RESEARCH ARTICLE

Quantitative Assessment of the Diagnostic Role of CDH13 Promoter Methylation in Lung Cancer

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Abstract

In order to explore the association between cadherin 13 (*CDH13*) gene promoter methylation and lung carcinoma (LC) risk, we carried out a meta-analysis with searching of PubMed, Web of Science. Ultimately, 17 articles were identified and analysised by STATA 12.0 software. Overall, we found a significant relationship between *CDH13* promoter methylation and LC risk (odds ratio=6.98, 95% confidence interval: 4.21-11.56, $p<0.001$). Subgroup analyses further revealed that LC risk was increased for individuals carrying the methylated *CDH13* compared with those with unmethylated *CDH13*. Hence, our study identified a strong association between *CDH13* gene promoter methylation and LC and highlighted a promising potential for *CDH13* methylation in LC risk prediction.

Keywords: *CDH13* - lung carcinoma - methylation

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Introduction

Lung cancer (LC) is the most frequent cancer worldwide still. There were more than 1.8 million new cases (13% of total cancer incidence) and almost 1.6 million deaths (20% of total cancer mortality), as estimated in 2012 (Bernard W. Stewart, 2014). Moreover, LC is the leading cause of cancer death in men in 87 countries and in women in 26 countries. Despite the advent of new diagnostic techniques, most LCs are detected at a late stage, and the 5-year survival rate of LC is less than 15% in the US (Jemal et al., 2011). Once tumor cells have spread, the long-term prognosis is poor since no curative treatments are available. However, the bottleneck in improving survival is early detection (Zhang et al., 2011). As an important mechanism for tumor suppressor gene inactivation in cancer, DNA hypermethylation could yield powerful biomarkers for early detection of LC, owning in comparable advantages over other traditional markers due to its stable chemical property, detection ability in remote patient media, quantitative signal , convenient low cost in detection, and so on (Li et al., 2012; Mikeska et al., 2012). Therefore, we believe that DNA methylation could become a powerful tool for LC diagnosis.

The cadherin 13 (*CDH13*) gene, a new member of the cadherin superfamily, was isolated recently and has been mapped to 16q24 (Takeuchi and Ohtsuki, 2001). The introduction of *CDH13* in human breast carcinoma cells reduced their invasive potential and markedly decreased their growth rate; in addition, it induced the reversion of morphology from an invasive type to a normal cell-like type (Lee, 1996). Abnormalities in the *CDH13* gene have been identified in human malignancies, including lung carcinomas (Sato et al., 1998). Moreover, *CDH13* expression is associated with tumorigenicity in non-small cell lung carcinoma (NSCLC) and frequently is silenced by promoter methylation of the *CDH13* gene in NSCLC (Toyooka et al., 2001; Hanabata et al., 2004; Kim et al., 2004).

In this article, we conducted a meta-analysis of the *CDH13* methylation on LC diagnosis. At the end, we found a strong association between *CDH13* gene promoter methylation and LC.

Materials and Methods

Search strategy, data extraction and statistical analysis

This pooled study involved searching a range of computerized databases, including PubMed, Web of Science for articles published in English by September 2014. The study used a subject and text word strategy with 'lung cancer or lung neoplasm or lung carcinoma', '*CDH13* or T-cadherin or cadherin 13 or H-cadherin', 'methylation or hypermethylation or epigenetic', as the primary search terms. Wildcard character of star, dollar or some other truncations were applied according to the rules of the databases to allow effective article collection.

Two independent reviewers screened the titles and abstracts derived from the literature search to identify relevant studies. The following types of studies were

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excluded: animal experiments, case reports, reviews or meta-analyses and studies of non-case-control 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 met the inclusion criteria: 1) the patients had to be diagnosed with LC; 2) the studies had to contain *CDH13* gene promoter methylation data from tissue, blood or serum; 3) the studies had to be case-control studies which included tissue-tissue, blood-blood or serum-serum in case and controls respectively. The reference sections of all retrieved articles were searched to identify further relevant articles. Potentially relevant papers were obtained and the full text articles were screened for inclusion by two independent reviewers. Decisions were made and disagreements about study selection were resolved by consensus or by involving a third reviewer. The following information was extracted from the studies: the first author's last name, publication year, original country of patients in the subjects, and the number of *CDH13* methylation of cases and controls in individuals, etc.

