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The relationship between *RASSF1A* promoter methylation and thyroid carcinoma

A meta-analysis of 14 articles and a bioinformatics of 2 databases (PRISMA)

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Abstract

Background: DNA promoter methylation can suppresses gene expression and shows an important role in the biological functions of Ras association domain family 1A (*RASSF1A*). Many studies have performed to elucidate the role of *RASSF1A* promoter methylation in thyroid carcinoma, while the results were conflicting and heterogeneous. Here, we analyzed the data of databases to determine the relationship between *RASSF1A* promoter methylation and thyroid carcinoma.

Methods: We used the data from 14 cancer–normal studies and Gene Expression Omnibus (GEO) database to analyze *RASSF1A* promoter methylation in thyroid carcinoma susceptibility. The data from the Cancer Genome Atlas project (TCGA) database was used to analyze the relationship between *RASSF1A* promoter methylation and thyroid carcinoma susceptibility, clinical characteristics, prognosis. Odds ratios were estimated for thyroid carcinoma susceptibility and hazard ratios were estimated for thyroid carcinoma prognosis. The heterogeneity between studies of meta-analysis was explored using H, I^2 values, and meta-regression. We adopted quality criteria to classify the studies of meta-analysis. Subgroup analyses were done for thyroid carcinoma susceptibility according to ethnicity, methods, and primers.

Results: Result of meta-analysis indicated that *RASSF1A* promoter methylation is associated with higher susceptibility to thyroid carcinoma with small heterogeneity. Similarly, the result from GEO database also showed that a significant association between *RASSF1A* gene promoter methylation and thyroid carcinoma susceptibility. For the results of TCGA database, we found that *RASSF1A* promoter methylation is associated with susceptibility and poor disease-free survival (DFS) of thyroid carcinoma. In addition, we also found a close association between *RASSF1A* promoter methylation and patient tumor stage and age, but not in patients of different genders.

Conclusions: The methylation status of *RASSF1A* promoter is strongly associated with thyroid carcinoma susceptibility and DFS. The *RASSF1A* promoter methylation test can be applied in the clinical diagnosis of thyroid carcinoma.

Abbreviations: AUC = area under the curve, DFS = disease-free survival, GEO = Gene Expression Omnibus, HR = hazard ratio, OR = odds ratio, OS = overall survival, *RASSF1A* = Ras association domain family 1A, SROC = summary receiver operating characteristics, TCGA = the cancer genome atlas project.

Keywords: GEO and TCGA, meta-analysis, methylation, promoter, *RASSF1A*

1. Introduction

Thyroid carcinoma, including papillary thyroid carcinoma, medullary thyroid carcinoma, and follicular thyroid carcinoma, is the most common endocrine malignant neoplasm worldwide.[1] In the United States, thyroid carcinoma accounts for 1.7% of all malignancies, corresponding to 2.6% of cancers in females and 0.85% of cancers in males, whereas in Japan the woman-to-man ratio may be 13.[2] Furthermore, many studies have demonstrated that the incidence of thyroid carcinoma is increasing for reasons remain unclear, but in part, could be related to epigenetic events.[3–5] Previous researches have shown that recurrence is a common event in thyroid carcinoma patients (15–30% of patients), especially in early-stage.[6,7] Therefore, it is very important to identify thyroid carcinoma patients at early recurrence so that more aggressive therapy and monitoring can be realized. As a common and important mechanisms for tumor suppressor gene inactivation in cancer, epigenetic alterations, such as aberrant promoter methylation, can yield powerful biomarkers for early detection of thyroid carcinoma.[8,9] Several revolutionary steps have been made to promote application of DNA methylation biomarkers in cancer screening.[10] Therefore, aberrant promoter methylation may be a powerful tool for thyroid carcinoma diagnosis.

RASS1FA is an important tumor suppressor protein in cells. It contain a *Ras* association domain, which can bind RAS proteins and may alter their function.[11–14] In doing so, Ras association

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domain family 1A (*RASSF1A*) affects multiple cellular processes.[15,16] However, several studies found that *RASSF1A* promoter region contains a CpG island A (737 bp) and its expression was decreased by its promoter methylation.[11,16,17] Two studies found that the frequency of *RASSF1A* promoter methylation in thyroid cancer was about 30% to 70%,[18–21] whereas other studies found the inverted.[22,23] Therefore, there lack a unified view of the methylation of *RASSF1A* promoter in thyroid carcinoma.

In this study, we used the data from 14 cancer–normal studies, Gene Expression Omnibus (GEO) and the Cancer Genome Atlas project (TCGA) databases to analyze the methylation of *RASSF1A* promoter in thyroid carcinoma susceptibility. Meanwhile, data from TCGA database were also used to analyze the methylation of *RASSF1A* promoter in thyroid carcinoma clinical characteristics (age status, genders, and pathologic tumor stages) and prognosis.

2. Materials and methods

2.1. Ethics statement

This study was approved by the First People's Hospital of Yunnan Province Ethics Committee. This study does not involve patients, so ethical approval was not required.

