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 Cochrane 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 (33.7%), 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 BRAFV600E 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, BRAFV600E 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, anti-apoptosis, 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 melanomas6,7 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
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 BRAFV600E 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 BRAFV600E 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 Cochrane 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 BRAFV600E 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, BRAFV600E 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.23 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 BRAFV600E 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 (I²). I²>50% suggested significant heterogeneity and so random effects model (DerSimonian–Laird method) was chosen; otherwise, fixed effects model (Mantel–Haenszel method) would be considered.28 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.

All the 22 studies reported the frequency of TERT promoter mutations, and 18 studies were available for analyzing the clinicopathological features and prognostic significance. 15 studies investigated the relationship of TERT promoter and BRAF\(^{V600E}\) mutations, and six of them evaluated the synergistic effect of both mutations.

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.](https://www.dovepress.com/)

**Abbreviation:** TERT, telomerase reverse transcriptase.
Table 1 Characteristics and methodological quality of 22 studies included in the meta-analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Study design</th>
<th>Number of centers</th>
<th>Number of cases</th>
<th>Histotype (years)</th>
<th>Sex (F/M)</th>
<th>TERT mutation Sample source</th>
<th>Sequencing method</th>
<th>Mutation type</th>
<th>Follow-up Cases</th>
<th>Duration (months)</th>
<th>Quality (NOS)</th>
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<td>TC</td>
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<td>NA</td>
<td>Surgical specimen</td>
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<td>C228T + C250T</td>
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<td>NA</td>
</tr>
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<td>Surgical specimen</td>
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<td>63.7</td>
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<td>74</td>
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<td>48</td>
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<td>Bae et al29</td>
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<td>Korea</td>
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<td>Single</td>
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<td>DTC</td>
<td>NA</td>
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<td>China</td>
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<td>Single</td>
<td>653</td>
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<td>59.79</td>
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<td>NGS</td>
<td>C228T + C250T</td>
<td>19</td>
<td>65.95</td>
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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.
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).

**Discussion**

The majority of thyroid cancer has excellent prognosis after thyroidectomy with/without radioiodine ablation. However, a small group of patients suffer from unfavorable outcome. 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. 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 BRAFV600E 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

<table>
<thead>
<tr>
<th></th>
<th>C228T</th>
<th>C250T</th>
<th>C228T or C250T</th>
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<tr>
<td></td>
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<td>Pooled</td>
</tr>
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<td></td>
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<tr>
<td>DTC</td>
<td>2,828</td>
<td>258</td>
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<td>PTc</td>
<td>2,443</td>
<td>220</td>
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<td>CPTc</td>
<td>723</td>
<td>77</td>
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<td>FVPTc</td>
<td>216</td>
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<td>TCPTc</td>
<td>62</td>
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<td>86</td>
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<tr>
<td>ATC</td>
<td>205</td>
<td>80</td>
<td>0.3885</td>
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<tr>
<td>Total</td>
<td>3,654</td>
<td>413</td>
<td>0.1126</td>
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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)
### Distant metastasis

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<th>OR (95% CI)</th>
<th>% weight</th>
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<tr>
<td>Liu et al(^\text{14})</td>
<td>13.61 (2.73–67.82)</td>
<td>10.56</td>
</tr>
<tr>
<td>Melo et al(^\text{16})</td>
<td>6.67 (3.30–13.49)</td>
<td>19.66</td>
</tr>
<tr>
<td>Xing et al(^\text{11})</td>
<td>10.68 (4.39–25.99)</td>
<td>17.51</td>
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<tr>
<td>Crescenzi et al(^\text{12})</td>
<td>54.60 (1.74–1,710.57)</td>
<td>3.45</td>
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<tr>
<td>Gandolfi et al(^\text{12})</td>
<td>4.90 (1.79–13.38)</td>
<td>16.21</td>
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<tr>
<td>Qasem et al(^\text{12})</td>
<td>1.91 (0.72–5.08)</td>
<td>16.48</td>
</tr>
<tr>
<td>Shi et al(^\text{12})</td>
<td>11.25 (2.53–50.59)</td>
<td>11.45</td>
</tr>
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<td>Bae et al(^\text{8})</td>
<td>280.14 (14.80–4,572.79)</td>
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<td>Overall (I(^2)=58.3%, P=0.019)</td>
<td>8.19 (4.11–16.32)</td>
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### Stage

