Meta-Analysis of The Association Between Gene Mutation and Tumor Spread Through Air Space in Lung Adenocarcinoma

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Research article

Keywords: lung adenocarcinoma, tumor, spread through air space, Meta-analysis

Posted Date: May 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-477599/v1

Abstract

Background A series of studies have shown that spread through air spaces (STAS) has a significant effect on surgical outcomes in patients with lung adenocarcinoma.

Objectives: The aims of this study are to evaluate the association between gene mutation and STAS in lung adenocarcinoma for preoperative prediction of STAS in lung adenocarcinoma, eventually, which could help us choose appropriate surgical type.

Methods The retrieval period was from 2015 to December 2020. The Newcastle-Ottawa quality assessment scale (NOS) was used for assessing the quality of each study. High-quality study was defined as those with ≥6 stars at the NOS. Statistical results were analyzed using the Stata16 statistical package.

Results A total of 12 studies were included, including 4790 patients. The results of meta-analysis showed that: In patients with lung adenocarcinoma, the expression of EGFR, KRAS, BRAF, ALK and ROS1 were correlated with the occurrence of STAS, with RR values (95%CI) of 0.75 (0.63~0.90), 1.173 (1.00~1.37), 2.116 (1.17~3.84), 1.71 (1.53~1.90), 1.565 (1.35~1.82), respectively.

Conclusion Mutations in KRAS, BRAF, ALK, and ROS1 increase the incidence of STAS in patients with lung adenocarcinoma. Mutations in EGFR decrease the incidence of STAS in patients with lung adenocarcinoma.

Introduction

Lung cancer is one of the most common malignant tumors in the world, and one of the main causes of death from malignant tumors [1]. For years, researchers have worked to detect cancer early and have saved countless lives. After lung cancer is identified, the classification and staging of lung cancer will have a great impact on the choice of surgical methods for patients, to ensure the survival time and quality of life of patients with lung cancer as far as possible. Therefore, precise diagnosis of a patient's cancer type before surgery is of Paramount importance. In the past, surgical modality has been defined by the TNM Staging System and patient tolerance. However, in 2015, Kadota [2] discovered a new mode of lung adenocarcinoma dissemination —— spread through air space (STAS). Kadota define it as spread of lung cancer tumor cells into air spaces in the lung parenchyma adjacent to the main tumor. And it was found that STAS significantly affected postoperative survival. In the same year, the World Health Organization (WHO) included STAS into the invasive mode of lung adenocarcinoma. [3] Although there is still some debate about STAS. [4-6] However, mainstream opinion has accepted the existence of STAS. Since then, a large number of researchers have investigated the effect of STAS on postoperative survival of lung adenocarcinoma, as well as the effect of different surgical procedures on postoperative survival of patients with the same number of stages but different STAS. The researchers found that STAS significantly affected patient prognosis. In a study of the relationship between STAS and prognosis in

patients with early lung adenocarcinoma , Kadota found that STAS was associated with postoperative recurrence of lung adenocarcinoma in the sublobectomy group , but STAS was not an adverse prognostic factor in the lobectomy group. [7] Ren in the stage of Ⅰ a lung cancer study, no matter you choose operation method, STAS positive patients of recurrent free survival （RFS） and overall survival （OS） were significantly better than the STAS negative patients. [8] It's looks like shows in patients with stage Ⅰ a adenocarcinoma of the lung, the tumor STAS is positive or negative for does not affect the choice of the surgery. But then Shiono in on the prognosis of patients with lung adenocarcinoma of Ⅰ a study found that if to subdivide thesub-lobectomy for segmentectomy and pulmonary wedge resection, then STAS in pulmonary wedge resection is OS significant prognostic factors, but not in the lung segment resection. [9] Therefore, accurately determining whether a tumor has STAS will determine the best surgical procedure for a patient before surgery. Currently, the only way to determine STAS is through postoperative pathological staining, which can only be known a few days after the patient's surgery. Obviously, although in theory, STAS can be used to guide a patient's approach to surgery, in practice, the presence or absence of a STAS can only be known after a patient's surgery. It is impossible to use information that is known only after an operation to guide an operation that has already been performed. To this end, many researchers are trying to find ways to determine whether tumor tissue has STAS before or during surgery. Walts, A. E. [10]tried to use Frozen Section Evaluation to determine STAS, but failed to find its effect. Some groups have attempted to use preoperative needle biopsies to determine whether patients have STAS, but preoperative needle biopsies have also proved unable to determine whether tumor tissues have STAS. However, after reading a large number of literatures, we found that there were significant differences in tumor gene expression between STAS positive patients and STAS negative patients in lung adenocarcinoma patients. If the association between tumor genes and STAS can be further clarified, we will be able to preoperatively determine whether the tumor has STAS through the genetic diagnosis of tumor samples obtained by puncture.