2.2. Search strategy, selection of studies, and data extraction

This pooled study involved searching a range of computerized databases, including Chinese National Knowledge Infrastructure, PubMed, Web of Science, and Google Scholar, for articles published in English or Chinese up to April 2017. The study used a subject and text word strategy with (*RASSF1* or Ras association domain family member 1 A or *RASSF1A* or *REH3P21* or *RDA32* or *NORE2A*) AND (methylation or hypermethylation or epigenetics) AND (thyroid cancer or cancerous goiter or carcinoma of thyroid or thyroid carcinoma or papillary thyroid carcinoma or papillary thyroid cancer or medullary thyroid carcinoma or follicular thyroid carcinoma) as the primary search terms. Additional studies were identified by hand searching references in original articles and review articles.

Two independent reviewers (JY and HN) screened the titles and abstracts to identify relevant studies. The following types of studies were excluded: animal or cell experiments, case reports, meta-analyses or reviews, studies of non-normal–cancer studies or studies with insufficient data or those proving inaccessible after making contact with the authors. The remaining articles were further examined to see if they satisfy the following criteria: the patients had to be diagnosed with thyroid cancer (papillary thyroid carcinoma or medullary thyroid carcinoma or follicular thyroid carcinoma); the studies should be contain *RASSF1A* promoter methylation data; the studies should be included cancer samples (blood/tissue) and normal samples (healthy blood/tissue, or adjacent cancer normal tissue, or noncancer samples). Decisions were made and any disagreements regarding decisions were resolved by discussion with YH and KY. Studies which met the prespecified selection standards were summarized in data extraction forms. The following information was extracted from the studies: first author's last name, year of publication, original country of patients, age (mean or median), proportion of TNM stage, gender proportion (male/female), methylation detection methods and primers, the number of *RASSF1A* promoter methylations in individual cases and normal controls in individuals, and more. Ethnicity was categorized as "Caucasian," "Asian," or "mixed population" when a study did not state which ethnic groups were included.

2.3. Meta-analysis and SROC analysis

The data we acquired were analyzed and visualized mainly using R (R version 3.3.2) software. The strength of *RASSF1A* promoter methylation in thyroid carcinoma susceptibility was measured by a pooled odds ratio (OR) with a 95% confidence interval (CI) and *P* value. When the pooled OR with a threshold of $P < .05$, the significant difference was made. Heterogeneity was tested using the I^2 and H statistic. If $H > 1.5$, $I^2 > 50\%$, and $P \leq .05$, there was a strong heterogeneity between studies and a random-effects model was taken.[24–26] Otherwise, a fixed-effects model was used when $H < 1.2$, $I^2 \leq 50\%$, and $P > .05$.[27] Tau-squared (τ^2) can determine how much heterogeneity was explained by subgroup differences. Sensitivity analyses were performed to assess the contributions of single studies to the final results. Generally, Begg test and Egger test were used to assess funnel plot asymmetry related to reporting publication bias.[28,29] When $Z < 1.96$ and $P > .05$ by Begg test or $P > .05$ by Egger test, we considered that publication bias did not existed. If bias exists, we use a conventional meta-trim method to re-estimate the effect size. A summary receiver operating characteristic (SROC) analysis was applied to test the diagnostic value of meta-analysis.[30,31] The SROC curve shows the performance of the diagnostic ability of *RASSF1A* promoter methylation to thyroid carcinoma susceptibility. The exact area under the curve (AUC) for the SROC function was used to assess the accuracy of the test.[30]

2.4. The extraction and analysis of GEO and TCGA data

DNA methylation information for thyroid carcinoma was collected from the GEO (Illumina Infinium Human Methylation 27 [HM27] Bead Array platform) (GSE51090) and TCGA (Illumina Infinium Human Methylation 450 [HM450] Bead Array platform) databases. There were 25,978 probes in HM27 and 485,577 probes in HM450. The methylation status of each probe was defined according to the beta-value (beta-value = intensity of the methylated allele/[intensity of the methylated allele + intensity of the unmethylated allele]). When a beta-value is greater than the empirical threshold of 0.3, the probe will be considered methylated.[32,33]

According to the UCSC database-Table Browser-assembly-Mar.2006 (NCBI 36/hg18), it is known that *RASSF1A* translation start site (TSS) locates at chr3: 50353240. We also found that *RASSF1A* promoter region contains 2 CpG islands: 1 includes 139 CpG sites (locates a chr3: 50349269–50350633), 1 contains 84 CpG sites (locates at chr3: 50352808–50353544). Therefore, 35 probes (cg26357744, cg06063729, cg14884256, cg07344955, cg11035216, cg10152523, cg13497155, cg19152024, cg21522636, cg27149285, cg06375085, cg26093954, cg22796393, cg15043975, cg08078366, cg06821120, cg21418575, cg02930432, cg09386807, cg00743929, cg20826201, cg23147362, cg06117233, cg07130266, cg24859722, cg13872831, cg00777121, cg04743654, cg12966367, cg08047457, cg25747192, cg21554552, cg27569446, cg25486143, cg06172942) in HM450 and 9 probes (cg06063729, cg06821120, cg06980053, cg11035216, cg15043975, cg26357744, cg00777121, cg08047457, cg21554552) in HM27 were taken as the object of our study.