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<tbody>
<tr>
<td>Liu et al(^\text{10})</td>
<td>2.27 (0.98–5.25)</td>
<td>10.24</td>
</tr>
<tr>
<td>Melo et al(^\text{10})</td>
<td>68.25 (24.32–194.48)</td>
<td>9.69</td>
</tr>
<tr>
<td>Xing et al(^\text{11})</td>
<td>4.27 (2.44–7.48)</td>
<td>11.44</td>
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<tr>
<td>Crescenzi et al(^\text{12})</td>
<td>7.00 (0.33–148.00)</td>
<td>3.10</td>
</tr>
<tr>
<td>De Biase et al(^\text{10})</td>
<td>1.62 (0.50–5.26)</td>
<td>8.66</td>
</tr>
<tr>
<td>Gandolfi et al(^\text{10})</td>
<td>4.24 (1.44–12.46)</td>
<td>9.11</td>
</tr>
<tr>
<td>Muzzo et al(^\text{18})</td>
<td>2.19 (1.01–4.74)</td>
<td>10.54</td>
</tr>
<tr>
<td>Qasem et al(^\text{18})</td>
<td>6.10 (2.74–13.58)</td>
<td>10.41</td>
</tr>
<tr>
<td>Bullock et al(^\text{17})</td>
<td>7.54 (1.92–29.63)</td>
<td>7.80</td>
</tr>
<tr>
<td>Myung et al(^\text{18})</td>
<td>12.40 (1.52–101.35)</td>
<td>5.14</td>
</tr>
<tr>
<td>Bae et al(^\text{8})</td>
<td>33.29 (1.95–569.63)</td>
<td>3.45</td>
</tr>
<tr>
<td>Jin et al(^\text{1})</td>
<td>3.16 (1.42–7.02)</td>
<td>10.43</td>
</tr>
<tr>
<td>Overall (I(^2)=75.8%, P=0.000)</td>
<td>5.39 (2.90–10.00)</td>
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</table>

### Recurrence/persistence

<table>
<thead>
<tr>
<th>Study ID</th>
<th>OR (95% CI)</th>
<th>% weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xing et al(^\text{11})</td>
<td>7.02 (3.93–12.55)</td>
<td>24.38</td>
</tr>
<tr>
<td>De Biase et al(^\text{10})</td>
<td>4.09 (0.45–36.96)</td>
<td>1.74</td>
</tr>
<tr>
<td>Muzzo et al(^\text{18})</td>
<td>4.25 (1.92–9.40)</td>
<td>18.94</td>
</tr>
<tr>
<td>Qasem et al(^\text{18})</td>
<td>2.31 (0.98–5.46)</td>
<td>28.23</td>
</tr>
<tr>
<td>Bullock et al(^\text{17})</td>
<td>1.69 (0.31–9.28)</td>
<td>6.82</td>
</tr>
<tr>
<td>Myung et al(^\text{18})</td>
<td>2.33 (0.65–8.36)</td>
<td>12.28</td>
</tr>
<tr>
<td>Jeon et al(^\text{16})</td>
<td>13.15 (0.48–360.96)</td>
<td>0.07</td>
</tr>
<tr>
<td>Sohn et al(^\text{10})</td>
<td>0.30 (0.01–6.85)</td>
<td>6.94</td>
</tr>
<tr>
<td>Overall (I(^2)=31.5%, P=0.176)</td>
<td>3.75 (2.58–5.45)</td>
<td>100</td>
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</tbody>
</table>