The strength of the association between the *CDH13* methylation and LC risk was measured by pooled odds ratio (OR) with its 95% confidence interval (CI). The significance of the pooled OR was determined by the Z test and $p<0.05$ was considered as statistically significant. Subgroup analysis was performed stratified by the study character of tissue sample and blood sample. The heterogeneity assumption was checked by chi-test based on Q-test (significance level of $p<0.10$) (Dickersin and Berlin, 1992). With a lack of heterogeneity among included studies, the pooled odds ratio estimates were calculated using the fixed-effects model (Mantele-Haenszel) (Mantel and Haenszel, 1959). Otherwise, the random-effects model (DerSimonian and Laird method) was used (DerSimonian and Laird, 1986). Sensitivity analyses were performed to assess the contributions of single studies to the final results. Begg's funnel plots were used to examine whether the results of a meta-analysis may have been affected by publication bias. Egger's test was implemented to testing for funnel plot asymmetry (M. Egger, 1997). All statistical analyses were performed using Stata statistical software.

Results

Study characteristics

After being selected in accordance with the inclusive criteria, and finally, 17 studies with data on the relationship between *CDH13* gene promoter methylation and LC were pooled for analysis (Table 1) (Toyooka et al., 2001; Zhong et al., 2001; Toyooka et al., 2003; Kim et al., 2004; Kim et al., 2006; Suzuki et al., 2006; Ulivi et al., 2006; Hsu et al., 2007; Kim et al., 2007; Tsou et al., 2007; Yanagawa et al., 2007; Brock et al., 2008; Feng et al., 2008; Jin et al., 2009; Zhang et al., 2011; Kontic et al., 2012; Zhai and Li, 2014). All these articles were written in English and were original study. The 17 studies published between 2001 and 2014.

In total, 1,786 LC tissues/serum and 1,134 normal counterpart tissues/serum were collected. There was 35.78 % of LC patients had the methylated *CDH13* allele with a frequency ranging from 18.64% to 98.46% in individual trials. However, there was 10.67% of normal had the methylated *CDH13* allele with a frequency ranging from 0.00% to 25.49% in individual trials. For histologic type, 15 studies focus on NSCLC, two studies focus on LC; for sample source, 14 and 1 literatures based on the investigation of tissue and plasma, two articles both on tissue and plasma, respectively; for ethnic group, there was 6 articles of the Asian, 3 of the Caucasus and 8 of Mixed-race.

Meta-analysis and subgroup analysis

The ORs for *CDH13* methylation in cancer tissues compared with that in normal controls were 6.98 (95%CI:

Table 1. Main Characteristics of the Studies Included in the Meta-Analysis

Author	Year	Country	Ropulation	Method	Sample	Histologic type	Control			Case			Total
							M	T	%	M	T	%	
Zhai et al.	2014	China	Asian	MSP	Blood	NSCLC	0	40	0.00%	24	42	57.14%	82
Kontic et al.	2012	Serbia	Caucasian	BSP	Tissue	NSCLC	15	65	23.08%	64	65	98.46%	130
Zhang et al.	2011	Germany	Caucasian	MSP	Tissue	NSCLC	8	78	10.26%	38	78	48.72%	156
Jin et al.	2009	Japan	Asian	QMSP	Tissue	NSCLC	2	63	3.17%	25	72	34.72%	135
Feng et al.	2008	USA	Mixed	chip	Tissue	NSCLC	0	49	0.00%	11	59	18.64%	108
Brock et al.	2008	USA	Mixed	MSP	Tissue	LC	24	104	23.08%	19	50	38.00%	154
Yanagawa et al.	2007	Japan	Asian	MSP	Tissue	NSCLC	7	101	6.93%	26	101	25.74%	202
Tsou et al.	2007	USA	Mixed	MSP	Tissue	LC	0	11	0.00%	37	55	67.27%	66
Kim et al.	2007	Korea	Asian	MSP	Tissue	NSCLC	7	88	7.95%	26	88	29.55%	176
Hsu et al.	2007	China	Asian	MSP	Tissue	NSCLC	6	36	16.67%	28	63	44.44%	99
Hsu et al.	2007	China	Asian	MSP	Blood	NSCLC	6	36	16.67%	21	63	33.33%	99
Ulivi et al.	2006	Italy	Caucasian	MSP	Tissue	NSCLC	0	15	0.00%	40	61	65.57%	76
Ulivi et al.	2006	Italy	Caucasian	MSP	Blood	NSCLC	0	15	0.00%	14	61	22.95%	76
Suzuki et al.	2006	Japan	Asian	MSP	Tissue	NSCLC	3	60	5.00%	40	150	26.67%	210
Kim et al.	2006	Korea	Asian	MSP	Tissue	NSCLC	26	102	25.49%	35	102	34.31%	204
Kim et al.	2004	Korea	Asian	MSP	Tissue	NSCLC	4	127	3.15%	29	85	34.12%	212
Toyooka et al.	2003	Mixed	Mixed	MSP	Tissue	NSCLC	5	84	5.95%	129	514	25.10%	598
Zhong et al.	2001	USA	Mixed	MSP	Tissue	NSCLC	6	35	17.14%	15	35	42.86%	70
Toyooka et al.	2001	USA	Mixed	MSP	Tissue	NSCLC	2	25	8.00%	18	42	42.86%	67