The strength of *RASSF1A* promoter methylation in thyroid carcinoma susceptibility and clinical characteristics (age status, gender, and pathologic tumor stage) was measured by Chi-squared test. A P value $< .05$ was considered significant. Overall survival (OS) and disease-free survival (DFS) curves were calculated using the Kaplan–Meier method and compared by log-rank testing. The receiver operating characteristics (ROC) curve of both specificity and sensitivity of the sets was also constructed.

3. Results

3.1. Study characteristics

The literature search yielded about 60 published articles using the above keywords. After excluding those articles according to the prespecified exclusion criteria, 14 studies were used to report a relationship between the *RASSF1A* promoter methylation and thyroid carcinoma[8,18–21,34–42] (Fig. 1). In total, 652 thyroid carcinoma tissues and 325 normal counterpart tissues were collected (Table 1). The frequency of *RASSF1A* promoter methylation was 49.23% in the thyroid carcinoma samples and 9.54% in the normal control samples. Among the 14 studies, the patients of 5 articles were from the United States, 5 studies were belong to China, and 4 studies were from Germany, Italy, Sweden, and Iran, respectively (Table 1). Here, the United States was categorized as "mixed population"; China and Iran were categorized as "Asian"; and Germany, Italy, and Sweden were as "Caucasian." For the experimental methods to explore *RASSF1A* promoter methylation status, 9 of 14 inclusions used methylation-specific polymerase chain reaction (MSP), 2 used quantitative MSP (QMSP), while 1 used combined bisulfite restriction analysis (COBRA) and 2 used MethylScreen technol-

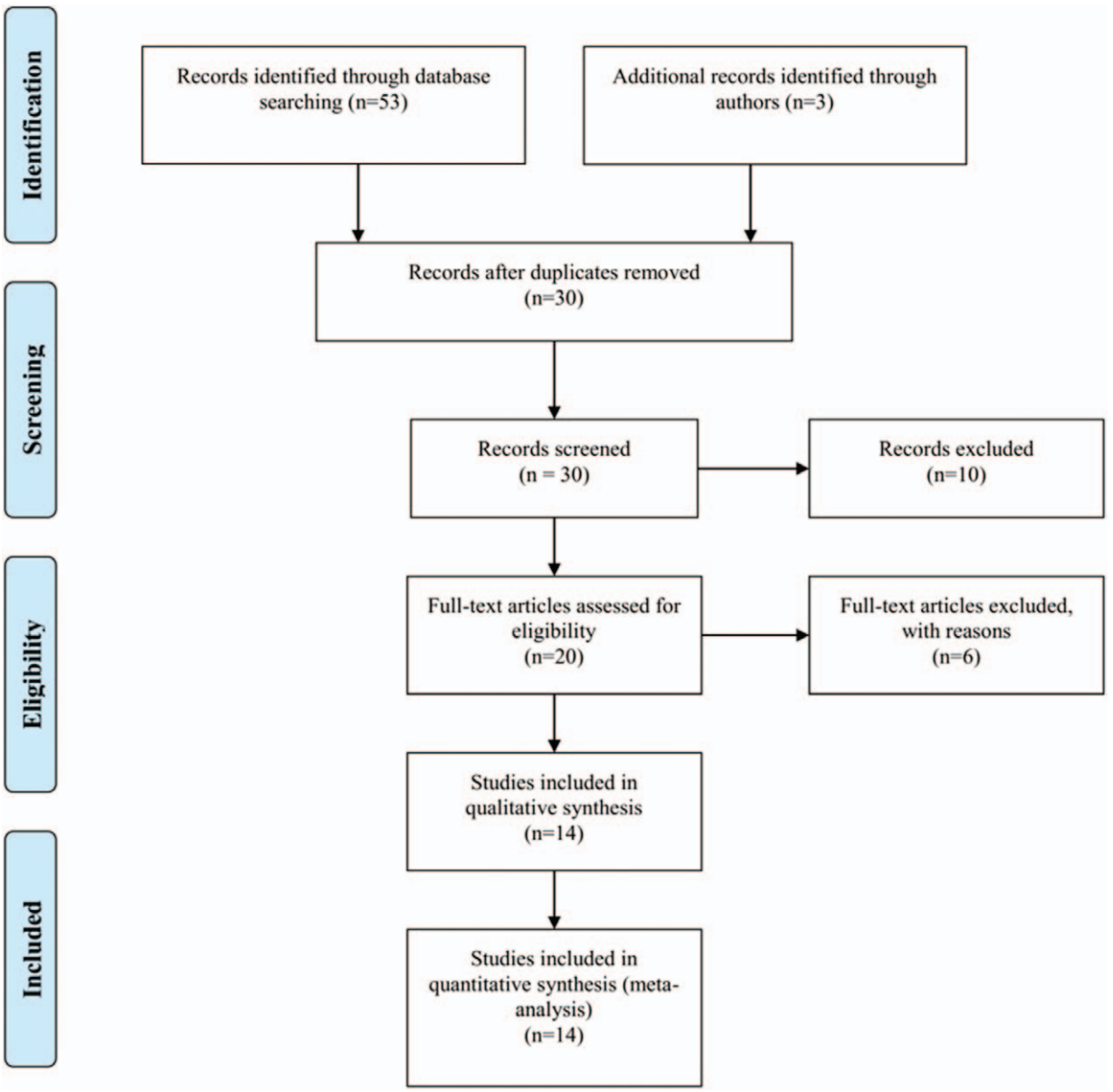

Figure 1. Flow chart shows study selection procedure and the distribution of the number of topic-related articles in the electronic database.

