## Original Article
# Methylation of sodium iodide symporter promoter correlated with aggressiveness and metastasis in papillary thyroid carcinoma: a meta-analysis

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**Abstract:** Background: Methylation of sodium iodide symporter promoter has been reported to increase the incidence of papillary thyroid carcinoma (PTC). In this meta-analysis stratified via methylation of sodium iodide symporter promoter, we evaluate the relationship between methylation of sodium iodide symporter promoter and PTC. The association between methylation with aggressiveness and metastasis potential of PTC is also discussed. Methods: We searched electronic databases for original articles and references of included studies both in English and Chinese from 1966 to 2014. Two reviewers selected the case-control study and extracted data from relevant literature independently. Results: Seven articles, including 360 cases and 268 controls, were involved in this meta-analysis. The prevalence of PTC in patients with methylated sodium iodide symporter promoter was significantly higher than those with non-methylated promoter (OR=7.36, 95% CI: 4.25-12.74, $P<0.001$). Stratified analysis showed that PTC patients with multiple lesions, capsule invasion and lymphatic metastasis had significantly higher rates of methylation (OR=2.22, 95% CI: 1.12-4.41, $P=0.02$; OR=2.14, 95% CI: 1.12-4.08, $P=0.02$; OR=3.56, 95% CI: 1.97-6.46, $P<0.0001$). But no relationship was found among the methylation of sodium iodide symporter and age, gender and size of tumor. Conclusions: The methylation of sodium iodide symporter promoter is related with PTC and its aggressive and metastatic potential. Due to the limited sample size, more clinical researches should be taken in the future.

**Keywords:** Sodium iodide symporter, methylation, papillary thyroid carcinoma, aggressiveness, metastasis

## Introduction

Papillary thyroid carcinoma (PTC), the most common type of thyroid cancer (75-80%), originates from thyroglobulin-producing follicular cells [1]. Postoperative radioactive iodine therapy is recommended to eradicate remaining thyroid tissue and the metastasis, and it is effective for most of PTC patients [2]. But there still exists 20-30% patients with failure therapy which may cause the recurrence, local or distant metastasis, and about 1% patients died [3]. As the increased prevalence of PTC, patients with recurrence, metastasis and even death increase year by year. Clearing the reason of failure therapy is imminent.

Sodium iodide symporter (NIS), a kind of glyco-protein locating in the basolateral membrane of thyroid follicular cell, is involved in the biosynthesis of triiodothyronine and thyroxine by transporting the iodine ions into the cavity of thyroid follicular cell. Therefore, radioactive iodine (iodine ions labeled with 131) can be largely took up by NIS and the released beta-ray destroys the thyroid cells. Once the ability of transport influenced, the iodine ions are unable to enter and this causes the failure of treatment. That is the reason why anaplastic thyroid cancer, which is unable to take up radioactive iodide, is invariably fatal. The factors that can influence the ability of transport include the following potential reasons: NIS cannot properly target to the plasma membrane [4, 5] and abnormal expression of NIS gene and protein [6-8]. Because of the human NIS (hNIS) promoter has CpG-rich regions, which associated with transcription; DNA methylation may be respon-
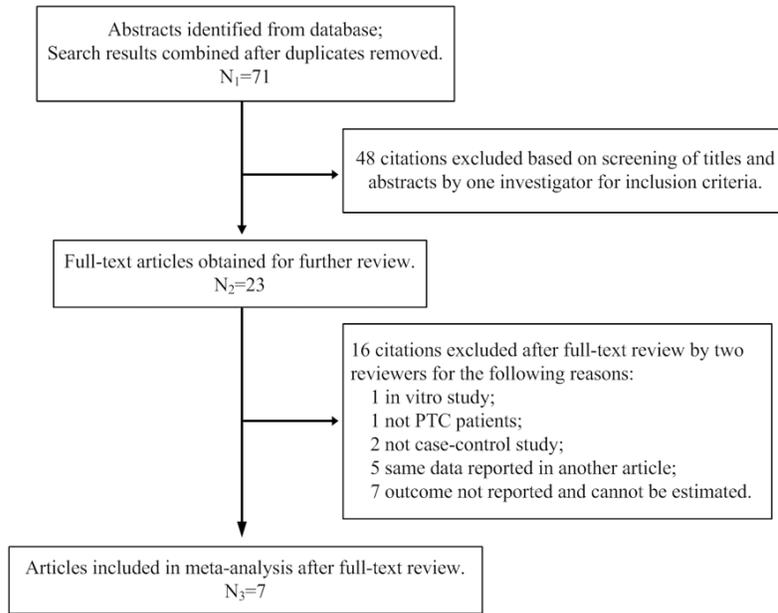
Figure 1. Literature search flow diagram.

