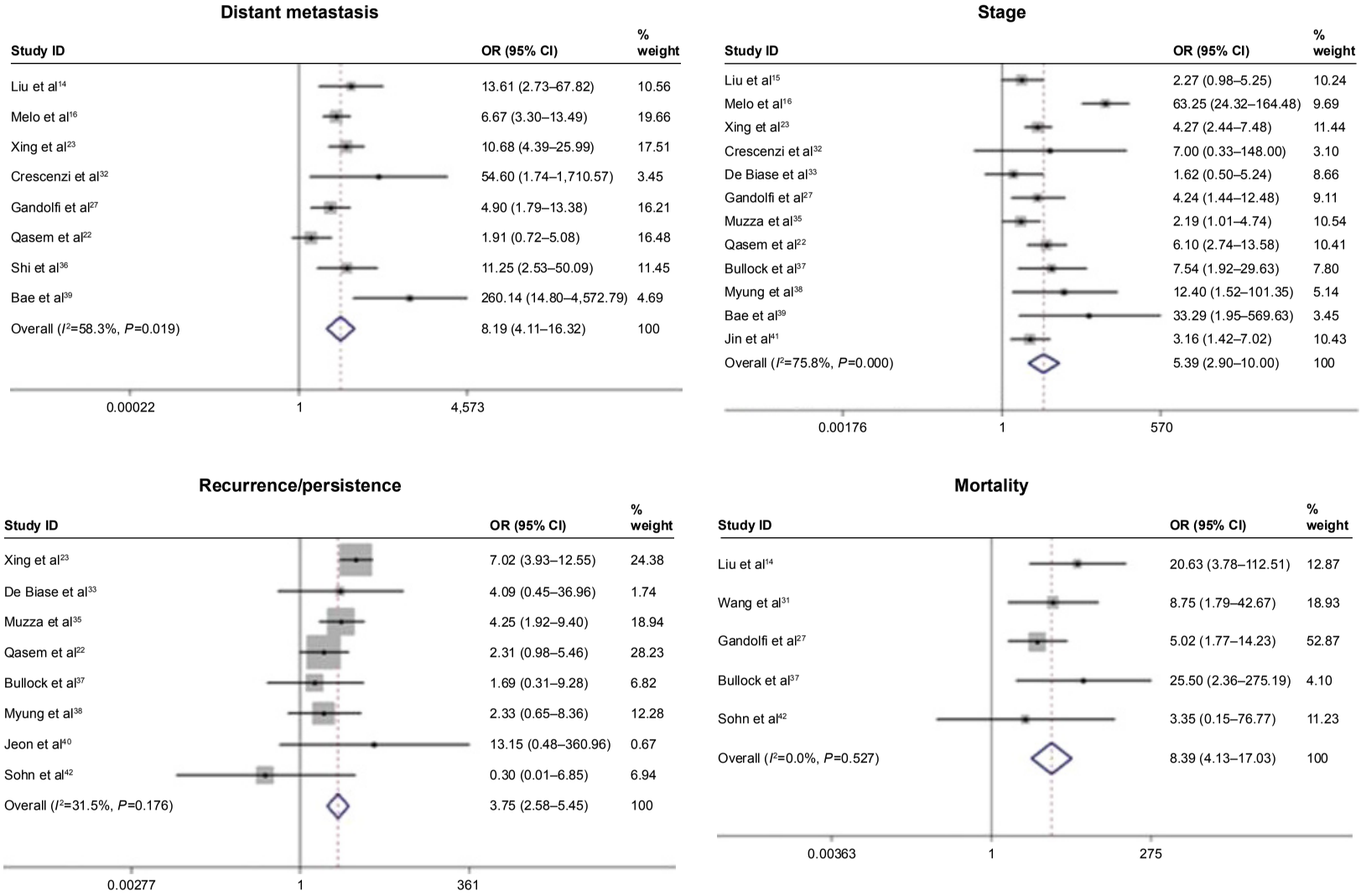


Figure 3 Forest plot showing the association of *TERT* promoter mutations with clinicopathological features and adverse outcomes.
Notes: Weights are from random effects analysis; Weights are from fixed effects analysis.
Abbreviations: TERT, telomerase reverse transcriptase; SMD, standardized mean difference; OR, odds ratio; CI, confidence interval; I^2, inconsistency index.