Methods

1.1 Inclusion and exclusion criteria

Inclusion criteria（1）The study design：Cohort study on the relationship between gene expression and STAS in lung adenocarcinoma patient. Due to the particularity of such studies, exposure factors and outcome indicators of patients can only be obtained after obtaining postoperative pathological tissue of patients with lung adenocarcinoma. So that：even retrospective studies on survival analysis of lung adenocarcinoma can still be regarded as cohort studies when studying the relationship between gene expression and STAS. （2）Patients：Patients undergoing radical surgery for lung adenocarcinoma. The tumor stage, race, nationality, sex, and age of the patient were not limited, and the patient had no comorbidities. （3）Exposure factor：The gene expression of lung adenocarcinoma tumors of patients was analyzed using tumor tissue samples obtained after radical surgery. （4）Outcome indicator：The final pathological results were positive or negative for STAS.

Exclusion criteria：（1）If a gene has been studied in only one literature, the gene is excluded. （2）Low quality literature with a NOS score of less than 6. （3）Studies in which raw data on relevant outcomes were not clearly presented.（6）Non-Chinese and English Literature. （7）Studies where the sample is duplicated with other studies.

1.2 Literature search strategy

We performed a systematic literature search through the following databases: PubMed, Embase, Web of Science, Cochrane Library, CNKI, Veep and Wanfang Data. The retrieval dates from the STAS discovery of 2015 to December 2020 and contains received, but unpublished, preprints. Use the ways that combined subject words and free words to search，while sacrificing accuracy to ensure comprehensiveness，The main search terms included: spread through air spaces，STAS，intra-alveolar tumor spread，air space invasion，lung adenocarcinomas, etc.

1.3 Data extraction

Two researchers will screen the literature respectively, and the third researcher will discuss and resolve the differences of opinions encountered. The data were extracted independently and cross-checked by two researchers. In cases of disagreement in the extraction of data, both parties re-examine the original literature together. During literature screening, first read the title and abstract to exclude the obviously irrelevant literature, and then read the full text of the literature to determine the literature with relevant research content. The gene types studied in the literature were extracted. If any genes were studied by only one literature, the Meta-analysis of these genes would be further excluded. Then we detailed data extraction was carried out for the remaining literatures.

1.4 Quality assessment

Quality was assessed using the Newcastle-Ottawa Scale (NOS). The NOS consists of three parts: selection (0-4stars), comparability (0–2 stars), and outcome assessment (0–3 stars). NOS stars of≥6 was regarded as high-quality studies.

1.5 Statistical analysis

Data analysis was performed using StataMP 16.0. The original data from all the original studies were extracted, and the rate ratio（RR）value and 95%CI were combined after heterogeneity test to evaluate the influence of different gene expression in lung adenocarcinoma patients on STAS. The heterogeneity amid studies was tested by H statistic and I^2 statistic. If I^2< 31%, then we consider these studies are considered homogeneous. If I^2>56%, we believe that there is heterogeneity among the studies. When 31%≤I^2≤56%, reference H statistic, if H<1.2, all studies are considered homogeneous, and if H>1.5, it is considered that there is heterogeneity among all studies. When 1.2≤H≤1.5, if the 95%CI of H value does not contain 1, it is considered that there is heterogeneity; otherwise, it is considered that all studies are homogeneous. When there was no heterogeneity, the fixed effect model was used to merge. When there was heterogeneity, the random-effects model was used for data consolidation, and subgroup analysis was

attempted to eliminate heterogeneity. Potential publication offset was evaluated by BEEG and EGGER test.

Results

2.1 Search results

A total of 1047 articles were searched:9 from CBM, 158 from Embase, 251 from PubMed, 194 from Web of science,130 from Wanfang date, 32 from Veep and 273 from CNKI. After carefully inspection of these articles, 12 Original studies[7, 11-21] were finally enrolled in our meta-analysis. The detail processes of study selection were showed in the flow diagram (Fig. 1). The clinical features and NOS scores of the included studies were shown in Table 1. In the end, 4790 patients (1752 STAS positive patients and 3038 STAS negative patients) were included in the analysis. The overall quality of the included literatures was high, with NOS scores above 6 stars.

