# Promoter methylation of eight tumor-suppressor genes and the risk of thyroid cancer: A meta-analysis protocol

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## Abstract

## Introduction

The incidence of thyroid cancer is increasing and histological test by itself cannot differentiate thyroid cancer from some benign nodules. Our immediate goal is to meta-analysis and determines the impact of promoter methylation of eight selected candidate TSGs on thyroid cancer and to identify the most important molecules in this carcinogenesis pathway.

## Methods and analysis

We will include observational studies evaluating the promoter methylation in patients with thyroid cancer. A comprehensive search was performed using PubMed, Scopus, and ISI Web of Knowledge databases, and eligible studies were included. The methodological quality of the included studies was evaluated according to the Newcastle Ottawa scale table and pooled odds ratios (ORs); 95% confidence intervals (CIs) were used to estimate the strength of the associations with Stata 12.0 software. Egger's and Begg's tests were applied to detect publication bias, in addition to the "metatrim" method.

### Ethics and dissemination

No ethical issues are predicted. These findings will be published in a peer-reviewed journal and presented at national and international conferences.

### Registration number

This systematic review protocol is registered in the PROSPERO International Prospective Register of Systematic Reviews, registration number (CRD42016033484).

### Strengths and limitations of this study

This systematic review, for the first time, will conduct to evaluate the prognostic and diagnostic accuracy of DNA methylation in patients with thyroid cancer using comprehensive search of several databases. The study screening, data extraction, and risk of bias assessment of the current study will be conducted by two researchers independently. We expect some potential heterogeneities between previous studies, including stage, and histological grade in patient samples.


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## Protocol

Planning to write a systematic review over candidate promoter methylation in Thyroid cancer
**Step 1.**

# Introduction

The National Cancer Institute indicated to the fact that in 2010 there would be 44,670 new cases of thyroid cancer with 1,690 deaths (1) . In comparison with other cancers, thyroid cancer usually occurs in younger people between the ages of 20 and 60 years (2, 3). Thyroid cancer is categorized to a number of histological types and subtypes based on cellular origins and characteristics (3, 4), however it is typically composed of two types of thyroid cells, Follicular thyroid cells and Para follicular C cells. In recent times, scientists have verified the involvement of genetic and epigenetic alterations in the development and management of cancers including thyroid cancer (5-13). More than genetic mutation (14) that changes the gene expression of genes (15, 16), alterations in the genome through DNA methylation or histone modification go ahead of the changes in the expression of target genes (17-19). More often than not epigenetic alterations are observed by aberrant gene methylation, an epigenetic hallmark of human cancers, including thyroid cancer (20, 21). DNA methylation is a process in which methyl groups are added to the DNA molecule, altering the activity of the DNA segment exclusive of changing the nucleotide sequence. Hypomethylation and over expression of may genes are observed in various cancers (22). Several studies have shown the association of gene promoter methylation in thyroid cancers (18, 23-26).

### Objectives

In this study, we will run a comprehensive meta-analysis of candidate genes associated with methylation in patients with thyroid cancer in order to shed a light on the most effective tumor suppressor gene as a progression risk factor towards thyroid carcinogenesis.

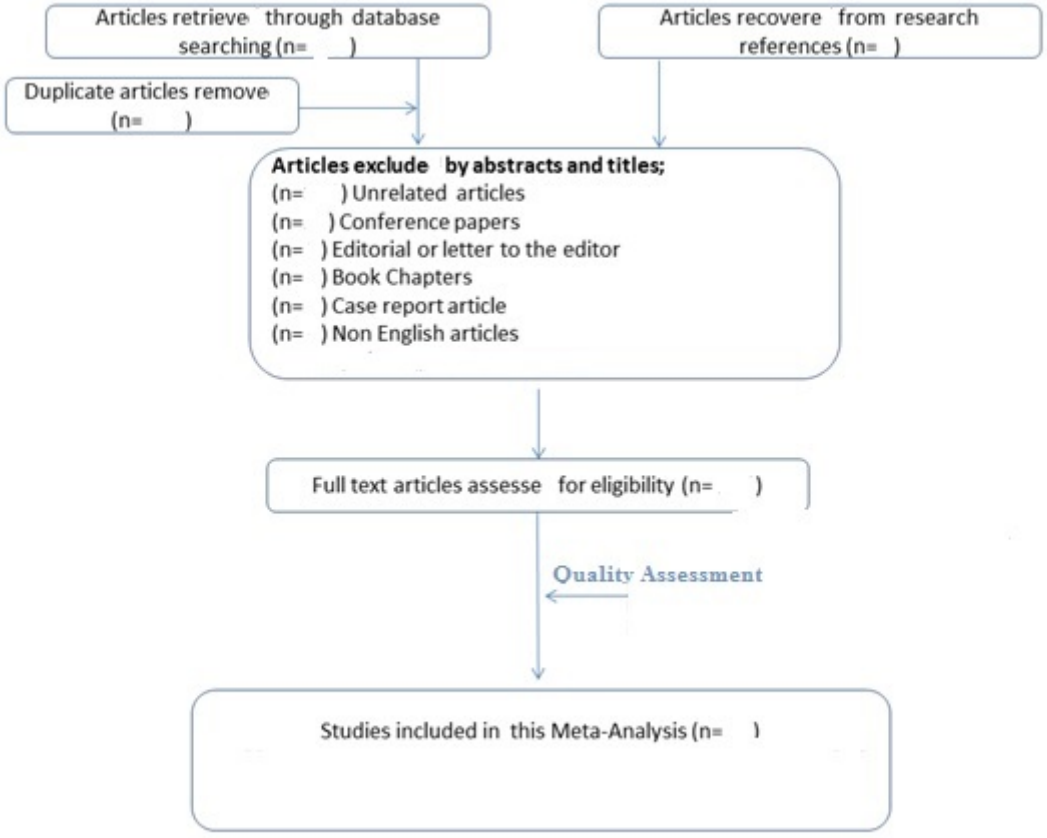
# Materials and Methods

The current meta-analysis is designed according to the latest version of the PRISMA checklist for meta-analysis guidelines.