Table 1

Characteristics of eligible studies considered in the report.

Author	Year	Country	Sample	Method	M/F	Mean age, y	TNM stage	M_C	T_C	M_N	T_N
Brown et al	2014	The United States	Tissue	MethylScreen	11/33	49	I–II	30	44	2	42
Kunstman et al	2013	The United States	Tissue	MethylScreen	11/30	49.5	I–IV	7	41	0	18
Santoro et al	2013	Italy	Tissue	MSP	18/26	56.95	I–IV	9	44	0	44
Qu et al	2012	China	Tissue	MSP	6/22	43	I–III	15	28	7	28
Brait et al	2012	The United States	Tissue	QMSP	10/33	NA	I–IV	38	44	3	15
Dai et al	2011	China	Tissue	MSP	15/35	40	I–III	30	50	7	32
Mohammadi-asl et al	2011	Iran	Tissue	COBRA	7/18	NA	NA	8	50	1	26
Tang et al	2010	China	Tissue	MSP	5/29	41	I–IV	19	34	8	34
Wang et al	2009	China	Tissue	MSP	23/53	44.83	NA	63	78	0	10
Lee et al	2008	Sweden	Tissue	MSP	6/15	45.33	I–IV	18	21	1	21
Peng et al	2006	China	Tissue	MSP	9/22	41.74	NA	18	31	0	9
Hoque et al	2005	The United States	Tissue	QMSP	NA	NA	NA	11	71	1	15
Nakamura et al	2005	The United States	Tissue	MSP	NA	NA	NA	28	78	0	27
Schagdarsurengin et al	2002	Germany	Tissue	MSP	NA	NA	I–IV	27	38	1	4

Subscript "C/N" means cancer/normal.

COBRA = combined bisulfite restriction analysis, M = the number of patients with methylation, M/F = male/female, MSP = methylation-specific polymerase chain reaction, NA = not available, QMSP = quantitative MSP, T = the total number.

ogy (Table 1). Three kinds of methylation detection primers or probes were found to be utilized for the 14 studies. The information of the 3 sets of primers is listed in Table 2.

DNA methylation information for thyroid carcinoma was collected from GEO and TCGA databases including methylation 27K (HM27) and 450K (HM450) datasets. We analyzed 9 different probes from HM27 dataset and 35 different probes from HM450 dataset overlap the *RASSF1A* promoter region (Fig. 2A). We collected 83 primary thyroid cancer samples and 8 adjacent normal samples from GEO database (GSE51090). In the data from TCGA database, we collected 507 thyroid cancer samples and 56 adjacent normal tissue samples (Table S1, http://links.lww.com/MD/B957). Among the 507 patients, the patient's age ranged from 15 to 89 years, the mean age was 47.16 ± 15.59. In addition, 134 males and 364 females (Table S1, http://links.lww.com/MD/B957) among these patients, the American Joint Committee on Cancer (AJCC) pathologic tumor stage ranged from I to IV (Tables 3 and S1, http://links.lww.com/MD/B957). We chose 486 patients to analyze the methylation of *RASSF1A* gene promoter in thyroid cancer DFS, and 498 patients were for thyroid cancer OS.

3.2. The methylation of RASSF1A promoter in thyroid carcinoma susceptibility and clinical characteristics

Based on the meta-analysis, the OR for *RASSF1A* promoter methylation in cancer samples compared with that in normal controls were 10.22 (95% CI = [6.63; 15.74], z = 10.54, $P < .0001$) in fixed-effects model and 10.82 (95% CI = [5.65; 20.72], z = 7.1861, $P < .0001$) in random-effects model pooled, demonstrating a statistically significant increasing in likelihood of methylation in thyroid cancer samples comparing to normal controls (Fig. 2B). For heterogeneity of the meta-analysis, the H = 1.31, $I^2 = 42.1\%$ (0%; 69.1%), $P = .0489$, suggesting a significant heterogeneity between the 14 studies. Therefore, meta-regression by random effect was taken. Meta-regression reveals that the

Table 2

Three kinds of primers of the present 14 studies.