sible for the altered expression of NIS. The decreased expression of NIS mRNA in thyroid cancer has been suggested to be secondary to methylation of the promoter [9-11]. Caillous et al. [10] also found that PTC patients with lower or without iodide-concentrating ability always accompanied with the methylated NIS. In addition, demethylation agents have been suggested to restore iodide transport in dedifferentiated thyroid cancer. But whether patients with PTC have higher rate of methylated NIS promoter was controversial. Some reported that NIS promoter methylation is a low frequency event in PTC patients and no statistical evidence was found [12, 13]. While other studies showed the significant difference of methylation in specimens from PTC patients comparing with controlled specimens [9, 14-17] Potential relationships among methylation and characteristics of tumor, especially invasiveness, were also found by some studies [12, 13, 15-17]. Both the number of studies in this field and the number of patients in each study were limited. So, we conducted a meta-analysis to evaluate the association between methylation of NIS promoter and PTC, the methylation with aggressive and metastatic potential of PTC.

## Methods

### Searching progress

We conducted a search of the following databases: PubMed, Embase, Cochrane library, Sinomed, CNKI, Wanfang, for case-control study of NIS promoter methylation status in patients with PTC. Databases were searched from the earliest data to 31 July 2014. Search terms used were [sodium-iodide symporter OR thyroid iodide transporter OR sodium iodide symporter OR sodium-iodide cotransporter OR Na-I-cotransporter OR NIS OR SLC5A5 OR solute carrier family 5, member 5] AND [methylation OR epigenomics OR methylated OR epigenetic*] AND [thyroid cancer, papillary OR thyroid neoplasms OR papillary thyroid carcinoma OR PTC OR thyroid carcinoma, papillary OR papillary carcinoma of thyroid OR ((thyroid OR papillary thyroid) and (carcinoma OR cancer OR neoplasm OR tumor))]. References of all eligible articles and related previous review were also hand searched.

Eligible studies met the following criteria: (i) published in English or Chinese; (ii) case-control study design; (iii) primary study comparing the NIS promoter methylation status of PTC tissue with none tumor tissue; (iv) samples in the PTC group were obtained from the lesions of PTC; (v) samples in the control group were collected from the benign tissue adjacent to the malignant tumors or the benign tissue from patient without thyroid cancer; (vi) the number of events, or odds ratios (OR) and 95% confidence intervals (CIs) that can indirectly calculated were reported.

### Study selection and data extraction

Two reviewers screened the abstracts and extracted data from included studies using data extraction forms, independently. Referred to the trial report and discussion between two reviewers were conducted to resolve the disagreements. The third reviewer would decide if an agreement still not reached. The following information should be extracted: (i) general characteristics of studies (including first authors' name, year of publication, age, sex ratio and sample size of two groups) and the inclusion criteria; (ii) original tissue of control