### Mortality

<table>
<thead>
<tr>
<th>Study ID</th>
<th>OR (95% CI)</th>
<th>% weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liu et al(^\text{10})</td>
<td>20.63 (3.78–112.51)</td>
<td>12.87</td>
</tr>
<tr>
<td>Wang et al(^\text{11})</td>
<td>8.75 (1.79–42.67)</td>
<td>18.93</td>
</tr>
<tr>
<td>Gandolfi et al(^\text{12})</td>
<td>5.02 (1.77–14.23)</td>
<td>52.87</td>
</tr>
<tr>
<td>Bullock et al(^\text{17})</td>
<td>25.50 (2.36–275.19)</td>
<td>4.10</td>
</tr>
<tr>
<td>Sohn et al(^\text{10})</td>
<td>3.35 (0.15–76.77)</td>
<td>11.23</td>
</tr>
<tr>
<td>Overall (I(^2)=0.0%, P=0.527)</td>
<td>8.36 (4.13–17.03)</td>
<td>100</td>
</tr>
</tbody>
</table>

**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; F, inconsistency index.
The image contains a forest plot showing the relationship of TERT promoter mutations and BRAF mutation. The plot is divided into three sections: C228T + C250T, C228T, and C250T.

**C228T + C250T**
- **Study ID**
  - Liu et al\(^{13}\)
  - Liu et al\(^{15}\)
  - Melo et al\(^{16}\)
  - De Biase et al\(^{13}\)
  - Dettmer et al\(^{44}\)
  - Gandolfi et al\(^{27}\)
  - Qasem et al\(^{22}\)
  - Bullock et al\(^{37}\)
  - Bae et al\(^{39}\)
  - Jeon et al\(^{40}\)
  - Jin et al\(^{41}\)
  - Sohn et al\(^{42}\)
- **OR (95% CI)**
  - Liu et al\(^{13}\): 1.20 (0.30–4.74)
  - Liu et al\(^{15}\): 4.83 (2.00–11.67)
  - Melo et al\(^{16}\): 1.13 (0.63–2.02)
  - De Biase et al\(^{13}\): 1.35 (0.50–3.63)
  - Dettmer et al\(^{44}\): 2.01 (0.39–10.41)
  - Gandolfi et al\(^{27}\): 1.89 (0.70–5.09)
  - Qasem et al\(^{22}\): 2.24 (1.08–4.66)
  - Bullock et al\(^{37}\): 1.01 (0.24–4.23)
  - Bae et al\(^{39}\): 1.27 (0.33–4.87)
  - Jeon et al\(^{40}\): 2.38 (0.09–62.70)
  - Jin et al\(^{41}\): 2.59 (0.97–6.93)
  - Sohn et al\(^{42}\): 4.00 (0.35–45.38)
  - Overall (\(I^2=0.0\%, P=0.510\)): 1.88 (1.41–2.51)
- **% weight**
  - Liu et al\(^{13}\): 5.24
  - Liu et al\(^{15}\): 8.74
  - Melo et al\(^{16}\): 30.11
  - De Biase et al\(^{13}\): 9.97
  - Dettmer et al\(^{44}\): 3.17
  - Gandolfi et al\(^{27}\): 8.05
  - Qasem et al\(^{22}\): 13.46
  - Bullock et al\(^{37}\): 5.31
  - Bae et al\(^{39}\): 5.62
  - Jeon et al\(^{40}\): 0.75
  - Jin et al\(^{41}\): 8.54
  - Sohn et al\(^{42}\): 1.04
  - Overall (\(I^2=0.0\%, P=0.510\)): 100

**C228T**
- **Study ID**
  - Liu et al\(^{13}\)
  - Liu et al\(^{15}\)
  - Xing et al\(^{23}\)
  - Gandolfi et al\(^{27}\)
  - Shi et al\(^{36}\)
  - Jeon et al\(^{40}\)
- **OR (95% CI)**
  - Liu et al\(^{13}\): 1.46 (0.65–3.28)
  - Liu et al\(^{15}\): 4.82 (1.84–12.60)
  - Xing et al\(^{23}\): 2.43 (1.41–4.18)
  - Gandolfi et al\(^{27}\): 2.48 (0.78–7.90)
  - Shi et al\(^{36}\): 2.85 (0.96–8.42)
  - Jeon et al\(^{40}\): 1.58 (0.06–41.03)
- **% weight**
  - Liu et al\(^{13}\): 25.22
  - Liu et al\(^{15}\): 13.35
  - Xing et al\(^{23}\): 41.13
  - Gandolfi et al\(^{27}\): 9.38
  - Shi et al\(^{36}\): 9.33
  - Jeon et al\(^{40}\): 1.58
  - Overall (\(I^2=0.0\%, P=0.601\)): 100