Author	Primer types	Forward primer 5′–3′	Reverse primer 5′–3′	Size, bp	Location to translation initiation site
Brown et al	Set III	NA	NA	775	−338 to +437
Kunstman et al	Set III	NA	NA	775	−338 to +437
Santoro et al	Set I	GTGTTAACGCGTTGCGTATC	AACCCCGCGAACTAAAAACGA	93	+214 to +308
Qu et al	Set II	GGGTTTTGCGAGAGCGCG	TAACAAACGCGAACCG	169	−203 to −34
Brait et al	Set II	GCGTTGAAGTCGGGGTTC	CCCGTACTTCGCTAACTTTAAACG	75	−85 to −10
Dai et al	Set I	GTGTTAACGCGTTGCGTATC	AACCCCGCGAACTAAAAACGA	93	+214 to +308
Mohammadi-asl et al	Set II	GGTTYGYGTTTGTTAGYGTTTAAAGTT	CTCAAACTCCCCCRACATAA	70	−203 to −133
Tang et al	Set II	GGGTTTTGCGAGAGCGCG	TAACAAACGCGAACCG	169	−203 to −34
Wang et al	Set I	GTGTTAACGCGTTGCGTATC	AACCCCGCGAACTAAAAACGA	93	+214 to +308
Lee et al	Set II	CGAGAGCGCGTTTAGTTTCGTT	CGATTAAACCCGTACTTCGCTAA	192	−195 to −3
Peng et al	Set I	GTGTTAACGCGTTGCGTATC	AACCCCGCGAACTAAAAACGA	93	+214 to +308
Hoque et al	Set II	GCGTTGAAGTCGGGGTTC	CCCGTACTTCGCTAACTTTAAACG	75	−85 to −10
Nakamura et al	Set I	GTGTTAACGCGTTGCGTATC	AACCCCGCGAACTAAAAACGA	93	+214 to +308
Schagdarsurengin et al	Set I	GTGTTAACGCGTTGCGTATC	AACCCCGCGAACTAAAAACGA	93	+214 to +308

Primer Set I = +214 to +308, primer Set II = −203 to −3, primer Set III = −338 to +437. cg06063729, cg06821120, cg06980053, cg11035216, cg15043975, cg26357744, cg00777121, cg08047457, and cg21554552 are covered by the replication region of Sets I–III primers.

NA = not available.

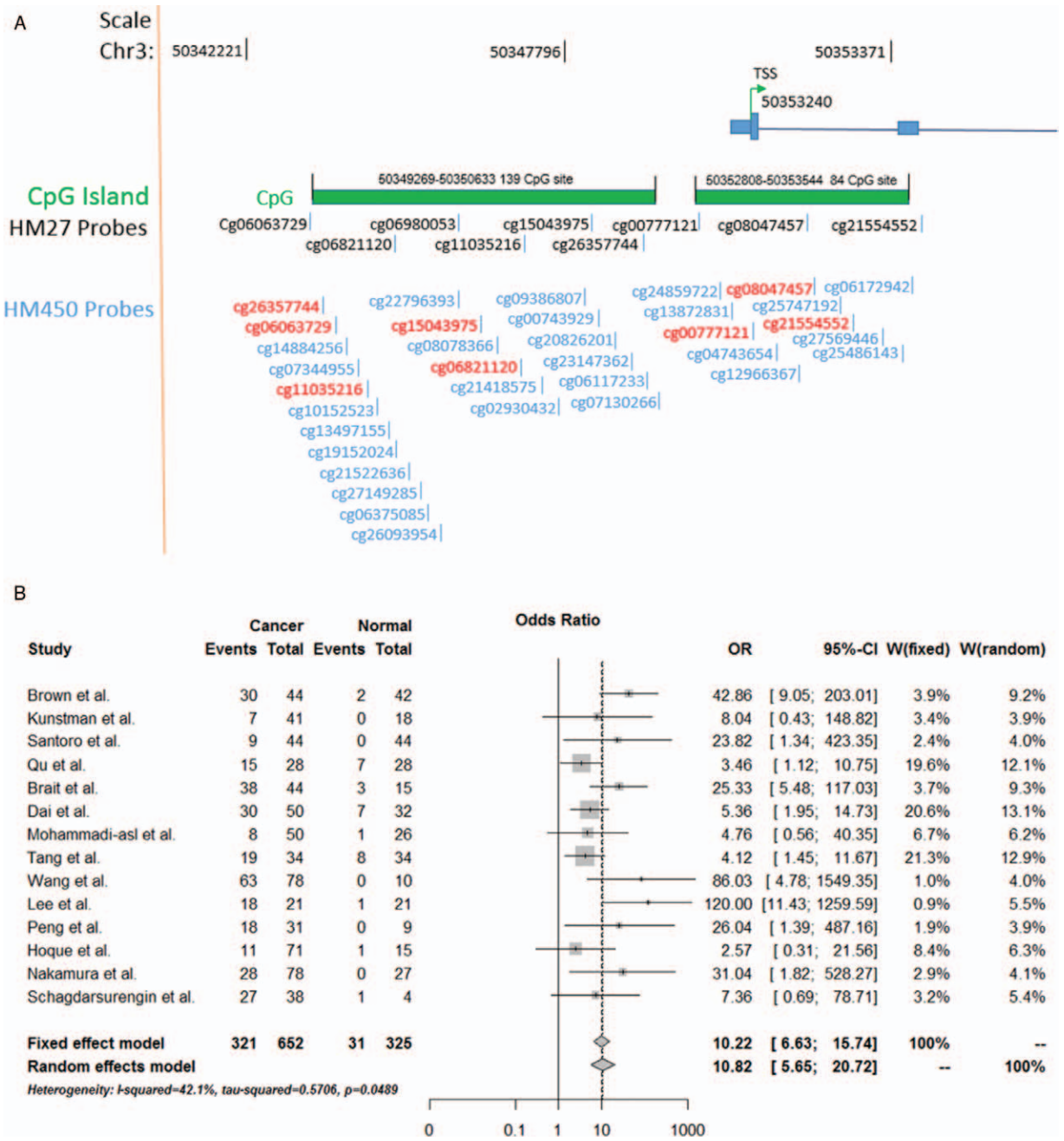

Figure 2. (A) DNA methylation probes matching *RASSF1A* promoter region CpG islands. (B) Meta-analysis for the methylation of *RASSF1A* promoter in thyroid carcinoma susceptibility based on random-effects model and fixed-effects model.