**Table 1.** General characteristics of studies

| Author, year | Age (year) | Sample size | | Male (%) | | Original of control | Test method | Quality assessment according to the NOS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PTC | Control | PTC | Control | | | Section | Comparability | Exposure | Total |
| Venkataraman, 1999 | - | 16 | 5 | - | | - | MSP | 3 | 1 | 2 | 6 |
| Smith, 2007 | 54.7 | 32 | 27 | 59.4 | 88.9 | A | MSP | 4 | 2 | 2 | 8 |
| Tang, 2007 | 41.0 | 34 | 34 | 14.7 | 14.7 | A | MSP | 4 | 2 | 2 | 8 |
| Yin, 2009 | - | 11 | 11 | 18.2 | 18.2 | A | MSP | 4 | 2 | 2 | 8 |
| Shi, 2009 | 44.0 | 60 | 20 | 20.0 | - | A | MSP | 3 | 1 | 2 | 6 |
| Xu, 2010 | 43.6 | 152 | 152 | 15.1 | 15.1 | A | qMSP | 4 | 2 | 2 | 8 |
| Liu, 2013 | 30.7 | 55 | 19 | 36.4 | 63.2 | H | MSP | 3 | 1 | 2 | 6 |

Footnotes: PTC: papillary thyroid carcinoma; A: autologous control, benign tissue adjacent to the malignant tumors; H: heterogeneous control, benign tissue from patient without thyroid cancer; MSP: methylation-specific polymerase chain reaction; qMSP: real-time fluorescent quantitative methylation specific polymerase chain reaction. NOS: Newcastle-Ottawa Scale.
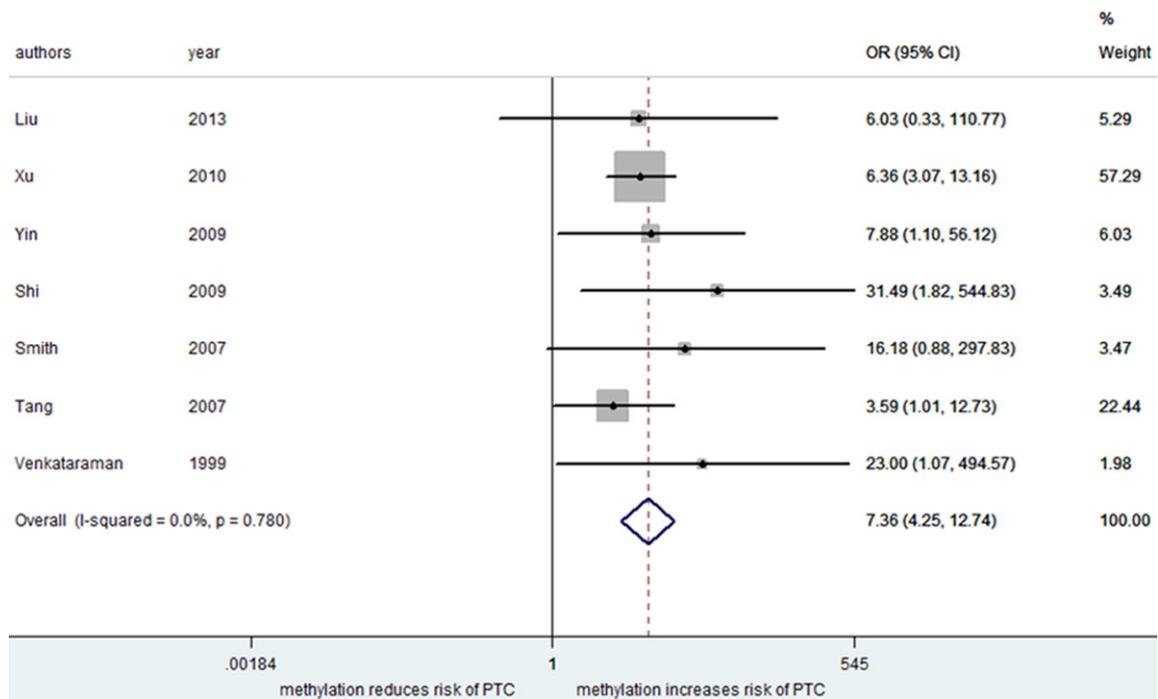


**Figure 2.** Forest plot of methylation of NIS promoter from studies of PTC to control.

group and test method; and (iii) type of outcome and measurement.