# Publication selection

This study (Prospero code: CRD42016033484) will conducted using PubMed, Scopus, and Web of Science search databases. Studies published between January 1, 2000 and October 1, 2016 will be considered. The following key words will be used: “methylation” or “hypermethylation” and “thyroid

cancer” or “thyroid neoplasm” or “thyroid tumor” or “thyroid carcinoma”. Additionally, the references of the selected articles and related review articles will be manually reviewed in order to identify any additional studies (figure 1).




**Step 2.**

Select relevant studies for Meta analysis

**Step 3.**

# Inclusion and exclusion criteria

All nominated studies will be reviewed by two authors separately. The purpose of our investigation is to identify definite gene promoter methylation in tumor tissue (fresh-frozen tissue, formalin-fixed paraffin-embedded [FFPE] samples, and plasma) from patients with thyroid cancer and normal sex- and age-matched controls. Normal adjacent tissue of thyroid cancer or guiterous samples are defined as normal adjacent tissue of thyroid cancer (NPTC), goiter, and nodular goiter (NG) samples. For normal controls, samples should be the same or similar tissue type as those collected from patients with thyroid cancer. Studies with insufficient data despite contacting the author will be excluded.

Data Analysis and quality assessmnets

**Step 4.**

# Data collection

The following information will be extracted from each study: first author, year of publication, country of research, type of sample, method for methylation determination, pathological stage, type of tissue (tumor and control), name of targeted gene(s), and frequency of promoter methylation in the target gene(s) in both tumor and normal thyroid tissues.

# Quality assessment of individual studies

The quality of each study will be  assessed according to the Newcastle-Ottawa Scale (NOS) assessment tool (27). Articles containing case control studies will be scored according to the selection, comparability, and exposure.

### Assessment of heterogeneity

To investigate heterogeneity, we will include study design (prospective or retrospective and year of publication), population characteristics (gender, ethnicity, age, disease types, and stage distribution), test characteristics (test type), and versions of reference standards as our study-level variables. We will test these study-level covariates in the bivariate model in the common threshold or add them to the Rutter and Gatsonis HSROC model to evaluate heterogeneity in test threshold, diagnostic accuracy, and the shape of curves. The likelihood ratio test will be used to determine the statistical significance of the covariates included in the models.

### Assessment of reporting bias

The publication bias will be assessed by funnel plots (ie, plots of study results against precision) and Begg’s and Egger’s tests.

# Statistical analysis and data synthesis

All included studies will be overviewed and will be presented in two separate tables. One table will provide details on study quality according to the *Newcastle-Ottawa* Quality Assessment Form tool. The other table will include study design, participants, test characteristics, and results. The following test characteristics will be extracted into 2 × 2 tables for all included studies, Study-specific estimations of sensitivity and specificity with 95% confidence intervals will be displayed in forest plots using review Manager (Version 5.2). These graphical displays will reveal the variations in accuracy among the studies and the different types and brands of the index test.

Final analysis

**Step 5.**

**Inferential statistics:**

All the included studies will be synthesis after systemic review. Study-specific Odds Ratio,

Sensitivity and Specificity estimates will be pooled using a fixed-effects model if no significant heterogeneity exists, otherwise a random-effects model will be applied. The extent of heterogeneity across studies will be checked using the chi-squared test and I-squared test; p=0.10 and/or I2>50% indicating significant heterogeneity. For evaluating source of heterogeneity, sub-group analysis and meta-regression will be used. The potential for publication bias was assessed using funnel plot, the Begg rank correlation method, and the Egger weighted regression method. A p-value of = 0.05 will be considered to

be statistically significant.

**Statistical analysis**

Stata 12.0 (Stata Corporation, TX, USA) will be used in the current meta-analysis. The odds ratios (ORs) and 95% confidence intervals (CIs) will be applied to evaluate the association between promoter methylation of the eight TSGs and the risk of thyroid cancer (28). Q-tests based on the $\chi^2$ statistic and $I^2$ statistic will be employed to investigate the heterogeneity among the studies (29-31). If substantial heterogeneity existed ($P$<0.05 for the Q statistic or $I^2$>50%), a random effect model will be applied to pool the ORs; if not, a fixed effect model was applied (32, 33). In addition, a meta-regression analysis will be performed to discover the underlying reasons for statistical heterogeneity. Sensitivity analysis will be carried out to measure the effects of single studies on the overall estimate by ignoring one study at a time. A funnel plot, the trim-and-fill method, Begg's test, and Egger's test will be applied to assess publication bias. All tests will be two-sided, and results with $P$ values of less than 0.05 were considered significant.

## Contributors

SMT was involved in study design and developed the search strategies. FKH was involved in study design, implementation, and analysis and also drafted the manuscript of the protocol and SMT revised it. MA and LT will also screen the potential studies, extract data and assess quality. Any discrepancies will be resolved by consensus between the two authors.

## Competing interests

The authors declare no conflict of interest.


## Funding

No.


## Data sharing statement

All recorded data from the data extraction process will be available on request to the extent that it is not included in the systematic review article.

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