4.21-11.56, $p<0.001$) in random effects model pooled, demonstrating a statistically significant increasing in likelihood of methylation in LC tissues comparing to controls (Figures and Table 2). In other words, compared with healthy person, LC patients had a 6.98-fold higher risk for *CDH13* methylation.

Subgroup analyses were conducted for different subtypes, which included histologic type (NSCLC and LC), sample source (tissue or blood), ethnic group (Asian, Caucasus and Mixed-race). Histological type ORs showed (Figure 1) that LC risk was increased for individuals carrying the methylated *CDH13* compared with those with unmethylated *CDH13* in NSCLC group (OR=7.33; 95%CI, 4.33-12.41) and LC group (OR=7.35; 95%CI, 0.29-183.86). Testing materials subgroup analyses showed (Figure 2) that the incidence of *CDH13* methylation in LC tissues was higher than that in normal tissues (OR=6.93; 95%CI, 4.07-11.80), and similar results was found in plasma sample (OR=10.75; 95%CI, 0.89-129.16). When stratifying for ethnic-specific (Figure 3), the increased risk of *CDH13* methylation in cases than controls was found in Asian populations (OR=5.51; 95 %CI, 2.87-10.57), Caucasian populations (OR=28.78; 95 %CI, 4.61-179.78) and mixed-race (OR=4.91; 95%CI, 2.34-10.31).

Table 2. Summary of *CDH13* Methylation and LC Risk

Subgroup	OR	[95% Conf. Interval]	Significance Test(S) Of OR=1	Begg's
Nsclc	7.333	4.332-12.413	0.000	0.039
Lc	7.346	0.293-183.864	0.000	0.317
Blood	10.745	0.894-129.159	0.000	0.005
Tissue	6.929	4.070-11.797	0.000	0.602
Asian	5.509	2.871-10.572	0.000	0.083
Caucasian	28.781	4.608-179.777	0.000	0.317
Mixed	4.914	2.342-10.312	0.000	0.174

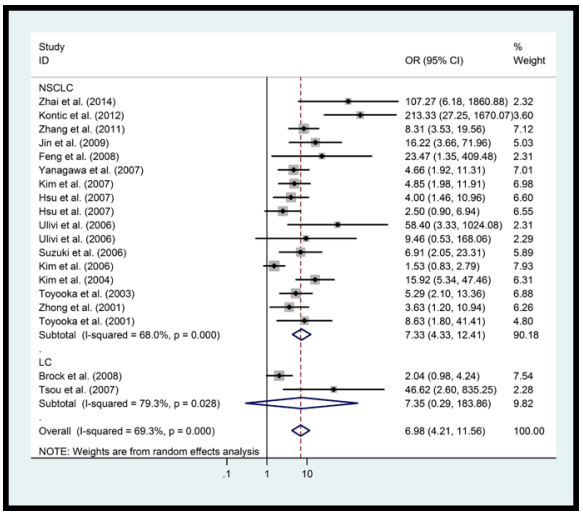

Figure 1. Forest Plot of Meta-Analysis for Association between *CDH13* Methylation and Lung Cancer. The forest plot displays the effect size and 95% CI for each study, subgroup and overall. The pooled odds ratios for risk are from fixed-effects models using STATA12.0