*Statistical analysis*

Gene-specific methylation status of NIS promoter was compared in the PTC and control specimens. The meta-analysis with fixed effects model was performed by computing OR and 95% CI for outcomes of dichotomous variables. The statistical analysis method used in the current study for each analysis was Mantel-Haenszel method. $I^2$ was calculated as an index of heterogeneity between studies. The computation formula shows below:

$$I^2 = {}^{Q-(K-1)}/_{Q} \times 100\% = {}^{Q-df}/_{Q} \times 100\% = max(0, {}^{Q-df}/_{Q} \times 100\%)$$

Q is the chi-square value of heterogeneity test. K is the number of studies in the analysis.

The degree of heterogeneity was divided by the level of $I^2$ as following: 0-25%, no heterogeneity; 25-50%, moderate heterogeneity; 50-75%, large heterogeneity; 75-100%, extreme heterogeneity [18]. Sensitive analysis and subgroup analysis should be performed to find out the source of heterogeneity if $I^2$ was higher than 50%. If no heterogeneity exists, the fixed effects model was performed. Otherwise, the

**Table 2.** Stratified analysis of the association between NIS methylation and characteristics of PTC

| Group | Number of study | Fixed effects model | | Heterogeneity | |
| --- | --- | --- | --- | --- | --- |
| | | OR (95% CI) | P | $I^2$ (%) | P |
| Age | 3 [12, 16-17] | 1.57 (0.85-2.92) | 0.15 | 0 | 0.37 |
| Gender | 3 [12, 16-17] | 1.31 (0.57-3.03) | 0.53 | 0 | 0.88 |
| Size | 2 [12, 16] | 1.32 (0.69-2.54) | 0.40 | 14 | 0.28 |
| Number of lesions | 2 [16-17] | 2.22 (1.12-4.41) | 0.02 | 0 | 0.71 |
| Capsule invasion | 2 [12, 16] | 2.14 (1.12-4.08) | 0.02 | 34 | 0.22 |
| Lymphatic metastasis | 5 [12-13, 15-17] | 3.56 (1.97-6.46) | <0.0001 | 15 | 0.32 |

random effects model was used. Funnel plots of primary outcome by visual inspection was used to assess the potential publication bias [19]. The Begg rank correlation and Egger linear regression test with the natural log of the OR versus its standard error were applied as well [19, 20].

*Quality assessment and risk of bias*

The quality assessment was assessed via Newcastle-Ottawa Scale (NOS) which consists of the following parameters: (i) selection (is the case definition adequate?; representativeness of the cases; selection of controls; definition of controls); (ii) comparability (comparability of cases and controls on the basis of the design or analysis); (iii) exposure (ascertainment of exposure; same method of ascertainment for cases and controls; non-response rate) [21]. Two reviewers determined these items, independently. Sensitive analysis was performed in studies with low quality. This meta-analysis was performed by using Stata SE.

*Role of the funding source*

The sponsor of this study had no role in study design, data collection, analysis, interpretation, or writing of this manuscript. The corresponding authors, Zhao JY, Wang HJ, Dong JJ and Liao L had full access to all the data and final responsibility for the decision to submit this report.

**Results**

*Search results and study characteristics*

The initial search strategy found 71 articles and 23 were selected for full-text review. Of these, 12 articles met the inclusion criteria and five duplicate published articles were removed. Finally, seven articles including a total of 360 cases and 268 controls were included in this meta-analysis. Searching progress is summa-

rized in **Figure 1** and the general characteristics of the seven trials are summarized in **Table 1**. Among the final seven studies, five were conducted in China [12, 14-17] and two in American [9, 13]. The sample sizes ranged from 11 to 152 in PTC group while 5 to 152 in control group. All the cases were pathologically confirmed as PTC. 5 trials [13, 14-17] reported that samples in the control group were collected from the benign tissue adjacent to the malignant tumors, one [12] was the benign tissue from patient without thyroid cancer, and one [9] did not show the details. The test methods of the methylation status were methylation-specific polymerase chain reaction (MSP) [9, 12-15, 17] and real-time fluorescent quantitative methylation specific polymerase chain reaction (qMSP) [16].