2.2 Results of Meta-analysis

2.2.1 The association between EGFR genotype and STAS in lung adenocarcinoma

A total of 11 original studies were included [11-21]. We combined these studies using random effects. In the original studies, 3 studies suggested no significant association between EGFR expression and STAS, 1 study suggested that patients with EGFR mutation had a higher risk of STAS positivity, and 7 studies suggested that patients with EGFR mutation had a lower probability of STAS positivity. This meta-analysis showed that among patients with lung adenocarcinoma, patients with mutant EGFR had a lower probability of STAS RR=0.75 95%CI 0.63~0.90 z=3.09 P=0.002 Fig. 2.

2.2.2 The association between KRAS genotype and STAS in lung adenocarcinoma

A total of 4 original studies were included [11, 12, 14, 17]. We combined these studies using fixed effects. In these original studies, all of the 3 papers believed that KRAS was not associated with STAS within the confidence interval of P >0.1. However, the results of this meta-analysis suggested that patients with KRAS gene mutation had a higher incidence of STAS RR=1.17 95%CI 1.00~1.37 z=1.99 P=0.047 Fig. 3. This may be related to the relatively low proportion of patients with KRAS mutation. If the sample size can be expanded, we believe that the original study will also get the same results as this meta-analysis.

2.2.3 The association between BRAF genotype and STAS in lung adenocarcinoma

A total of 2 original studies were included [14, 17]. We combined these studies using random effects. Meta-analysis results showed that BRAF mutation patients had a significantly increased incidence of STAS RR=2.12 95%CI 1.17~3.84 z=2.47 p=0.013 Fig. 4

2.2.4 The association between ALK genotype and STAS in lung adenocarcinoma

A total of 7 original studies were included [7, 11, 12, 14-16, 21]. We combined these studies using random effects. Meta-analysis results showed that patients with ALK gene mutation had a higher incidence of STAS than patients with ALK wild type.RR=2.03 95%CI 1.56~2.66 z=5.21 p<0.001 Fig. 5.1However, during the merger process, we found that the heterogeneity could be eliminated only by eliminating the results of Zhang, Z. [16]. After the exclusion of Zhang's study, we combined using fixed effect, and still concluded that patients with ALK gene mutation had a higher probability of STAS. RR=1.71 95%CI 1.53~1.90 z=9.74 p<0.001 Fig. 5.2

2.2.5 The association between PD-L1 genotype and STAS in lung adenocarcinoma

A total of 3 original studies were included [13, 14, 18]. We combined these studies using fixed effects. In the original study, HU, S Y's study[14] believed that among lung adenocarcinoma patients, the probability of PD-L1 mutation patients developing STAS was significantly lower than that of PD-L1 wild-type patients. However, the results of this meta-analysis indicated that there was no significant correlation between PD-L1 and the incidence of STAS in lung adenocarcinoma patients. RR=1.07 95%CI 0.83~1.37 z=0.52 p=0.602 Fig. 6

2.2.6 The association between ROS1 genotype and STAS in lung adenocarcinoma

A total of 4 original studies were included [11, 12, 15, 16]. We combined these studies using fixed effects. In the original study, three studies suggested that patients with ROS1 gene mutation had a higher incidence of STAS[11, 12, 15]. The combined results of this meta-analysis showed that patients with ROS1 gene mutation had a higher incidence of STASRR=1.57 95%CI 1.35~1.82 z=5.80 p<0.001 Fig. 7

2.3 Sensitivity analysis and publication bias

Begg's funnel plot and Egger's linear regression test were used to study the publication offset of the included studies with the combined number of literatures greater than or equal to 4. All content within a confidence interval of P>0.1 can be considered to have no publication offset (Table. 2). In this analysis, it was found that the research conclusions of Zhang, Z. [16] on the relationship between ALK and STAS occurrence were greatly deviated from other studies, which had a great impact on the results of data merging.(Fig .8) Therefore, the heterogeneity could be eliminated if this paper was excluded. However, whether this study is excluded or not, it can be concluded that patients with ALK gene mutation have a higher incidence of STAS. We believe that the heterogeneity may be related to the selection of reagents

Discussion

Since 2015, when Kadota discovered that STAS can significantly affect patients' surgical outcomes, there have been numerous studies on STASThere were also a series of meta-analyses on prognosis, which confirmed that STAS did have a significant impact on the prognosis of patients, but no meta-analysis on the relationship between gene mutations and STAS was explored. Moreover, if the association between gene mutation and the occurrence of STAS can be further confirmed, on the one hand, it can provide

guidance for the molecular mechanism of the occurrence of STAS in the future, and on the other hand, it can provide guidance for the surgical methods of patients. It has very important research significance and clinical value.

