

The RET fusion gene and its correlation with demographic and clinicopathological features of non-small cell lung cancer: a meta-analysis

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Keywords: clinicopathological features, demographic features, fusion, meta-analysis, non-small cell lung cancer, RET, targeted therapy

Abbreviations: NSCLC, Non-Small Cell Lung Cancer; EGFR, Epidermal Growth Factor Receptor; GDNF, Glial cell line-derived Neurotrophic Factor; ARTN, Artemin; NRTN, Neurturin; PSPN, Persephin; PI3K, Phosphatidylinositol 3-kinase; TKIs, Tyrosine Kinase Inhibitors; RT-PCR, Real-Time Polymerase Chain Reaction; OR, Odds Ratio; CIs, Confidence Intervals; ADC, Adenocarcinoma; NADC, Non-adenocarcinoma; SCLC, Small-cell lung cancer.

Purpose. The RET fusion gene is a novel oncogene observed in a subset of NSCLC in recent years. Nevertheless, the results of epidemiological studies concerning the gene remain unclear. Thus, a meta-analysis was conducted to evaluate the correlation of RET fusion gene with demographic and clinicopathological features of NSCLC. *Methods.* PubMed, Embase, and Web of Science databases were searched to identify eligible studies. The association of RET fusion gene occurrence with gender, age, smoking status, histology type and tumor stage were analyzed in meta-analysis. Subgroup analysis according to patients' location (Asian and non-Asian) was also conducted. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated to assess the correlation. *Results.* Nine studies with a total of 6,899 NSCLC patients met the inclusion criteria. A total of 84 patients with RET fusion gene were detected. The RET fusion gene was identified at significantly higher frequencies in female (OR = 0.55, 95%CI = 0.35–0.85) than male patients and in young (<60) patients (OR = 0.43, 95%CI = 0.19–0.99) than old patients (≤ 60), particularly in patients from Asian. A significant higher frequency was also identified in non-smokers (OR = 0.28, 95% CI = 0.16–0.49), and in patients with lung adenocarcinomas (OR = 3.59, 95%CI = 1.50–8.56). Additionally, no association between RET fusion gene and the TNM stage of tumor was observed. *Conclusion.* RET fusion gene occurred predominantly in Asian females with younger age, in non-smokers, and in lung adenocarcinomas patients. This subset of NSCLC patients might be good candidates for personalized diagnostic and therapeutic approaches.

Introduction

Non-Small Cell Lung Cancer (NSCLC) accounts for approximately 80% to 85% of lung cancers and is a heterogeneous disease with multiple underlying causative genetic mutations.¹ With the progress of molecular oncology, targeted gene therapy has become a prospective field in NSCLC treatment. Epidermal growth factor receptor (EGFR) mutation is the most common molecular driver alteration that occurred in 20 to 30 percent of NSCLC patients.² The treatment strategies blocking EGFR pathway in EGFR-mutant lung cancer, which effectively repress tumor by inhibition of driver oncogenes, establish a remarkable example of molecular targeted therapies.³ Other molecular alterations, such as KRAS, BRAF mutations and ALK translocations have also been defined as “driver mutations” in lung cancer successively.⁴ Compare with traditional chemotherapy and

radiotherapy, patients with certain mutant gene will benefit from targeted therapy owing to specificity and minimal toxic side-effects.⁵ However, nearly half of NSCLC patients still don't have explicit gene mutations.⁶ Thus, there is a continuing need for the exploration of potential driver mutations and to promote more effective diagnostic and individualized therapeutic strategies.

RET (located on chromosome 10q11.2) is a proto-oncogene that encodes a receptor tyrosine kinase and acts as a common receptor for members of the glial cell line-derived neurotrophic factor family (GDNF) ligands, including GDNF, artemin (ARTN), neurturin (NRTN), and persephin (PSPN).⁷ It has a classic receptor tyrosine kinase structure consisting of 3 domains: an extracellular ligand-binding domain, a transmembrane domain and an intracellular tyrosine kinase domain.⁸ The extracellular binding of receptor and ligand promotes the dimerization of RET and activates intracellular tyrosine kinase domain by

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Submitted: 03/10/2015; Accepted: 04/26/2015

<http://dx.doi.org/10.1080/15384047.2015.1046649>

phosphorylation. The downstream signal is transduced through multiple pathways like Ras/RAF and phosphatidylinositol 3-kinase (PI3K), and physiologically regulates cell survival, differentiation, proliferation and migration.⁸⁻¹⁰

Since the beginning of 2012, a series of researches have identified the fusion of RET gene with a couple of other genes such as KIF5B, CCDC6, NCOA4, and TRIM33 in lung cancer.¹⁰⁻¹³ The mutual exclusivity of this transforming gene to other major driver mutations, like EGFR, KRAS, ALK and BRAF, suggests its potential as a novel driver oncogene.¹⁴ Among several types of fusions, the KIF5B-RET is the most common one that formed by a pericentric inversion on chromosome 10. The inversion leads to a coiled coil domain, which induces autophosphorylation of RET tyrosine kinase, thus activating uncontrolled signal transduction and resulting in tumorigenesis.¹⁵

To verify whether RET was a feasible target for tumor inhibition, strategies have been used to suppress the activity of RET both in pre-clinical experiments and small clinical trials. In several pre-clinical studies, the growth of RET fusion-positive cells could be inhibited by the multitargeted tyrosine kinase inhibitors (TKIs) with activity against the RET kinase, like sunitinib, sorafenib, vandetanib and alectinib.^{10,11,16,17} While in 3 independent phase II trials, RET inhibitors including cabozantinib and vandetanib were used to treat a total of 7 RET fusion-positive patients of lung adenocarcinoma.^{14,18,19} As a result, 4 had confirmed partial responses and 3 had prolonged stable disease, indicating the therapeutic potential of RET inhibitors as targeted drugs tailored for RET fusion-positive patients.