*Quality of included studies*

The quality assessments of all the seven studies were performed via NOS and showed in **Table 1**. The studies in this meta-analysis were high quality (NOS scores equal to six or higher than six). The most common selection bias was the selection of control form heterogeneous control, which induced the comparability bias of case and control. In terms of exposure bias, all the studies reported the same test method and nonresponsive rates both in case and control group.

*Methylation of NIS promoter in PTC vs. control*

Seven studies included this index with a total of 628 participants, 360 cases and 268 controls, respectively. **Figure 2** shows the estimated pooled OR associated with PTC to methylation of NIS promoter. There is no heterogeneity detected ($I^2$=0%, *P*=0.78), so the fixed effects model was used. The pooled OR from all seven studies was 7.36 (95% CI: 4.25-12.74, *P*<0.001), that meant the prevalence of PTC in
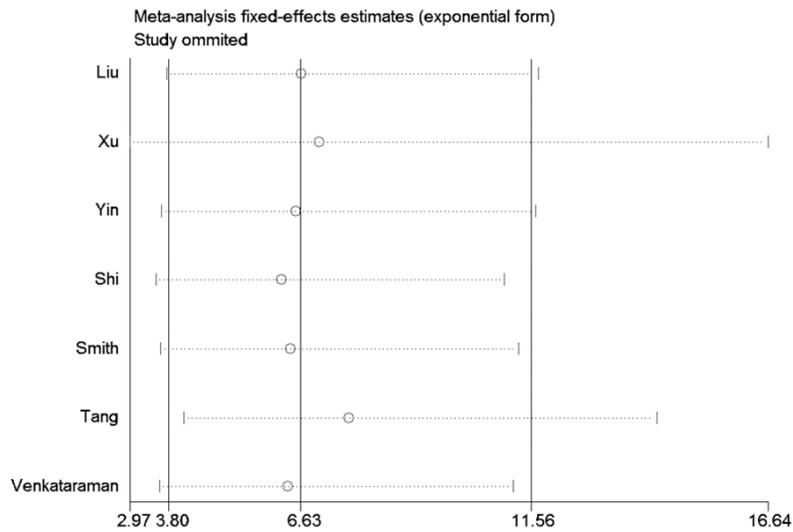
**Figure 3.** Forest plot of sensitivity analysis by removing each study in each turn.
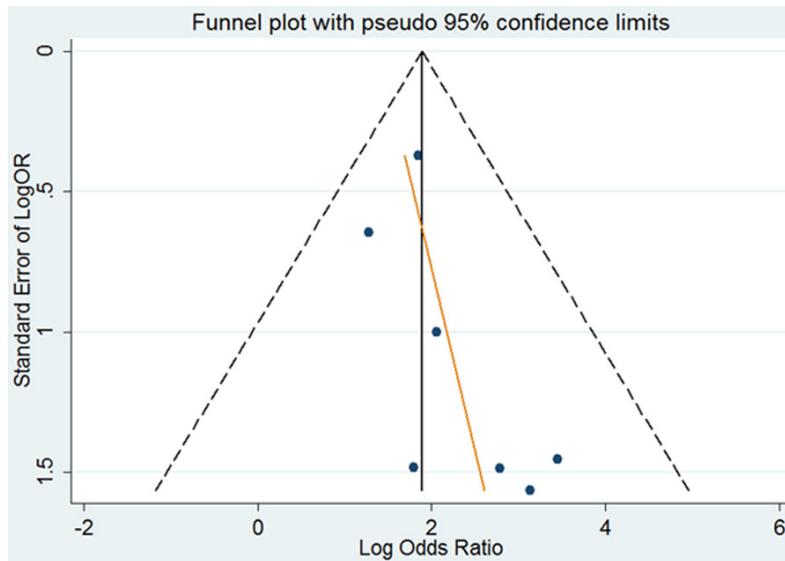


**Figure 4.** Funnel plot of publication bias.

the group of methylated NIS promoter was highly increased than non-methylated group. The same outcome was found both in patients of China (OR=6.71, 95% CI: 3.79-11.87, $P<0.00001$, $I^2$=0%, $P$=0.71) and American (OR=18.66, 95% CI: 2.19-159.33, $P$=0.007, $I^2$=0%, $P$=0.87), respectively.