Table 3

The methylation of *RASSF1A* gene promoter in thyroid carcinoma clinical characteristics.

	N	Methylation, n/%	OR	95% CI	*P**
Age ≤ 50	290	126/43.45	1.81	1.26–2.60	.001
Age > 50	208	121/58.17			
Stage I–II	333	154/46.25	1.51	1.03–2.10	.033
Stage III–IV	163	92/56.44			
Female	364	176/48.35	1.20	0.81–1.70	.359
Male	134	71/52.99			

CI = confidence interval, N = the number of patients, n/%, number/frequency, OR = odds ratio.
* From *t* test or Wilcoxon rank-sum test.

primer sets are an important heterogeneity source. It explains 100% of overall heterogeneity, as the subgroup analyses demonstrate the same result (Fig. S1A, http://links.lww.com/MD/B956). However, other factors, such as ethnicity and detection methods, fail to explain heterogeneity (Fig. S1B and C, http://links.lww.com/MD/B956). We then performed bias analysis and sensitivity analysis of the 14 articles. The visual assessment of the Begg test ($Z=1.5876$, $P=.1124$) and Egger test ($t=2.2957$, $df=12$, $P=.04051$) did not reveal any evidence of obvious asymmetry of funnel plot (Fig. S2A, http://links.lww.com/MD/B956). Therefore, there does not appear to be any publication bias in the 14 studies. Sensitivity analyses were conducted to determine the effect of omitting a single study on the overall effect, the overall ORs were between 8.99 (95% CI=[4.93;16.40]) and 12.58 (95% CI=[6.36; 24.9]) in the random-effects method, which suggested that combined OR was consistent and reliable (Fig. S2B, http://links.lww.com/MD/B956).

Using data obtained from GEO and TCGA databases, we were able to compare the frequency of *RASSF1A* promoter methylation in thyroid carcinoma samples and normal control samples. Among the 83 thyroid carcinoma samples and 8 normal control samples from GEO database, there was 70 (84.34%) patients has *RASSF1A* gene promoter methylation and none in normal control. We then analyzed the methylation status of *RASSF1A* promoter in TCGA database, and found a higher frequency methylation in thyroid carcinoma samples (49.51%) than normal control (1.79%). Therefore, as similar to the meta-analysis result, a significant difference was found in *RASSF1A* promoter methylation of thyroid carcinoma samples and normal control by GEO (OR=6.39) and TCGA databases (OR=53.93) ($P<.0001$; Fig. 3A and B).

SROC analysis was assessed in the meta-analysis of diagnostic tests. The pooled sensitivity, specificity, and AUC of the *RASSF1A* promoter methylation test in the meta-analysis were 0.51 (0.36–0.67), 0.94 (0.86–0.97), and 0.87 (0.83–0.89), respectively, which revealed that this meta-analytic method represents a good quantitative approach to summarize the performances of diagnostic tests (Fig. S3A, http://links.lww.com/MD/B956). We also used ROC curve as the diagnostic tests for the *RASSF1A* promoter methylation in GEO and TCGA databases. The AUC was 0.922 for GEO data and 0.739 for TCGA data, suggesting a fair ability for thyroid carcinoma diagnosis by these 2 databases (Fig. S3B and C, http://links.lww.com/MD/B956).

DNA methylation is thought to be linked to certain clinical characteristics, such as age status and pathologic tumor stage. Therefore, using TCGA data, we conducted further analysis based on age status, gender status, and pathologic tumor stage. Significant differences were found between the OR=1.81 ($P=.001$) of the younger (age $\leq$ 50) and older (age $>$ 50) (Table 3). The patients with stage III to IV had a significantly bigger OR=1.506 than that stage I to II patients ($P=.033$) (Table 3), suggesting that advanced thyroid carcinoma has a high frequency of *RASSF1A* gene promoter methylation. However, there is nonsignificantly between the OR=1.204 ($P=.359$) of males and females in thyroid carcinoma patients (Table 3).