However, RET fusion genes occur in only <2% of NSCLC patients, even when the histological type is limited to lung adenocarcinoma, which makes it difficult for clinicians to screen fusion-positive patients.^{11,16,20,21} So exploring the specific features of RET-positive patients may help to target people at high risk and narrow the scope of gene test. Heretofore, several studies have reported the distribution of the RET fusion gene in NSCLC patients, however, the results remain uncertain. For example, Tsuta et al. demonstrated that RET rearrangements were significantly more common in younger patients and tended to occur in non-smokers, while Cai et al. revealed that there were no statistically significant difference in age, sex and smoking status.^{21,22} Therefore, we performed a meta-analysis to quantify the association of RET fusion genes with demographic factors and clinicopathological characteristics of NSCLC, which may facilitate the selection of cases for the check of RET rearrangements and further treatment of RET inhibitors.

Results

Study characteristics

The selection steps were summarized in the flow diagram shown in **Figure 1**. A total of 826 relevant articles were retrieved from PubMed, EMBASE, and Web of Science databases and 298 were excluded because of duplication. After screening the titles and abstracts, 475 articles were excluded due to a lack of significant relevance. The remaining 53 articles were further

reviewed in detail by full texts. Finally, 9 studies including 6,899 patients were qualified for our meta-analysis.^{10-12,20-25}

Main features of 9 eligible studies were summarized in **Table 1**. The median age was reported in 7 studies and the average is 61.4. For patients' location, 7 studies were conducted in Asians, and one study was conducted in patients of USA, while one study included patients from Japan, USA, and Norway. Of the 6,899 patients, 84 had RET fusion genes, giving an overall frequency of 1.2%. Specific information of each subtype of variants was given in 8 studies, including 3 types of fusions: KIF5B-RET fusions (85.6%, 59/69), CCDC6-RET fusions (13.0%, 9/69), and NCOA4-RET fusions (1.4%, 1/69). The detection method was real-time polymerase chain reaction (RT-PCR).

Overall analysis of RET fusion genes and demographic features of patients with NSCLC

Gender

The overall analysis of the association between RET fusion gene and gender was conducted in 9 studies including 6,492 patients, showing that female patients with NSCLC had a higher RET fusion rate (1.7%, 51/3,015) than male patients (0.9%, 31/3,477). The pooled OR was 0.55 (95% CI = 0.35–0.85, $P = 0.007$). For heterogeneity test, chi-squared was 6.74 ($P = 0.565$), $I^2 = 0\%$, showing there was no heterogeneity between studies (**Fig. 2A**).

Age

Three studies with 1,841 patients were analyzed for the association between RET fusion gene and age with the same age-grouping criterion (60 years). Of patients younger than 60 y old, 2.0% (19/957) were RET fusion positive, higher than 1.0% (8/884) of patients 60 y or older. The pooled OR was 0.43 (95% CI = 0.19–0.99, $P = 0.046$), indicating that younger patients were more likely to carry the RET fusion gene. No obvious heterogeneity was observed (chi-square was 0.11, $P = 0.946$, and $I^2 = 0\%$) (**Fig. 2B**).

Smoking

The overall analysis of the association between RET fusion gene and smoking history showed that non-smokers had a significantly higher rate than smokers with a pooled OR of 0.28 (95% CI = 0.16–0.49, $P < 0.001$). Among non-smokers, the RET fusion rate was 2.0% (66/3,221), 4 times that of smokers (0.5%, 16/3,268), coming from 9 studies including 6,489 patients. Between-study heterogeneity could be ignored (chi-squared was 6.01, $P = 0.538$, $I^2 = 0\%$) (**Fig. 2C**).

Overall analysis of RET fusion genes and clinicopathological characteristics of patients with NSCLC

Histology type

Six studies were analyzed for the association between RET gene fusions and histology type. Three studies were excluded because they specifically selected pulmonary adenocarcinoma (ADC) patients for their original researches. Overall, patients

with ADC had a significant higher fusion rate of 1.4% (57/3980), compared with 0.3% (4/1278) in non-adenocarcinoma(NADC) patients. The pooled OR was 3.59 (95% CI = 1.50–8.56, P = 0.004). No obvious heterogeneity was detected (chi-square was 2.16, P = 0.827, I² = 0%) (Fig. 3A).

TNM stage

No statistical significance was observed in correlation between the gene and TNM stage (based on the AJCC classification). The RET fusion rate did not vary much between stage I or II NSCLC and stage III or IV (1.1% Vs 1.6%) with a pooled OR of 0.64 (95% CI = 0.34–1.2, P = 0.164). Between-study heterogeneity could be ignored (chi-squared was 0.39, P = 0.983, I² = 0%) (Fig. 3B).