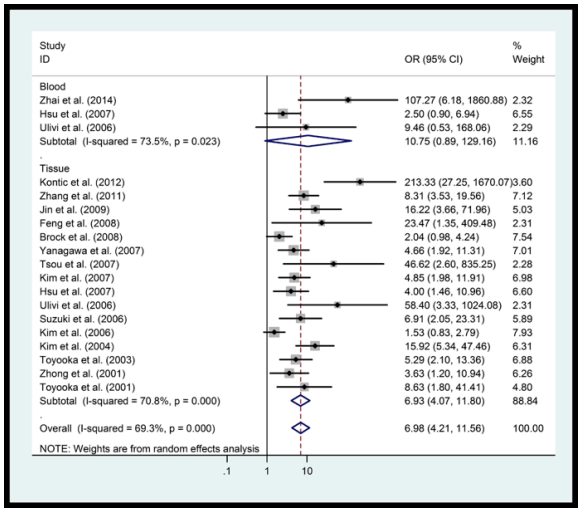

Figure 2. Forest Plot of Meta-Analysis for Association between *CDH13* Methylation and Lung Cancer. The forest plot displays the effect size and 95% CI for each study, subgroup and overall. The pooled odds ratios for risk are from fixed-effects models using STATA12.0

Sensitive analysis

Sensitive analyses were conducted to determine whether modification of the inclusive criteria of the meta-

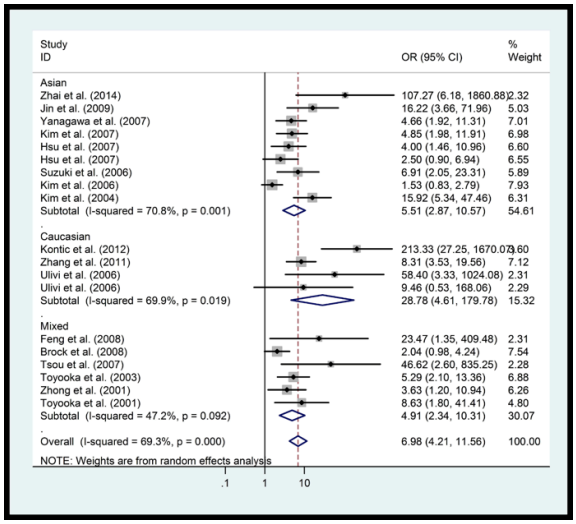

Figure 3. Forest Plot of Meta-Analysis for Association between *CDH13* Methylation and Lung Cancer. The forest plot displays the effect size and 95% CI for each study, subgroup and overall. The pooled odds ratios for risk are from fixed-effects models using STATA12.0

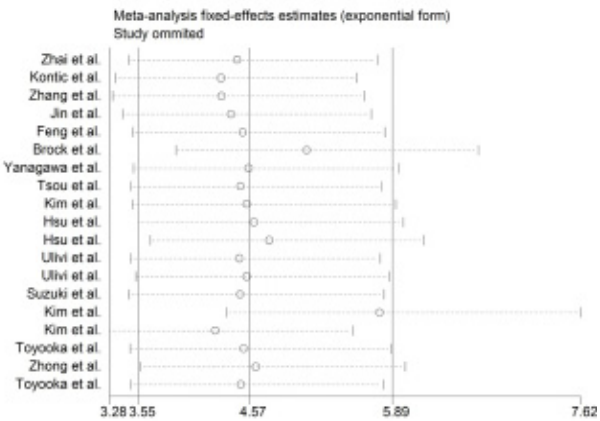

Figure 4. Sensitivity Analysis of the Summary Odds Ratio Coefficients on the Relationships between *CDH13* Promoter Methylation and the Lung Cancer Patients