Previous studies on the relationship between genetic mutations and the occurrence of STAS have drawn inconsistent and even completely opposite conclusions. Therefore, a meta-analysis had to be carried out to combine the results. In this analysis, we combined the association between EGFR and STAS, resulting in moderate heterogeneity. We attempted to perform subgroup analysis by region and subgroup analysis by tumor number stage, but the heterogeneity was not eliminated. We hypothesized that the heterogeneity may be due to the different sensitivities of the reagents used among the groups. In the process of merging the association between ALK and STAS, a moderate degree of heterogeneity also appears. The heterogeneity can be eliminated only by eliminating the results of Zhang ,Z[16]，RR=1.71 95%CI 1.53~1.90 z=9.74 p<0.001。According to our analysis, the main reason is that the probability of ALK mutation itself is small. Specific to grouping, the number of ALK mutations is only single digits, so it is inevitable that some randomness will produce great interference to the results.

No publication bias was found in this study. From the results, negative results were also included in these papers, indicating that the possibility of publication bias was very small. The results are relatively reliable.

There are still some shortcomings in this study. The population in this meta-analysis is mainly Asian, and there are few studies on other ethnic groups. Therefore, it is not clear whether the combined results are different among different ethnic groups. In addition, the number of studies on some genes is small, which also leads to the conclusion being greatly influenced by randomness. Secondly, this study only included the results of two languages, English and Chinese, which may lead to a certain degree of risk of language deviation.

Conclusion

The results of this meta-analysis showed that tumors with KRAS, BRAF, ALK and ROS1 gene mutations had a higher probability of STAS. Tumors with mutant EGFR had a lower incidence of STAS. The occurrence of STAS is independent of PD-L1. Among them, the probability of STAS positive in patients with lung adenocarcinoma with ROS1 gene mutation was twice as high as that in patients with ROS1 negative lung adenocarcinoma. The occurrence of STAS may be related to tumor genes and external environment. Mutations of different tumor genes or different external environment may independently or synergically affect the occurrence of STAS, and the specific mechanism is expected to be further studied in the future. Therefore, if a series of genes such as KRAS, BRAF, ALK and ROS1 are found to be mutated by preoperative genetic testing in patients with early lung adenocarcinoma, while EGFR is wild type, lobectomy can be considered, which can significantly improve the postoperative survival rate of patients. Otherwise, sub-lobectomy can be considered, which will not significantly reduce the postoperative survival rate, but can reduce the loss of lung function and improve the postoperative quality of life of patients.

Abbreviations

STAS: Spread through air spaces;

NOS: The Newcastle-Ottawa quality assessment scale;

RFS: Recurrent free survival;

OS : Overall survival;

RR: Rate ratio;

Declarations

Ethics approval and consent to participate Not applicable

Consent for publication Not applicable

Availability of data and materials The data that support the findings of this study are available in Pubmed at 10.1097/PAS.0000000000001285 10.3760/cma.j.issn.0529-5807.2017.05.004 10.1186/s12885-020-07200-w 10.1016/j.jtcvs.2018.04.126 10.1016/j.lungcan.2019.03.005 10.1016/j.lungcan.2018.07.020 10.21037/jtd-20-1820 10.1097/PAS.0000000000000409 10.1016/j.athoracsur.2018.01.037 10.1093/icvts/ivw211 10.21037/jtd-20-1820 10.1148/radiol.2018180431.

sources of funding None

Authors' contributions GW and BL worked together to collect the original study data and conduct a quality assessment GW did the data analysis was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Acknowledgements Not applicable

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Tables

Table. 1 The clinical features and NOS scores of the included studies.

author	year	region	stage	Male/Female	N[a]□STAS+ /STAS-□	NOS
Sun, P L[11]	2017	China	□~□	139/149	288□178/110□	8□
Jia, M[12]	2020	China	□~□	150/153	303□183/120□	8□
Toyokawa, G[13]	2018	Japan	□~□	153/174	327□191/136□	6□
Hu, S Y[14]	2018	China	□-□	163/337	500□134/366□	7□
Lee, J S[15]	2018	Korea	□-□	158/158	316□160/156□	7□
Kadota, K[7]	2019	Japan	□-□	380/355	522□225/297□	7□
Zhang, Z[16]	2020	China	□a	276/486	762□83/679□	7□
Warth, A[17]	2015	Germany	□-□	333/236	569□288/281□	8□
Toyokawa, G[18]	2018	Japan	□	134/142	276□153/123□	7□
Shiono, S[19]	2016	Japan	□	149/169	318□47/271□	6□
Liu, Z[20]	2021	China	□a	92/241	333□18/315□	6□
Kim, S K[21]	2018	Korea	□~□	129/147	276□92/184□	7□

a□That number was the number of patients included in the study, but not the number of patients whose genes were ultimately tested.