Subgroup analysis of demographic features

Subgroup analysis was conducted according to patient' location to see whether the demographic characteristics of RET fusion genes varied in NSCLC patients from different regions. Studies were divided into Asian group and non-Asian group. The study of Kohno et al. that included patients from Japan, USA and Norway were also split into Asian and non-Asian groups and were combined with the others. As a

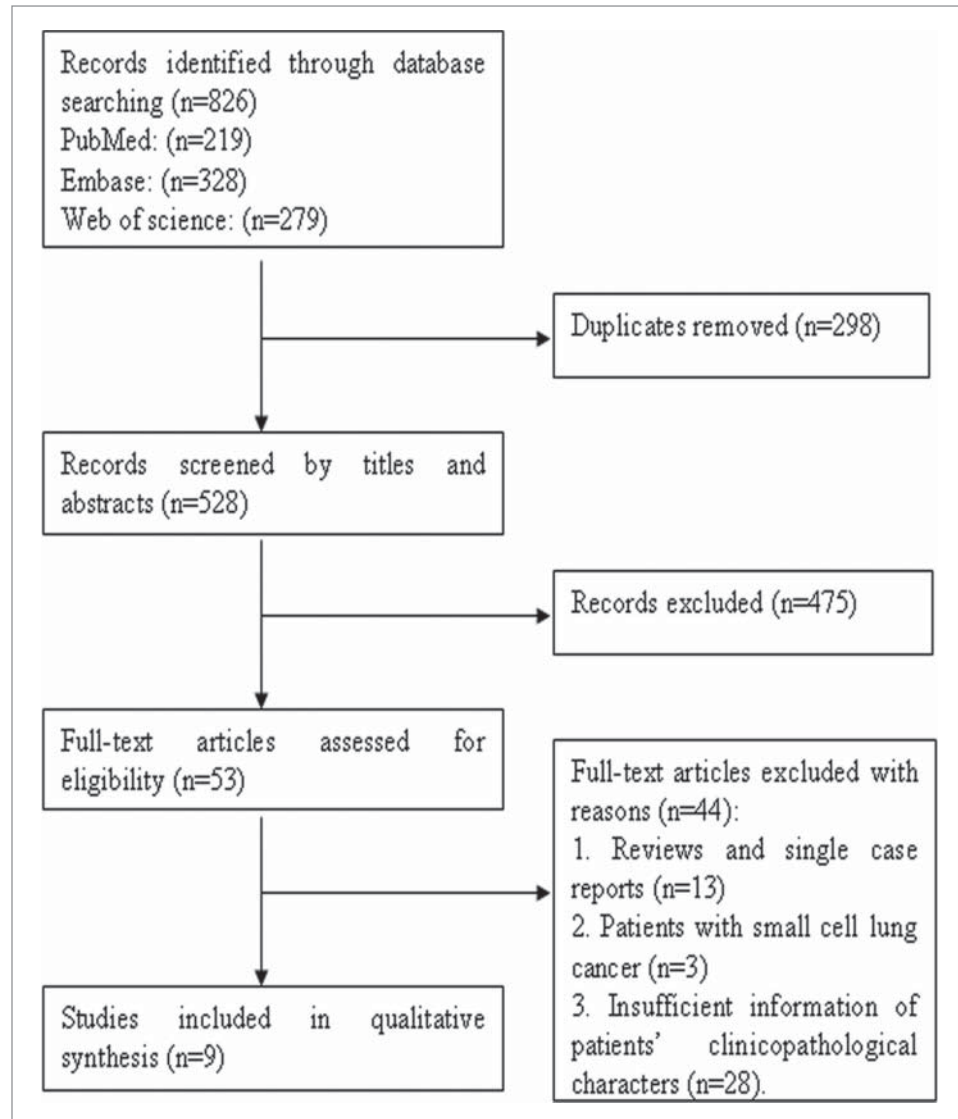


Figure 1. Flow diagram of studies selection procedure and specific reasons for exclusion in the meta-analysis.

Table 1. Main features of studies included in the meta-analysis

Author	Year	Nation	Cases	Age	Total	RET Fusion Positive				Gender		Age		Smoking		Histology		TNM Stage	
						K	C	N	T	M	F	≥60	<60	Y	N	ADC	NADC	I+II	III+IV
						P/N	P/N	P/N	P/N	P/N	P/N	P/N	P/N	P/N	P/N	P/N	P/N	P/N	P/N
Takeuchi	2012	Japan	1529	NA	14	12	2	0	0	4/880	10/632	NA	NA	3/995	11/517	14/1108	0/407	1/116	3/387
Yokota	2012	Japan	371	NA	3	3	0	0	0	0/160	3/107	NA	NA	0/90	3/177	3/267	0/101	2/210	1/57
Suechara	2012	USA	69	67.8	1	1	0	0	0	0/29	1/39	1/55	0/13	0/64	1/4	1/68	0/0	NA	NA
Wang	2012	Chinese	936	59.7	13	9	3	1	0	4/288	7/334	3/299	8/323	2/223	9/399	11/622	2/301	5/383	6/239
Kohno	2012	J,U,N*	433	62.3	7	7	0	0	0	4/215	3/211	NA	NA	1/265	6/158	7/426	0/0	NA	NA
Cai	2013	Chinese	392	60	6	6	0	0	0	3/208	3/178	NA	NA	0/148	6/238	4/227	2/159	3/222	3/164
Soo Yoo	2013	Korean	156	63.8	3	2	1	0	0	0/105	3/48	NA	NA	0/102	3/51	3/101	0/52	NA	NA
Tsuta	2014	Japan	1874	63.1	22	19	3	0	0	11/1054	11/798	NA	NA	7/1000	15/852	22/1598	0/254	16/1480	6/358
Pan	2014	Chinese	1139	59.4	15	—	—	—	—	5/507	10/617	4/522	11/602	3/365	12/759	15/1124	0/0	NA	NA

Abbreviations: Age, median age; K,KIF5B-RET; C,CCDC6-RET; N,NCOA4-RET; T,TRIM33-RET; P/N, positive expression/negative expression; M, male; F, female; Y, smoker; N, non-smoker; ADC, adenocarcinoma; NADC, non-adenocarcinoma NSCLC; NA, not available or not applicable; RT-PCR,real-time polymerase chain reaction; FISH, fluorescent in situ hybridization; J,U,N, Japan, USA, Norway.