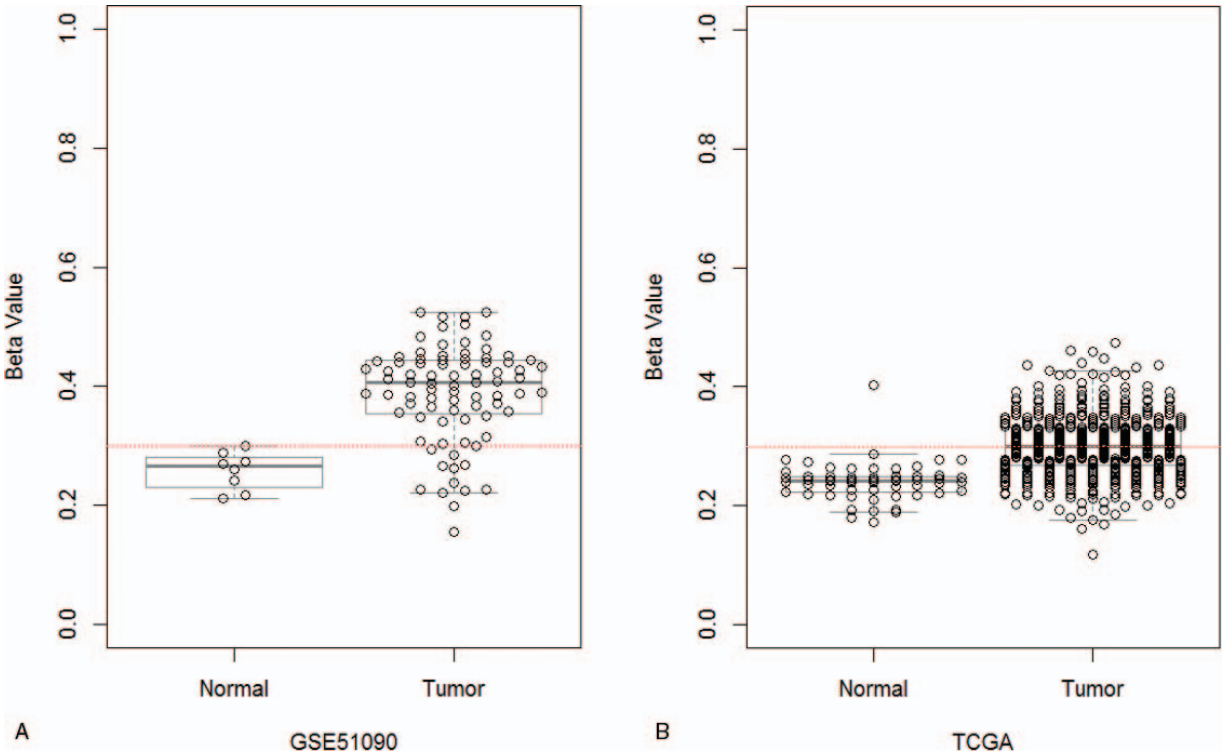

Figure 3. The relationship between *RASSF1A* promoter methylation and thyroid carcinoma susceptibility using the GEO and TCGA databases. (A) The relationship between *RASSF1A* promoter methylation and thyroid carcinoma susceptibility using the GEO database. The $P=3.88\times10^{-8}$ by *t* test. (B) The relationship between *RASSF1A* promoter methylation and thyroid carcinoma susceptibility using TCGA database. The $P=2.01\times10^{-20}$ by *t* test. Red-dotted line indicates beta-value=0.3. GEO = Gene Expression Omnibus, TCGA = the Cancer Genome Atlas project.

3.3. The methylation of RASSF1A promoter in thyroid carcinoma prognosis

The methylation of the *RASSF1A* promoter in cancers prognosis has been research by several studies.[43–45] However, the role of the *RASSF1A* promoter methylation in the prognosis of thyroid carcinoma was not known. Here, the data extracted from TCGA project were conducted to evaluate the relationship between the *RASSF1A* promoter methylation and prognosis as defined by OS and DFS in all thyroid carcinoma patients (Table S1, http://links.lww.com/MD/B957). The hazard ratio (HR) of 486 thyroid carcinoma patients analyzed for DFS was 2.63 (95% CI=[1.28; 5.38], $P=.0116$). Among these 486 thyroid carcinoma patients, 30 patients were recurrence and 21 with *RASSF1A* promoter methylation (Table S1, http://links.lww.com/MD/B957). Therefore, this result demonstrates that thyroid carcinoma patients with the *RASSF1A* promoter methylation have higher chance of recurrence after surgery or other treatment (such as chemotherapy and combined treatment) than that unmethylation patients (Fig. 4A). In addition, the HR was found to be 1.26 for TCGA data (95% CI=[0.45; 3.62], $P=.6635$) (Fig. 4B) for OS when we used 498 thyroid carcinoma patients analyzed by the Kaplan–Meier method, which suggests that with the *RASSF1A* promoter methylation is not associated with the OS of thyroid carcinoma patients.

4. Discussion

RASSF1A has been reported as an important tumor suppressor in numerics for cancers, such as breast[46] and lung cancers.[47] Although, in thyroid carcinoma, several studies found that the frequency of *RASSF1A* gene methylation in cancer was significant higher than normal control, 2 studies found the frequency of *RASSF1A* gene methylation in cancer samples was not or lower than in benign samples.[22,23] Considering this opposite view in the relationship between *RASSF1A* promoter methylation and thyroid carcinoma, we carried out this study, containing meta-analysis and bioinformatics analysis, to evaluate the relationship between *RASSF1A* promoter methylation and thyroid carcinoma.