Table. 2 Begg’s funnel plot and Egger’s linear regression test

gene	literature quantity	method	z	P	method	t	P
EGFR	11	Begg	1.56	0.12	Egger	-1.54	1.58
KRAS	4	Begg	-0.34	1.00	Egger	0.66	0.58
ALK	7	Begg	1.50	0.13	Egger	1.25	0.27
ROS1	4	Begg	-0.34	1.00	Egger	-0.23	0.84

Figures

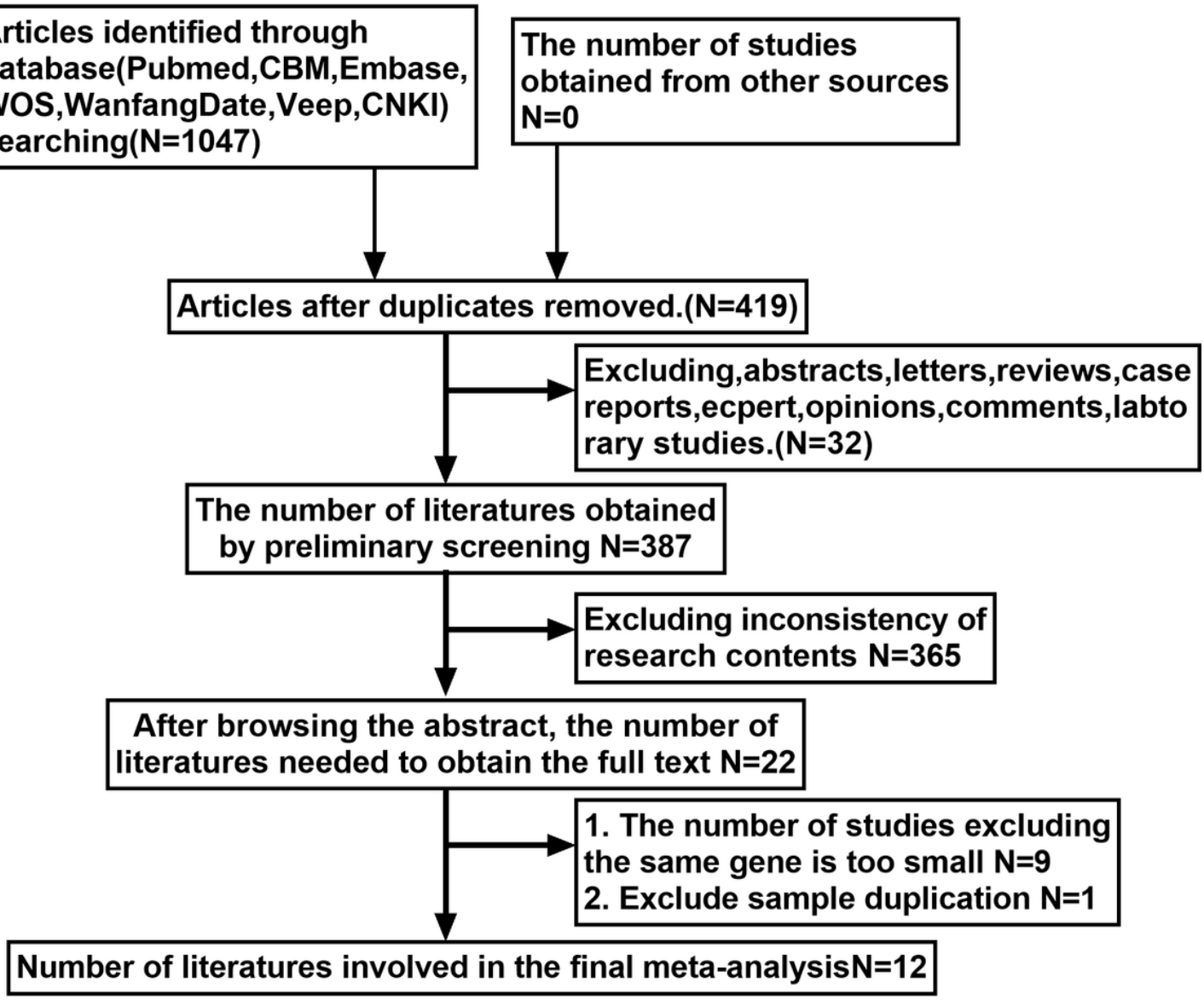

Figure 1

A total of 1047 articles were searched:9 from CBM, 158 from Embase, 251 from PubMed, 194 from Web of science,130 from Wanfang date, 32 from Veep and 273 from CNKI. After carefully inspection of these articles, 12 Original studies7, 11-21 were finally enrolled in our meta-analysis. The detail processes of study selection were showed in the flow diagram (Fig. 1). The clinical features and NOS scores of the included studies were shown in Table 1. In the end, 4790 patients (1752 STAS positive patients and 3038 STAS negative patients) were included in the analysis. The overall quality of the included literatures was high, with NOS scores above 6 stars.