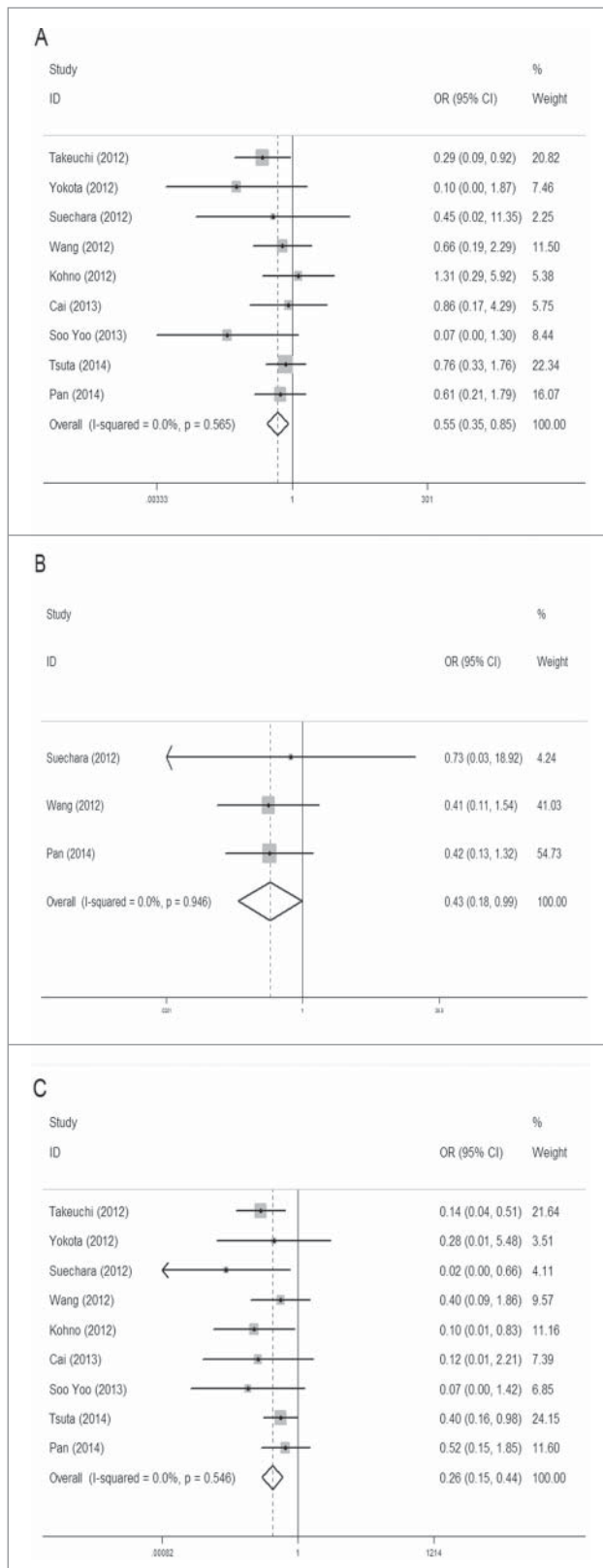


Figure 2. Meta-analysis of the association between RET fusion genes and demographic features: Gender: females vs. males (A); Age: younger (<60) vs. elder (≥60) (B); Smoking history: non-smokers vs. smokers (C).

result, the occurrence frequency of RET fusion genes were still significantly higher in females than in males in Asian group (1.7% vs. 0.9%) with OR = 0.57, 95%CI = 0.36–0.90, P = 0.017, but not in non-Asian group (OR = 1.09, 95%CI = 0.11–10.71, P = 0.94) (Fig. 4A). The same went with the factor of age, that younger patients of Asian group had a higher incidence of gene fusions than elder patients (2.0% vs. 0.8%) with OR = 0.41, 95%CI = 0.17–0.99, P = 0.047, while no statistical difference was detected in non-Asian group (OR = 0.73, 95%CI = 0.03–18.92, P = 0.850) (Fig. 4B). The result of subgroup analysis in smoking status accorded with overall analysis, showing that RET fusions were identified at high frequencies in non-smokers than in smokers both in Asian group (2% vs. 0.5%) with OR = 0.29, 95%CI = 0.16–0.50, P < 0.001 and non-Asian group (8.3% vs. 0.6%) with OR = 0.07, 95%CI = 0.01–0.75, P = 0.028 (Fig. 4C).

Sensitivity analysis

Sensitivity analysis was conducted by successively omitting each individual study and pooling the remaining studies. The pooled ORs and CIs were not altered significantly by each omitted study, which validated the credibility of outcomes. (Fig. 5)

Publication bias

We evaluated the publication bias using Begg's funnel plot. The shapes of the funnel plots did not have apparent asymmetry in all meta-analyses, suggesting the absence of significant biases (Fig. 6). This was confirmed by results of Egger's test (Table 2), demonstrating that our results were statistically robust.

Discussion

In this meta-analysis of 6,899 NSCLC patients, 84 patients were positive in RET fusions, giving an overall frequency of 1.2%. Among 69 RET mutations that classified subtype of variants, KIF5B-RET fusion gene was the major type, accounting for the largest proportion (86%, 59/69). Overall, the frequency and the distribution of mutant types of RET fusion genes in NSCLC patients were similar to other reports in the literature.