*Stratified analysis*

Stratified the studies by age (45 years or older vs. younger than 45 years), gender (female vs. male) and size of tumor (diameter larger than 2 cm vs. 2 cm or smaller) separately, and it

showed that no association was found between NIS methylation and these characteristics (**Table 2**). Stratification of the studies by the number of lesions (multiple vs. single) showed that no heterogeneity existed and the pooled OR by fixed effects model was 2.22 (95% CI: 1.12-4.41, $P$=0.02). That means PTC patients with multiple lesions have higher opportunity of methylation. 54 patients with PTC in 2 studies [12, 16] were classified by the capsule invasion status (invasion vs. without invasion). Heterozygosity test showed the moderate heterogeneity ($P$=0.22, $I^2$=34%) and fixed effects model was used to merge OR values. The pooled data was 2.14 (95% CI: 1.12-4.08, $P$=0.02), which means PTC patients with capsule invasion has higher rate of methylated NIS promoter. 5 studies [12, 13, 15-17] reported the details of lymphatic metastasis in PTC patients and no heterogeneity was found by heterozygosity test ($P$=0.32, $I^2$=15%). Pooled data from all five studies with fixed effects model showed that higher prevalence of NIS promoter methylation in group of PTC patients with lymphatic metastasis than PTC patients without (OR=3.56, 95% CI: 1.97-6.46, $P<0.0001$). In summary, PTC patients with higher opportunity of local or distant metastasis have higher rate of methylated NIS promoter.

*Sensitivity analysis*

The data of pooled OR and 95% CI by removing one study in each turn was showed in **Figure 3**. Random effects model was used to instead of fixed effects model, and pooled OR and corresponding 95% CI switched from 7.36 (95% CI: 4.25-12.74, $P<0.001$) to 6.63 (95% CI: 3.80-

11.56, *P*<0.001). Both of these two sensitivity analysis methods indicated the robustness of the main result.

Funnel plot was used to analysis the publication bias. And a symmetrical funnel plot was shown in **Figure 4** which indicated no publication bias existed. The Egger linear regression and Begg rank correction test also detected the same result (Egger: *P*=0.143; Begg: *P*=0.133). In summary, all the seven studies included in this meta-analysis have no publication bias.

## Discussion

Thyroid cancer has an increasing incidence over the last three decades and PTC accounts for approximately 80% of all thyroid cancer [22-24]. Radioactive iodide has been shown to have a positive impact on patients with PTC. But there still exist some PTC patients without significantly uptake of iodine [25]. Some other patients showed the lower iodine ions uptake in the progress of metastasis and radiation treatment [26]. Treatment of patients with those conditions is a big challenge.

Sodium iodide symporter (also known as SLC5A5), an intrinsic membrane glycoprotein involving in iodide uptake, plays a crucial role in iodine metabolism and its function has been widely used in the diagnosis and treatment of benign and malignant thyroid diseases. Some discoveries reported that the level of messenger RNA (mRNA) coding the NIS is decreased in thyroid cancer when compared with control [7, 8]. Other studies showed the same results in the protein level of NIS [6, 10]. The number of positive cells (immuohistochemistry using NIS antibodies) was significantly higher in well-differentiated cancer than in poorly differentiated cancer [26, 27]. NIS expression in metastatic lesions was different from the primary tumor. Park et al. [28] found that levels of the NIS mRNA expression in metastasized lymph nodes were lower, or even absent, than those in primary tumors. Thus, lost expression of NIS is one of the most important hallmarks of thyroid cancer and it maybe the cause of the worse outcomes for some patients [29]. Though, the explanation for the decreased iodide uptake in PTC does not lie simply in lower expression of NIS but probably involves a more complex change ultimately affecting NIS expression.