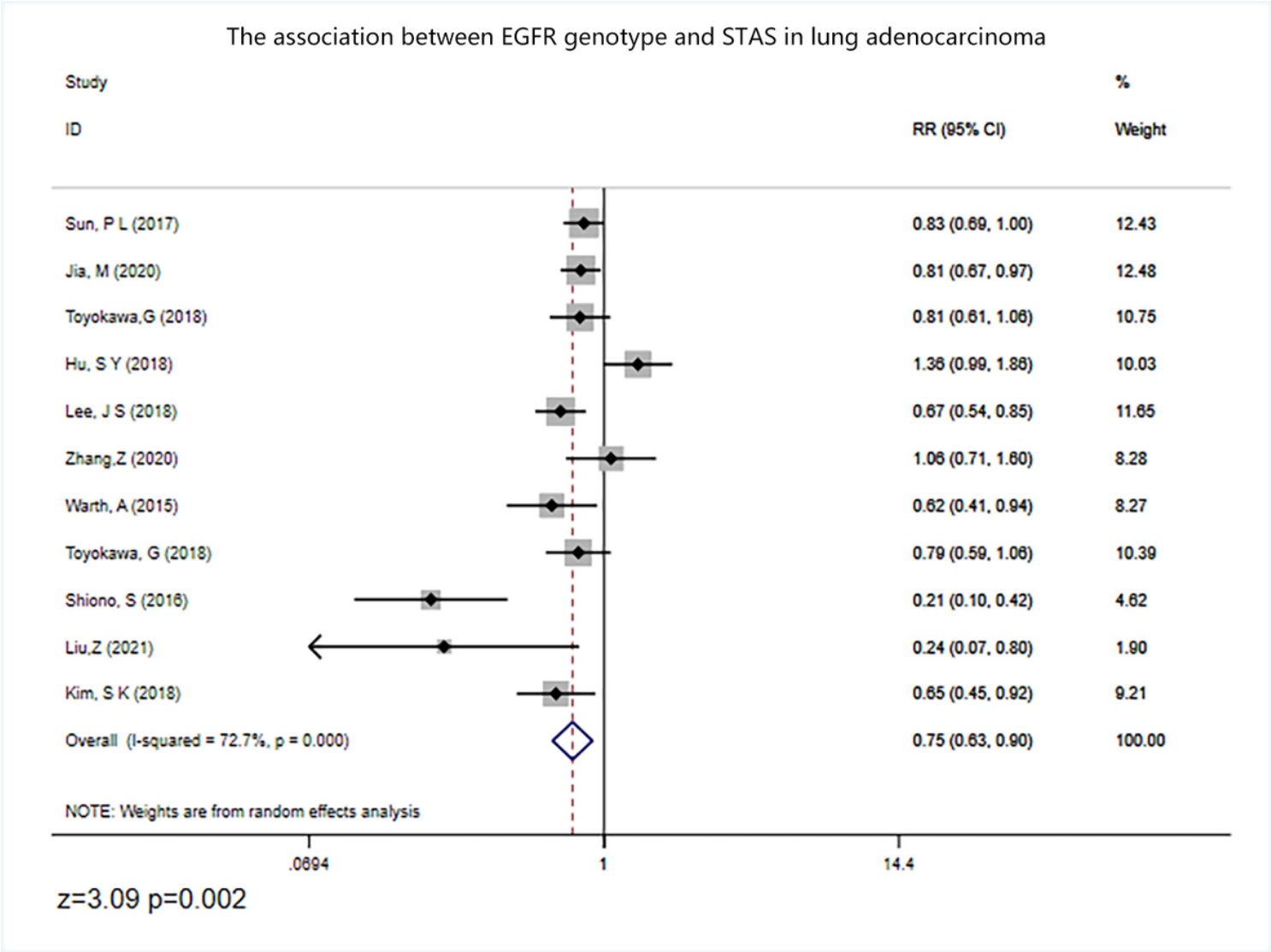

Figure 2

A total of 11 original studies were included 11-21. We combined these studies using random effects. In the original studies, 3 studies suggested no significant association between EGFR expression and STAS, 1 study suggested that patients with EGFR mutation had a higher risk of STAS positivity, and 7 studies suggested that patients with EGFR mutation had a lower probability of STAS positivity. This meta-

analysis showed that among patients with lung adenocarcinoma, patients with mutant EGFR had a lower probability of STAS（RR=0.75（95%CI 0.63~0.90 z=3.09 P=0.002 Fig. 2）.