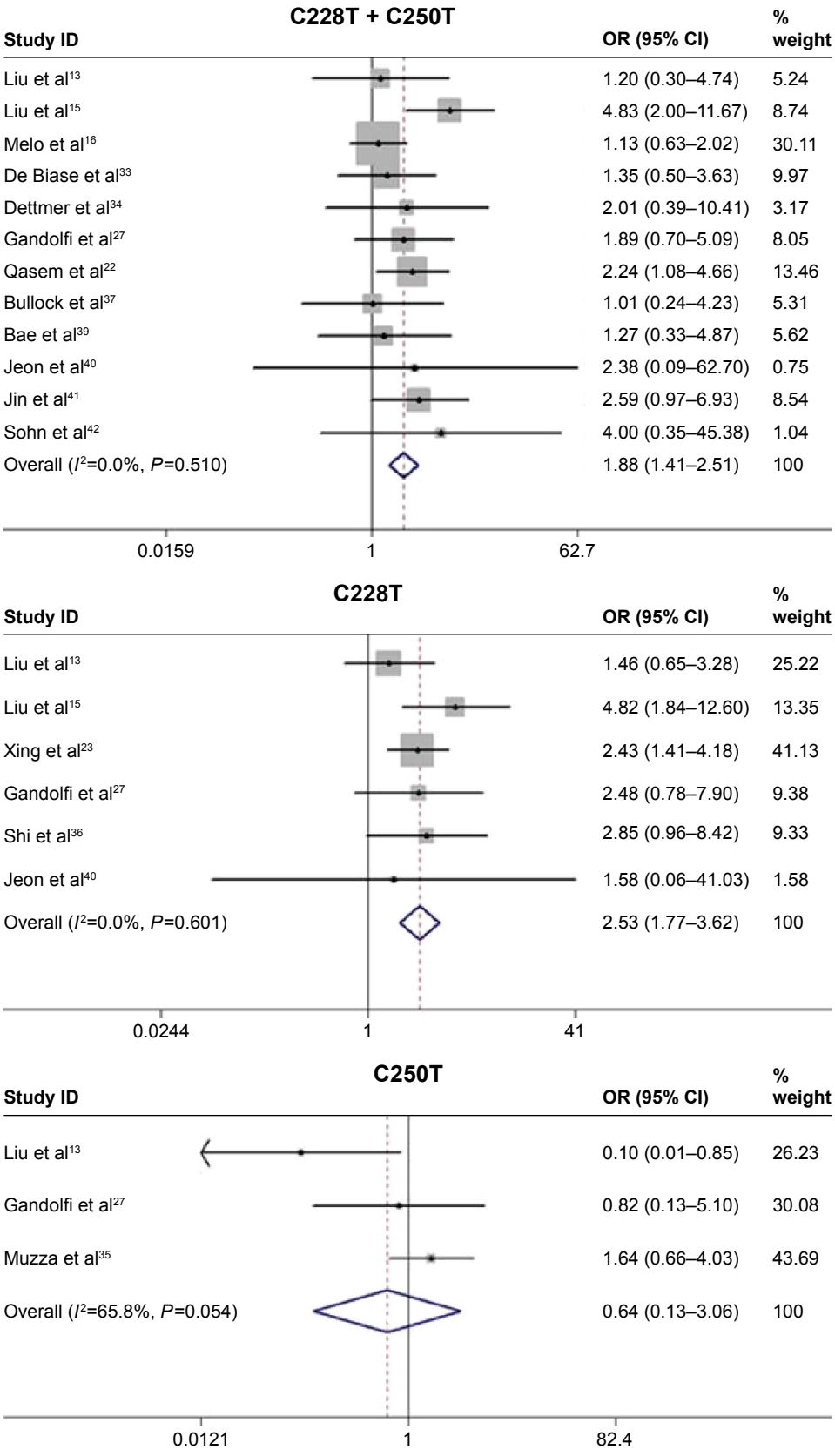


Figure 4 Forest plot showing the relationship of *TERT* promoter mutations and *BRAF* mutation.
Note: Weights are from random effects analysis.
Abbreviations: TERT, telomerase reverse transcriptase; OR, odds ratio; CI, confidence interval; I^2, inconsistency index.

Table 3 The synergetic effect of *TERT* promoter and *BRAF* mutations in clinicopathological features and adverse outcomes

Variable	No of studies	*TERT* + *BRAF* vs *TERT*			*TERT* + *BRAF* vs *BRAF*		
		No of cases	OR (95% CI)	Heterogeneity, I^2 (%)	No of cases	OR (95% CI)	Heterogeneity, I^2 (%)
Age	5	170	0.43 (–0.18–1.03)	61.8	1,121	0.77 (0.40–1.15)	70.2
Gender	5	170	3.71 (1.66–8.29)	0	705	2.38 (1.59–3.56)	38.0
Tumor size	3	88	0.80 (0.24–1.35)	0	873	0.69 (–0.26–1.64)	92.6
Extrathyroidal extension	3	108	5.85 (2.14–16.01)	16.6	759	5.76 (3.45–9.63)	0
Vascular invasion	2	73	0.57 (0.10–3.39)	57.5	223	0.57 (0.097–3.39)	0
Lymph node metastasis	5	125	2.08 (0.97–4.49)	0	646	1.58 (1.01–2.47)	0
Distant metastasis	2	82	1.47 (0.13–16.82)	73.1	248	13.07 (2.57–66.59)	58.7
Stage	5	150	7.90 (3.22–19.37)	0	1,032	4.22 (2.71–6.58)	25.8
Recurrence/persistence	3	94	2.65 (0.41–17.29)	57.1	421	8.50 (4.20–17.19)	4.0
Mortality	2	32	1.38 (0.32–5.98)	0	113	8.14 (2.38–27.89)	29.7