To date, several groups have investigated the relationship between the expression of RET fusion genes and demographic or clinicopathological features of NSCLC. Generally, translocations are observed in a higher percentage in young and adenocarcinoma patients.²⁶ However, there are discrepancies among other factors like sex, age, tumor size and stage. Our study, which contained a larger sample size, supported that RET fusion genes were significantly associated with gender, age, smoking status and histology type, but not with TNM stage. Since there might be a potential regional disparity in NSCLC patients with RET fusions like the variance in EGFR mutations, subgroup analysis of demographic features was also conducted among Asians and non-Asians.²⁷ It was not conducted in histology type and TNM stage on account of limited data of non-Asian group.

An important finding of our study is that female NSCLC patients were more likely to carry the RET fusion genes than

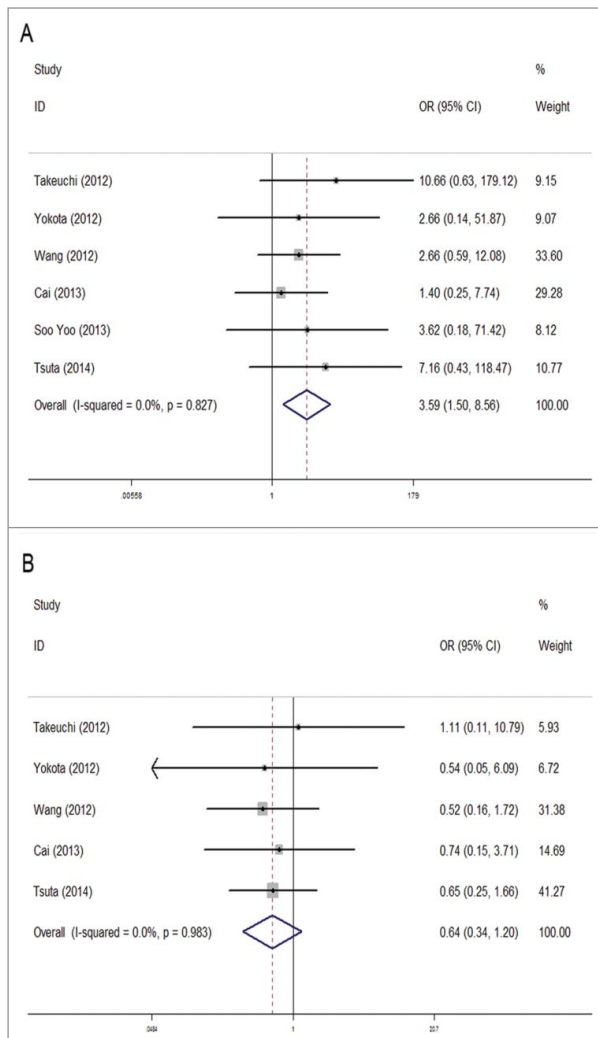
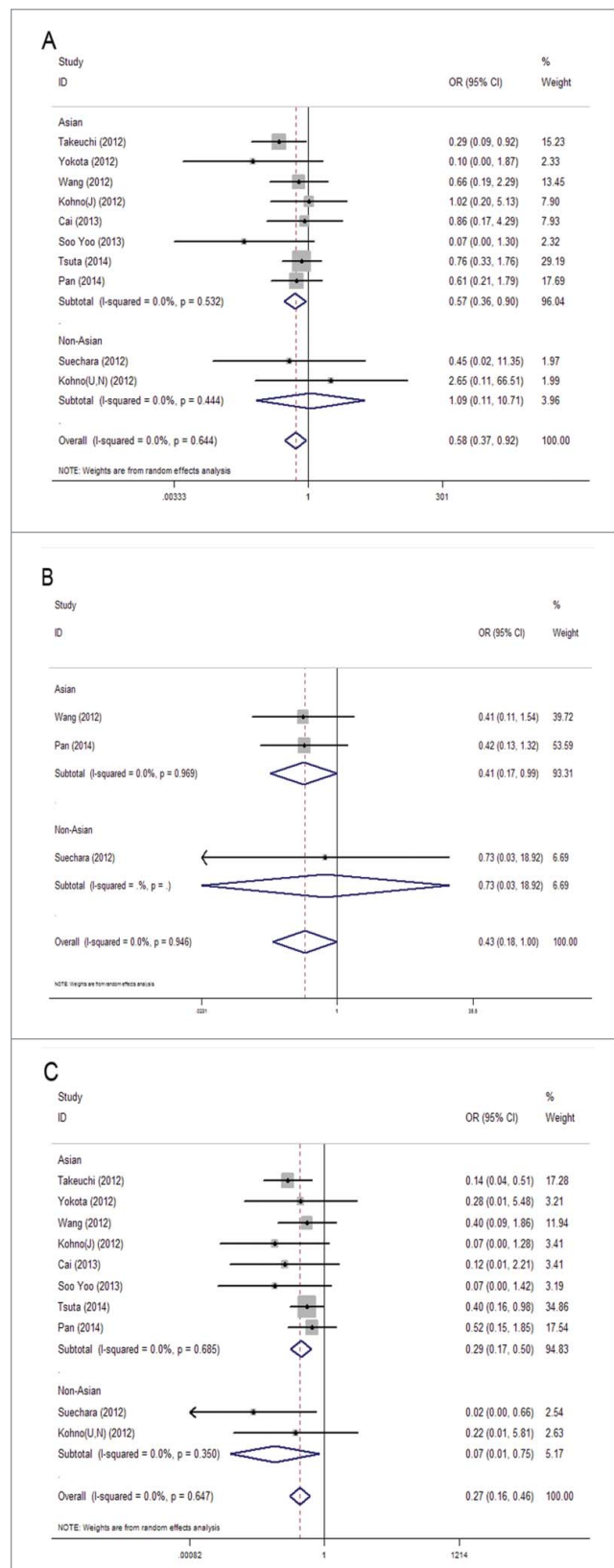