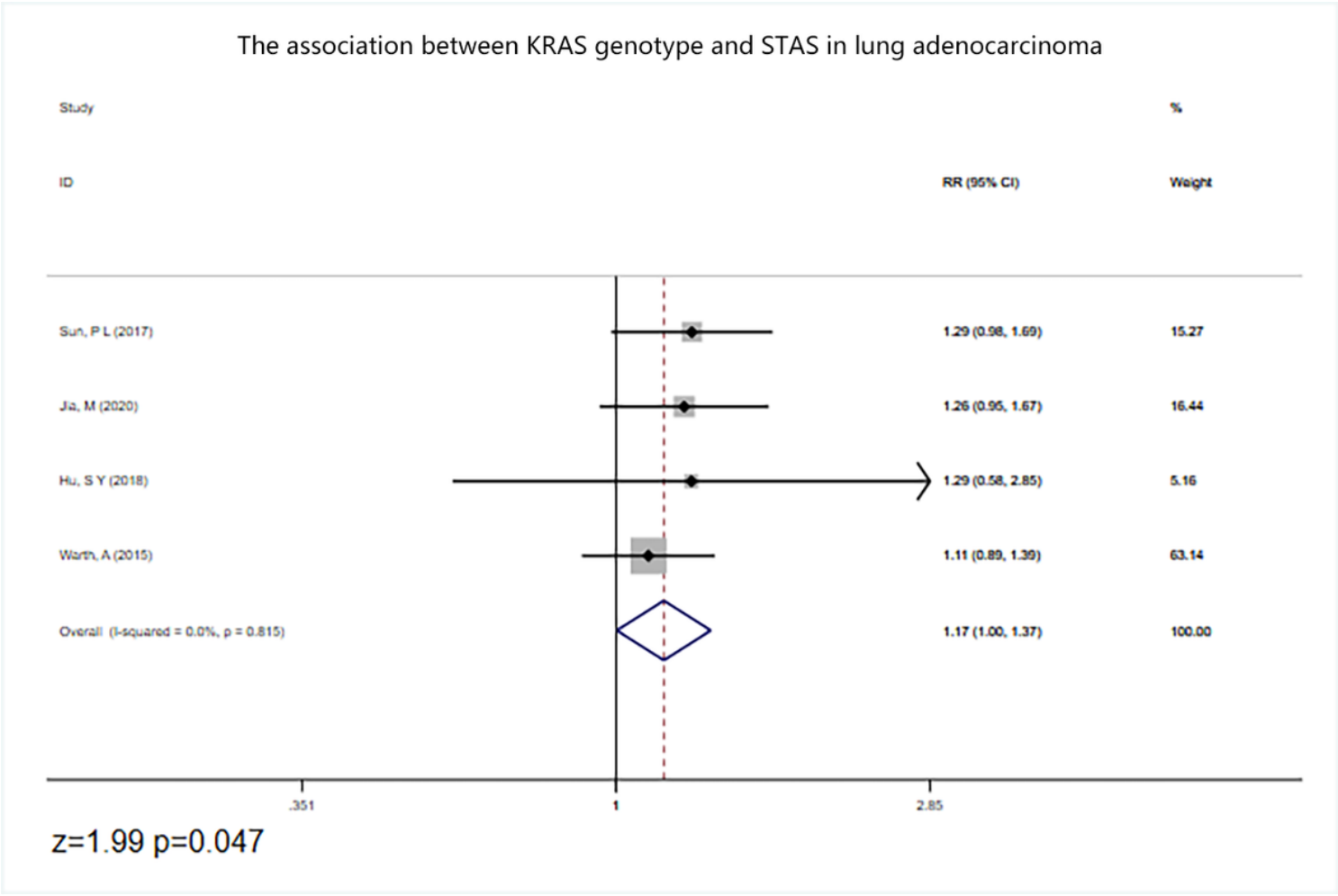

Figure 3

A total of 4 original studies were included 11, 12, 14, 17. We combined these studies using fixed effects. In these original studies, all of the 3 papers believed that KRAS was not associated with STAS within the confidence interval of P >0.1. However, the results of this meta-analysis suggested that patients with KRAS gene mutation had a higher incidence of STAS（RR=1.17 95%CI 1.00~1.37 z=1.99 P=0.047 Fig. 3）. This may be related to the relatively low proportion of patients with KRAS mutation. If the sample size can be expanded, we believe that the original study will also get the same results as this meta-analysis.