Recent researches have been extended to the molecular level and epigenetic silencing of related genes is one of the most commonly studies. It has been suggested that epigenetic abnormal may play a crucial role in the earliest steps in tumorigenesis [30, 31]. The mechanisms of epigenetic silencing include DNA methylation; covalent modification of chromatin, noncoding RNAs and et al. [32] Abnormal DNA methylation has been shown to play an important role in tumorigenesis and progression in many tumors, including thyroid cancer [33].

DNA methylation is an epigenetic change and linked to gene expression. The CpG islands, areas rich in phosphate-linked pairs of cytosine and guanine residues, are mainly in promoter areas and usually unmethylated. DNA methylation often occurs in CpG islands. Cytosine methylation in the CpG islands can regulate gene expression in transcription level, inducing the related gene silence which may causes the lower or absent expression [9-11]. Hypermethylation of CpG islands in the promoters has been suggested to be associated with tumorigenesis and the development of cancer [34]. Thereby, the mechanism of abnormal expression of NIS maybe associated with the abnormal methylated NIS promoter, resulting in a decreased ability to concentrate iodine. In addition, it has been reported that PTC patients have higher incidence of methylated NIS promoter [9, 14-17]. So, the methylation of NIS promoter maybe related to tumorigenesis of PTC. The results in this meta-analysis confirm this hypothesis either patients in China or America.

Further analysis showed that PTC patients with higher potential of metastasis and aggressiveness (multiple lesions, capsule invasion and lymphatic metastasis) have higher rate of methylation. But it had no relationship with age, gender or size of tumor. The results and mechanisms of these relationships are still controversial and uncertain. Fan et al. [35] proposed that methylation of CpG islands is a gradual process, which means every cytosine in the CpG islands methylates gradually. The gene expression was absent if the rate of methylated 5-CpG was higher than 60%. If not, the expression was lower than normal [36]. With the process of methylation, the gene and protein expression of NIS decreased gradually and ultimately

disappeared, and PTC cells changed to the cells that do not uptake iodine completely. These might be the reason why patients with distant metastasis have failure therapy.

This meta-analysis showed that methylation of NIS promoter is significantly related with PTC and significant correlation is also found between methylation with invasiveness and metastasis. Attempts have been made with demethylating agents (such as 5-fluoro-2'-deoxycytidine, zebularine, 5-azacytidine (azacitidine) and 5-aza-2'-deoxycytidine (decitabine)) to target reverse the gene silencing and improve the ability of iodide-concentrating [37]. Therefore, our study paves the way for the application of demethylating agents in patients with PTC, especially patients with high aggressive and metastatic potential which may cause the low radioactive iodine uptake.

In the analysis of methylation of NIS promoter in PTC comparing with control, heterozygosity tests showed no heterogeneity detected. Stratified analysis showed that heterogeneity was moderate in the subgroup of capsule invasion and others showed no heterogeneity ($I^2$: 0-25%). For NOS scores of these included studies were all equal to six or higher than six, the methodological quality was good. Sensitivity analysis, by removing one study in each turn and change of analysis model, supported the robustness of the main result. All the test of publication bias, including funnel plot, Egger linear regression and Begg rank correction test, suggested no publication bias was found. But the number of patients both in each study and pooled data were limited. So, more clinical researches with larger sample, higher quality and strictly case-control study should be taken in the future.

## Conclusions

To summarize, our meta-analysis indicated that methylation of NIS promoter plays a crucial role in the increased occurrence of PTC. And it is also correlated with aggressive and metastatic potential of PTC. This may provide a destination for future investigations of epigenetic prediction and therapy for thyroid cancer, especially in patients with high potential of metastasis and aggressiveness.

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## Disclosure of conflict of interest

None.

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