Figure 3. Meta-analysis of the association between RET fusion genes and clinicopathological characteristics: Histology type: adenocarcinoma vs. non-adenocarcinoma (A); TNM stage: stage I + II vs. stage III + IV (B).

males, which has not been declared previously. Reasons for this remain unclear, and it may be associated with different lifestyles, smoking habits and endocrine factors between females and males. However, non-Asian patients did not show this tendency, and no obvious difference of gender was found according to the subgroup analysis. The discrepancy may be due to different environmental and dietary factors in Asian and Non-Asian regions and/or cohort sizes. The meta-analysis also suggested that non-smokers had an increased incidence RET fusion genes (4 times that of smokers), which accorded with most previous studies.^{12,21} Interestingly, genotype-related sensitivity has also been proven in

Figure 4. Subgroup meta-analysis of the association between RET fusion genes and demographic features in Asians and Non-Asians: gender (a), age (b), smoking history (c). Studies are divided into Asian and Non-Asian group and plotted in order of publication years. Kohno (J) refers to Japanese patients in the study, and Kohno (U, N) refers to United States and Norway patients in the study.

never smokers of other subgroups of lung cancer like EGFR mutated lung cancer and EML4-ALK-positive lung cancer, suggesting that lung cancer in non-smokers is likely to be an



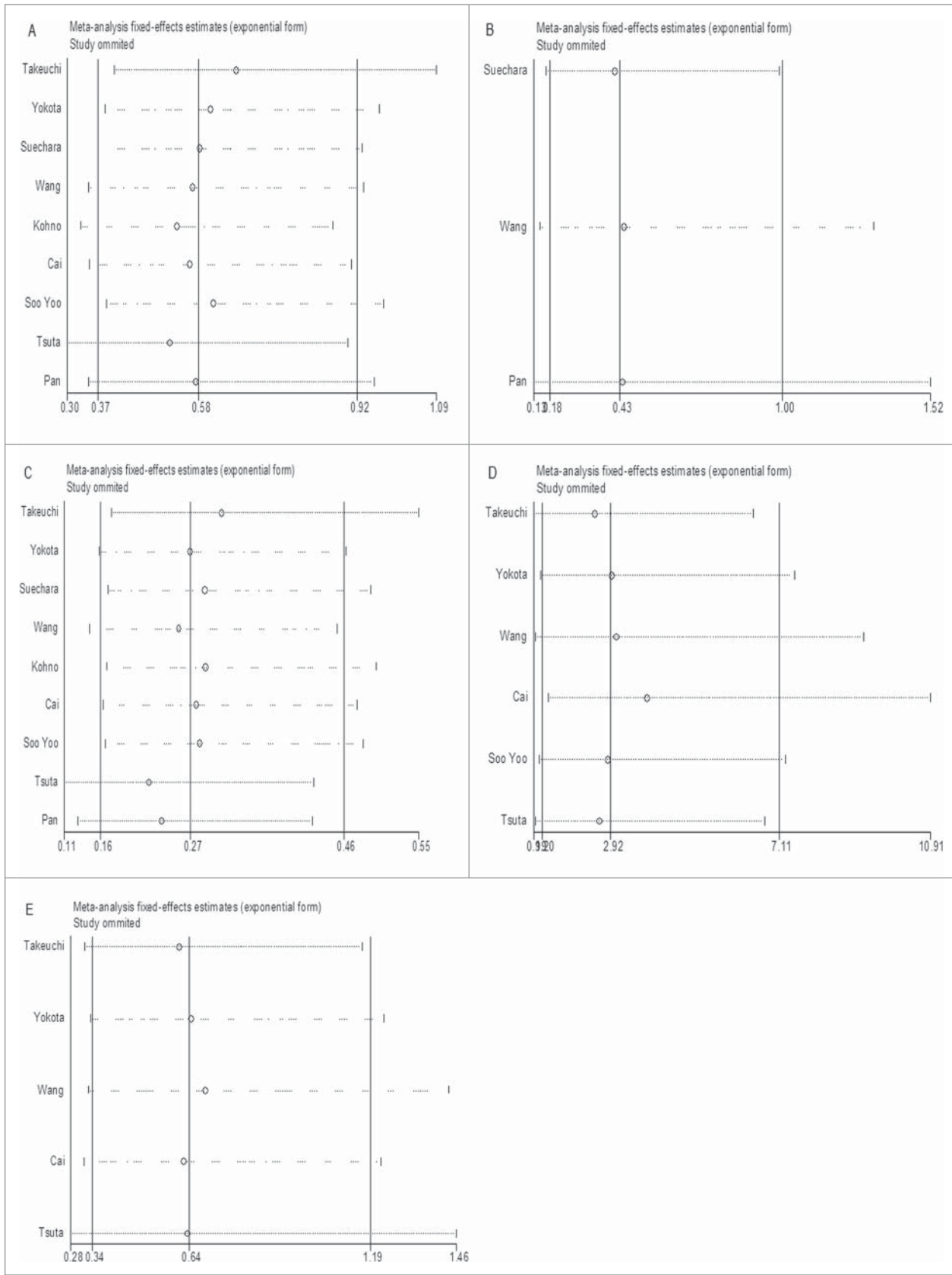


Figure 5. For figure legend, see next page.