**C250T**
- **Study ID**
  - Liu et al\(^{13}\)
  - Gandolfi et al\(^{27}\)
  - Muzza et al\(^{35}\)
- **OR (95% CI)**
  - Liu et al\(^{13}\): 0.10 (0.01–0.85)
  - Gandolfi et al\(^{27}\): 0.82 (0.13–5.10)
  - Muzza et al\(^{35}\): 1.64 (0.66–4.03)
- **% weight**
  - Liu et al\(^{13}\): 26.23
  - Gandolfi et al\(^{27}\): 30.08
  - Muzza et al\(^{35}\): 43.69
  - Overall (\(I^2=65.8\%, P=0.054\)): 100

**Note:** Weights are from random effects analysis.

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

**Figure 4** Forest plot showing the relationship of TERT promoter mutations and BRAF mutation.
Table 3 The synergetic effect of TERT promoter and BRAF mutations in clinicopathological features and adverse outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>No of studies</th>
<th>TERT + BRAF vs TERT</th>
<th>TERT + BRAF vs BRAF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of cases</td>
<td>OR (95% CI)</td>
<td>I² (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>5</td>
<td>170</td>
<td>0.43 (–0.18–1.03)</td>
</tr>
<tr>
<td>Gender</td>
<td>5</td>
<td>170</td>
<td>3.71 (1.66–8.29)</td>
</tr>
<tr>
<td>Tumor size</td>
<td>3</td>
<td>88</td>
<td>0.80 (0.24–1.35)</td>
</tr>
<tr>
<td>Extrathyroidal extension</td>
<td>3</td>
<td>108</td>
<td>5.85 (2.14–16.01)</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>2</td>
<td>73</td>
<td>0.57 (0.10–3.39)</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>5</td>
<td>125</td>
<td>2.08 (0.97–4.49)</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>2</td>
<td>82</td>
<td>1.47 (0.13–16.82)</td>
</tr>
<tr>
<td>Stage</td>
<td>5</td>
<td>150</td>
<td>7.90 (3.22–19.37)</td>
</tr>
<tr>
<td>Recurrence/persistence</td>
<td>3</td>
<td>94</td>
<td>2.65 (0.41–17.29)</td>
</tr>
<tr>
<td>Mortality</td>
<td>2</td>
<td>32</td>
<td>1.38 (0.32–5.98)</td>
</tr>
</tbody>
</table>

Abbreviations: TERT, telomerase reverse transcriptase; OR, odds ratio; CI, confidence interval; I², inconsistency index.

reliable diagnostic approach for thyroid cancer. Another study conducted by Liu and Xing also achieved brilliant results. 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, while the rapidly renewing tissues have alternative mechanisms for telomere elongation and are less dependent on TERT activation. 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. 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. A previous study found that BRAF<sup>V600E</sup> mutation had no significant value for indeterminate nodules classified as follicular neoplasm/suspicious for follicular neoplasm (FN/SFN). 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<sup>V600E</sup> 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<sup>V600E</sup> mutation. The mechanism underlying the synergetic effect of BRAF and TERT promoter mutations remains uncertain. Vinagre et al and Bullock et al demonstrated that BRAF and TERT promoter mutations can increase the expression of each other, which may be achieved by activation of MAPK pathway and regulation of ETS transcriptional factors. 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. Therefore, the functions of C228T and C250T mutations seemed to be more significant than promoter mutations.

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.

Acknowledgments
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Disclosure
The authors report no conflicts of interest in this work.

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