In this study, higher methylation of the *RASSF1A* promoter has been found to frequently occur in thyroid carcinoma samples than normal control samples. Meanwhile, higher frequency of *RASSF1A* promoter methylation was also found in older and advanced stage patients, suggesting that *RASSF1A* promoter methylation may be an early event in thyroid tumorigenesis and carcinoma development. The HR for DFS was 2.63 (95% CI=[1.28; 5.38], $P=.0116$), which suggests that *RASSF1A* promoter methylation is associated with the DFS of thyroid carcinoma patients. Previous researches have shown that recurrence is a common event in thyroid carcinoma patients,[6,7] so the methylation of *RASSF1A* promoter in thyroid carcinoma has a higher probability of recurrence.

There was some heterogeneity in the present meta-analysis, and primer sets were the most important heterogeneity sources from meta-regression analysis. In addition, the opposite result with us by previous research due to it used the benign thyroid tumor samples as the control.[22] Therefore, considered the pooled sensitivity (0.51), specificity (0.94), and AUC (0.87) of the *RASSF1A* methylation test in the present meta-analysis, *RASSF1A* methylation status may be a good biomarker in thyroid carcinoma diagnosis. Many studies have shown that aberrant methylation of the promoter genes plays a potential role in the formation and progression of thyroid cancer.[48,49] Meanwhile, in the absence of genome-wide methylation changes case, the promoter methylation of *RASSF1A* gene may serve as an important methylation event with a potential driving effect on the early stages of thyroid neoplasia formation. Aberrant methylation of the tumor suppressor gene promoter, containing *RASSF1A* promoter, may lead to a further abnormal change of genome methylation in the late stages of thyroid cancer.[50,51]

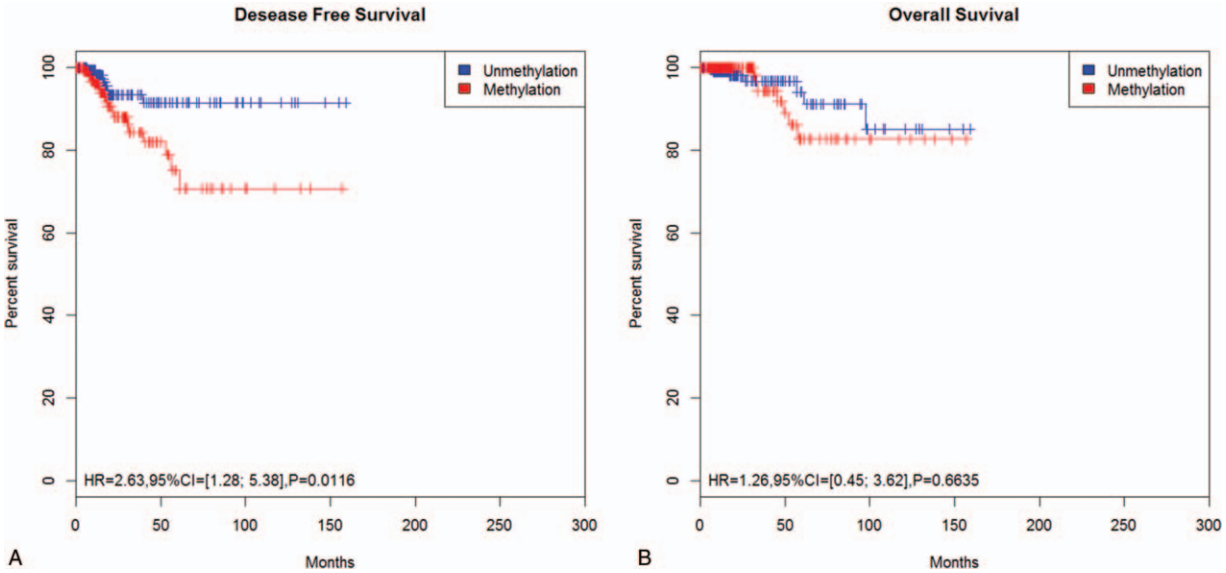

Figure 4. Association of thyroid carcinoma patient survival and *RASSF1A* promoter methylation status by the Kaplan–Meier method. (A) Disease-free survival curves by methylation status of *RASSF1A* promoter. The number of censored cases methylation and unmethylation was 244 and 242, respectively. (B) The Kaplan–Meier survival analysis of overall survival showing the association between thyroid carcinoma and *RASSF1A* methylation status. The number of censored cases methylation and unmethylation was 251 and 247, respectively.

Therefore, any single tumor suppressor gene promoter methylation change in thyroid cancer may occur as a random event on the abnormal regulation of genome-wide epigenetics.

5. Conclusion

In conclusion, this quantitative assessment provides a strong evidence that the methylation status of the *RASSF1A* promoter is strongly associated with thyroid cancer susceptibility and patient prognosis. Meanwhile, *RASSF1A* promoter methylation is strongly associated with an advanced stage and older patients. Therefore, methylation of the *RASSF1A* promoter can be a promising diagnostic assay for the clinical diagnosis of thyroid cancer.

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