assemblage of molecularly defined subsets.^{3,28,29} Risk factors for non-smokers in lung cancer have been described in several studies, including second-hand smoking, occupational exposure to carcinogens, hormonal element and cooking or heating fumes. Other factors reported are infections, dietary factors et al.^{30,31}

We also found a statistical difference between RET fusion genes and age, similar to Tsuta's result, demonstrating that RET fusion genes occurred preferentially in younger patients.²¹ It has been suggested that familial susceptibility and Mendelian inheritance may result in the early onset of lung cancer, which may account for the early onset of the lung cancer in younger patients.^{32,33} Moreover, the influence of different age-grouping criterion should be taken into consideration. For example, Suechara et al. divided the patients into the younger and older groups by 60 years, while Cai et al. divided by 65 years, thus we failed to combine these data, which affected the sample size and diminish the power of test in meta-analysis.^{20,22} Subgroup analysis also showed a significant correlation with younger age in Asian group, but not in non-Asian group. The discrepancies could be possibly explained that only one study was available in non-Asian group, so the authenticity of the conclusion remained to be confirmed.

For clinicopathological characteristics, the meta-analysis revealed that RET fusion genes occurred more frequently in ADC patients compared with NADC patients, which was consistent with former reports.^{11,12} In recent study, Dabir et al. found that small-cell lung cancer(SCLC) cells had a higher RET expression compared with adenocarcinoma lung cells, which deserves further investigation about the emerging role for RET in SCLC.³⁴ Besides, in concordance with previous reports, we did not detect obvious correlation between RET fusions and TNM stage.^{12,21,22} As to other factors like tumor size or differentiation, meta-analysis was not conducted because we failed to have the access to sufficient original data. The prognostic value of the RET fusion gene in NSCLC was also investigated in some studies. Takeuchi demonstrated that patients carrying fusion genes, including ALK, ROS1 and RET fusions, may have a better prognosis than patients without fusion genes, while the survival analysis of Cai's study showed the opposite result.^{7,22} Therefore, more studies with precise data are required to illustrate these aspects.

Indeed, the discovery of driver oncogene in NSCLC has dramatically changed today's therapeutic landscape, and the development of molecular profiling technology provides the potential of tailored medical care. The recognition of specific characteristics of RET-positive patients may help to narrow the scope of gene screening and target people for the individualized treatment of RET inhibitors. For example, we should put more emphasis on female adenocarcinoma patients with no smoking history since they are more likely to carry RET fusion genes and probably be sensitive to RET inhibitors.

Overall, the present meta-analysis is the first study to systematically illustrate the correlation of RET fusion genes with demographic and clinicopathological features of NSCLC. We carried out extensive searches to identify studies, and detected no significant heterogeneity or publication bias among eligible studies, indicating that our results were statistically robust and credible. Nevertheless, some limitations of this meta-analysis could not be ignored. On the one hand, our study was univariate analysis, therefore, potential interactions may exist between study variables, like gender and smoking status, for the overwhelming majority of females did not have a smoking history. On the other hand, some other characteristics like tumor size, differentiation and prognosis were not analyzed in our study due to the lack of original data. Further studies are warranted to provide more complete information.

In conclusion, this meta-analysis firstly revealed the significant association between the RET fusion gene and gender, along with age, smoking status and histology type, but not stage. Asian females with younger age, non-smokers and lung adenocarcinomas patients are more likely to carry the RET fusion gene. Our study may have implications for clinicians to select cases with fusion genes and to target patients for better responses to RET inhibitors in NSCLC treatment.

Materials and Methods

Search strategy

PubMed, EMBASE, and Web of Science databases were searched using the keywords "RET fusion" or "RET rearrangement" and "lung cancer" or "pulmonary tumor" or "NSCLC" or "non-small cell lung cancer" with species restricted to human. The last search time was February 1, 2015. Titles and abstracts were screened to identify reports which examined the association of RET fusion genes with demographic and clinicopathological features in NSCLC patients. Reference lists of original articles and review articles were also manually searched for additional eligible studies.

Selection

The inclusion criteria were applied to identify eligible studies: (a) human-based investigations; (b) pathologically confirmed non-small cell lung cancer; (c) the demographic features and clinicopathological characteristics of patients were provided; (d) the availability of data to allow the estimation of the odds ration (OR) with a 95% CI; (e) published in English.

Data extraction

Studies were independently assessed by 2 investigators to extract the following data: surname of the first author, year of

Figure 5. (See previous page). Sensitive analysis of the effect of individual studies on the pooled OR for association between RET fusion genes and gender (A), age (B), smoking history (C), histology type (D) and TNM stage (E). The vertical axis in the middle indicates the overall OR, and the 2 vertical axes on both sides indicate the 95% CI. Every hollow round indicates the pooled OR when the left study was omitted in a meta-analysis with a random model. The two ends of every broken line represent the respective 95% CI.