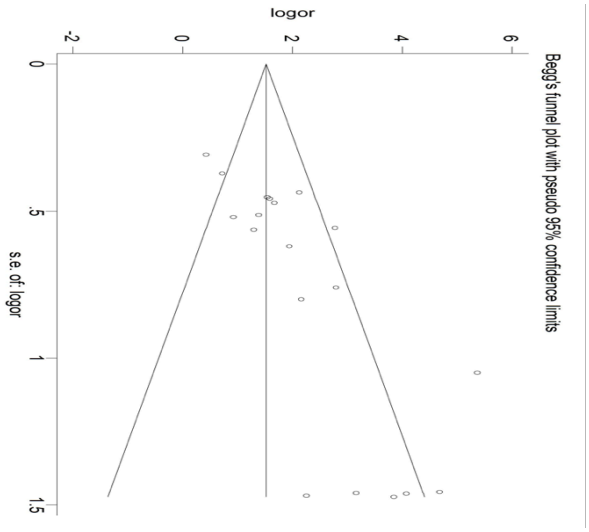

Figure 5. Funnel Plot (with pseudo 95 % Percent confidence limits) for Publication Bias Test. The natural logarithm of odds ratio (OR) and its standard error were used in the funnel plot. The circles correspond to the log OR from individual trials, and the diagonal lines show the expected 95 % CI around the summary estimate

analysis affected the final results. When we excluded the studies of (Kim et al., 2006), there was almost no difference between the remaining 16 studies (Figure 4). Therefore, our results were statistically robust.

Bias diagnosis

Begg's funnel plot and Egger's test were carried out to assess the publication bias of the studies. Here, we only used the Begg's funnel plot and for overall, the shapes of Begg's funnel plot revealed obvious symmetry (Figure 5). Although, our results showed significant publication bias in testing materials and histological type group, the ethnicity group did not provide any evidence of publication bias (Table 2). Hence, we made a conclusion that Begg's funnel plot detected any publication bias of our studies.

Discussion

The *CDH13* gene has been reported as an important tumor suppressor in colorectal cancer (Dong et al., 2005), and the aberrant of *CDH13* methylation had been reported in numerics for cancers, such as hepatocellular carcinoma (Riou et al., 2006), cervical neoplasia (Feng et al., 2007), breast cancer and LC (Sato et al., 1998). However, the diagnostic role of the methylation status of the *CDH13* gene in LC lacks quantitative assessment. We therefore performed an integrated analysis to quantify the ability for the *CDH13* gene promoter methylation test in LC diagnosis, and a significant association was identified between *CDH13* methylation and LC (OR = 6.98, $p<0.001$), suggesting a strong association of the methylation of *CDH13* gene promoter with LC. Three subgroup studies were filled when trim and fill tests were performed to eliminate the influence of publication bias on the random effects model, and the overall OR was still significant, although it was slightly smaller than that in the crude meta-analysis (Table 2), which revealed that *CDH13* methylation status is a good biomarker in LC diagnosis.

Meta-analysis has been widely applied in SNP-disease risk association studies because SNPs have specific genome location. Meta-analysis is also gradually starting to boom in the realm of DNA methylation. Since the late 1980s, various studies have shown that the same genetic/epigenetic alterations, such as DNA methylation, in the primitive tumors were also found in the circulating DNA of the patients with tumors(Esteller et al., 1999). Interestingly, in our study, the OR of the serum subgroup was greater than that of the tissue group of the *CDH13* methylation test for serum was greater than that for tissue in our meta-analysis, which indicated that the *CDH13* methylation test should be a promising serum biomarker for LC diagnosis.

It must be pointed that some limitations may affect the objectivity of our meta-analysis, such as smoking, gender, age and the TNM stage which are differences in *CDH13* methylation between cases and controls. Therefore, a meta-analysis including more high-quality designed epidemiology studies and a stratified analysis targeting different status are necessary in the future in this field.

In conclusion, this integrated analysis of the pooled data provides strong evidence that the methylation status of the *CDH13* promoter is strongly associated with LC risk. Therefore, the *CDH13* methylation test could be a promising diagnostic biomarker which could be applied in the clinical diagnosis of lung adenocarcinoma with remote non-invasive media detection.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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