Abbreviations: TERT, telomerase reverse transcriptase; OR, odds ratio; CI, confidence interval; I^2, inconsistency index.

reliable diagnostic approach for thyroid cancer.[46] Another study conducted by Liu and Xing also achieved brilliant results.[47] However, almost all of the studies were based on Americans and Europeans, except one from Saudi Arabia. This meta-analysis included five additional studies from Asia, which may be complementary because of the different genetic background among ethnicities.

TERT promoter mutations were exclusively present in FCDTC. Previous researchers propose that *TERT* promoter mutations usually exist in malignancies originating from terminally differentiated cells with low self-renewing capacity,[9] while the rapidly renewing tissues have alternative mechanisms for telomere elongation and are less dependent on TERT activation.[48] In addition, this study showed that *TERT* promoter mutations were absent in normal tissues or benign lesions; thus they can serve as biomarkers having high specificity for malignancy. However, the diagnostic efficiency may be severely limited by the low prevalence of *TERT* promoter mutations in DTC.[30] Liu and Xing and Crescenzi et al, respectively, evaluated the feasibility of *TERT* promoter mutations in preoperative FNAB and core needle biopsies and found it can improve the diagnostic efficiency for indeterminate nodules.[30,32,34] A previous study found that $BRAF^{V600E}$ mutation had no significant value for indeterminate nodules classified as follicular neoplasm/suspicious for follicular neoplasm (FN/SFN).[49] In this meta-analysis, the frequencies of *TERT* promoter mutations in FTC and FVPTC, the main components of malignant FN/SFN nodules, were found to be 17.03% and 8.09%, respectively. Therefore, *TERT* promoter mutations may be helpful to diagnose thyroid cancer in FN/SFN nodules.

TERT promoter mutations tended to aggregate in aggressive histological types (ATC, PDTC, and TCPTC) and were significantly associated with high-risk features and adverse outcome. Furthermore, the coexistence of $BRAF^{V600E}$ and *TERT* promoter mutations indicated more aggressive tumor and worse prognosis, and the influence of *TERT* promoter mutations seemed to be more significant than $BRAF^{V600E}$ mutation. The mechanism underlying the synergetic effect of *BRAF* and *TERT* promoter mutations remains uncertain.[15,24,25,29,30,39,50] Vinagre et al and Bullock et al demonstrated that *BRAF* and *TERT* promoter mutations can increase the expression of each other,[17,37] which may be achieved by activation of *MAPK* pathway and regulation of ETS transcriptional factors.[50] Li et al found that C250T mutation alone was insufficient to drive the transcription of *TERT* gene and required noncanonical NF-κB signaling for stimulus responsiveness at the same time.[51] Therefore, the functions of C228T and C250T mutations were distinct, which partially explained the result that no significant association was found between *BRAF* and C250T mutations. Therefore, *TERT* promoter mutations can distinguish not only malignancy but also aggressive cases that need more positive therapeutic approach and vigilant monitoring. Some researchers also reported that patients with *TERT* promoter mutations tended to suffer from radiotherapeutic resistance;[39,52] so *TERT* promoter mutations might also be potential predictors for therapeutic efficiency.

Limitations

There were some limitations in this meta-analysis. First, most of the studies were retrospectively designed which may cause potential selection bias to better-documented patients and larger tumors since they were more available for collection and analysis. Second, heterogeneity was present in some analyses probably due to confounding factors such as sample size, ethnicity, patients' age, tumor size, sample source, and so on. Besides, most of the aggressive variables are interrelated; so the results should be interpreted cautiously.

Conclusion

This meta-analysis confirmed that *TERT* promoter mutations were more frequent in aggressive histological types of thyroid cancer. And they were likely to present in older patients and males and strongly associated with larger tumor size, extrathyroidal extension, vascular invasion, lymph node metastasis, distant metastasis, advanced tumor stage, disease recurrence/persistence, and mortality. *TERT* promoter mutations seemed to coexist with *BRAF* mutation, which contributed to more aggressive tumor and worse prognosis. Therefore, *TERT* promoter mutations have the potential to serve as biomarkers assisting preoperative diagnosis, risk stratification, prognostic prediction, and individualizing therapeutic option or follow-up design of thyroid cancer.

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Disclosure

The authors report no conflicts of interest in this work.

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