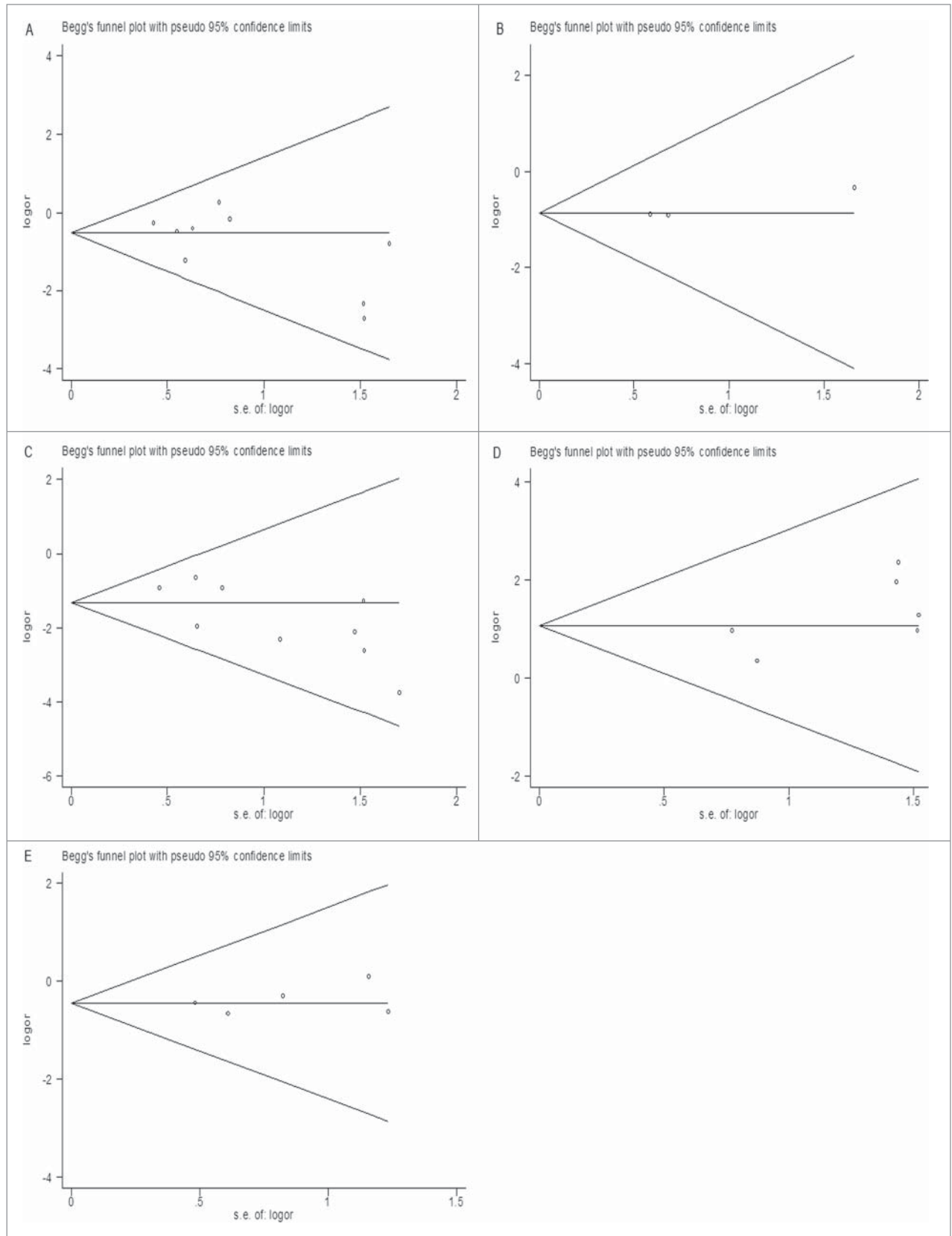


Figure 6. The funnel plot of studies included in the overall analysis of the association between RET fusion genes and gender (A), age (B), smoking history (C), histology type (D) and TNM stage (E). The funnel plot displays log OR against its standard error (s.e.) for each study included in the overall analysis. The horizontal line indicates the summary estimate of the OR, with the oblique lines indicate the expected 95% CI for a given standard error.

Table 2 Summary of publication bias test

	Begg's test		Egger's test	
	Z	P	t(bias)	P
RET fusion genes and gender	0.94	0.348	-1.58	0.159
RET fusion genes and age	0.52	0.602	3.36	0.184
RET fusion genes and smoking	1.56	0.118	-2.53	0.39
RET fusion genes and histology type	0.75	0.452	1.73	0.158
RET fusion genes and TNM stage	0.24	0.806	0.79	0.485

publication, study location, sample size, RET mutation status (the numbers of cases with RET mutation, the mutation types), demographic features (age, gender, smoking history), clinicopathological characteristics (histological types, clinical TMN stage) and assay method. Each study was examined fully to eliminate duplicates. Disagreements were resolved by discussion among 3 authors.

Data pooling and statistics

STATA 12.0 (STATA Corp., College Station, TX USA) was used to perform statistical analysis. For each study, the crude ORs and 95% CIs were calculated using χ^2 or Fisher's exact tests for fourfold table. To evaluate the overall strength of the

association between RET fusions and clinical characteristics, the pooled OR was calculated to estimate the effective value. The chi-square -based Q test and Higgin's I^2 statistic were performed to assess the heterogeneity between studies. When strong heterogeneity existed (Q test with $P < 0.05$ and I^2 statistic with values $> 50\%$), the pooled OR was calculated using a random effects model (The DerSimonian and Laird method). Otherwise, a fixed effects model (Mantel-Haenszel method) was more appropriate. Sensitivity analysis was conducted to confirm the credibility of the results in this meta-analysis, by successively omitting each individual study and pooling the remaining studies. We evaluated the publication bias using Begg's funnel plot and tested it with Egger's test. P value < 0.05 was considered statistically significant.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

Financial support was provided by project NO. 124119a6200 supported by Shanghai Science and Technology Committee.

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