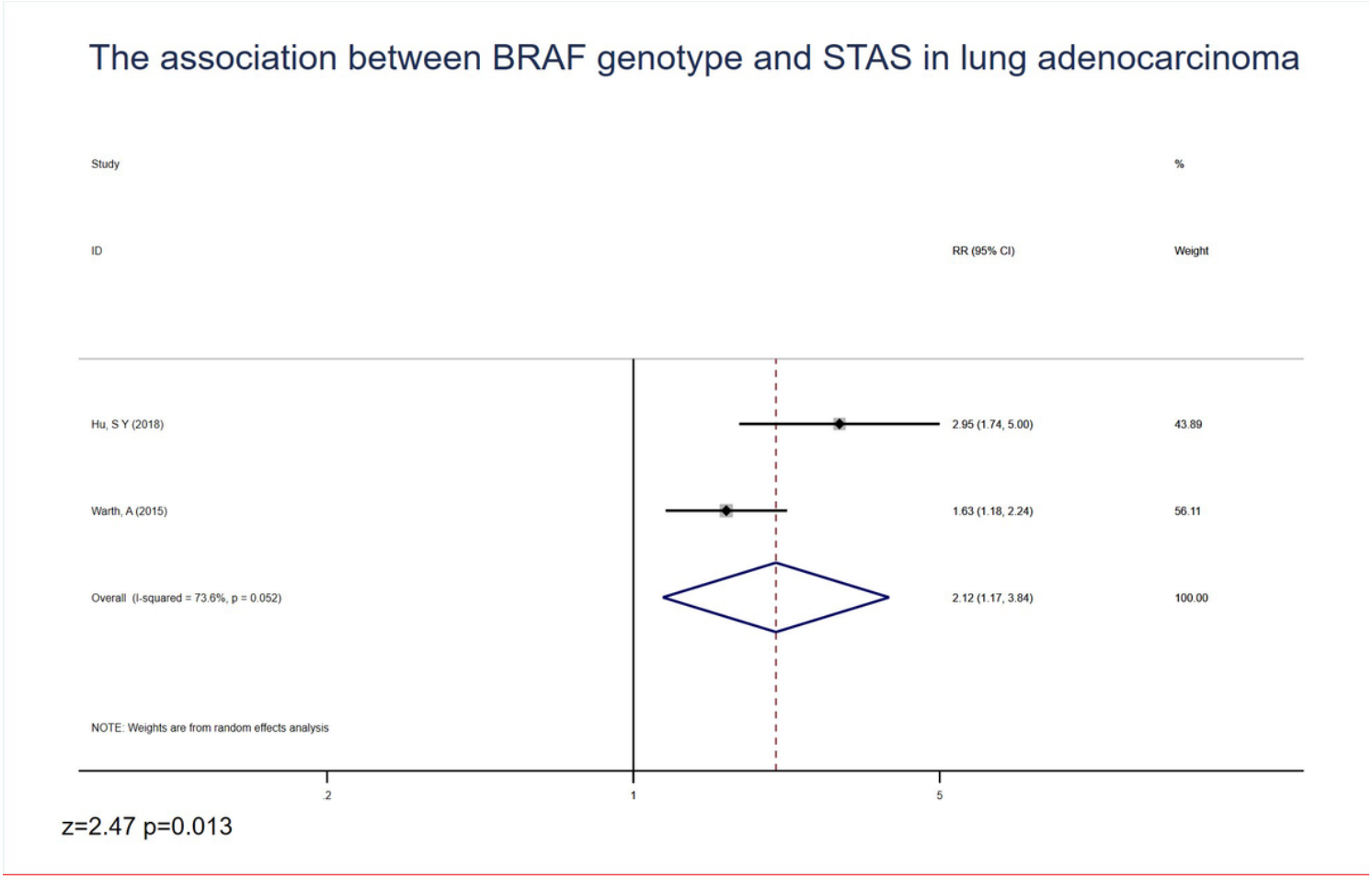

Figure 4

A total of 2 original studies were included 14, 17. We combined these studies using random effects. Meta-analysis results showed that BRAF mutation patients had a significantly increased incidence of STAS （RR=2.12 95%CI 1.17~3.84 z=2.47 p=0.013 Fig. 4)

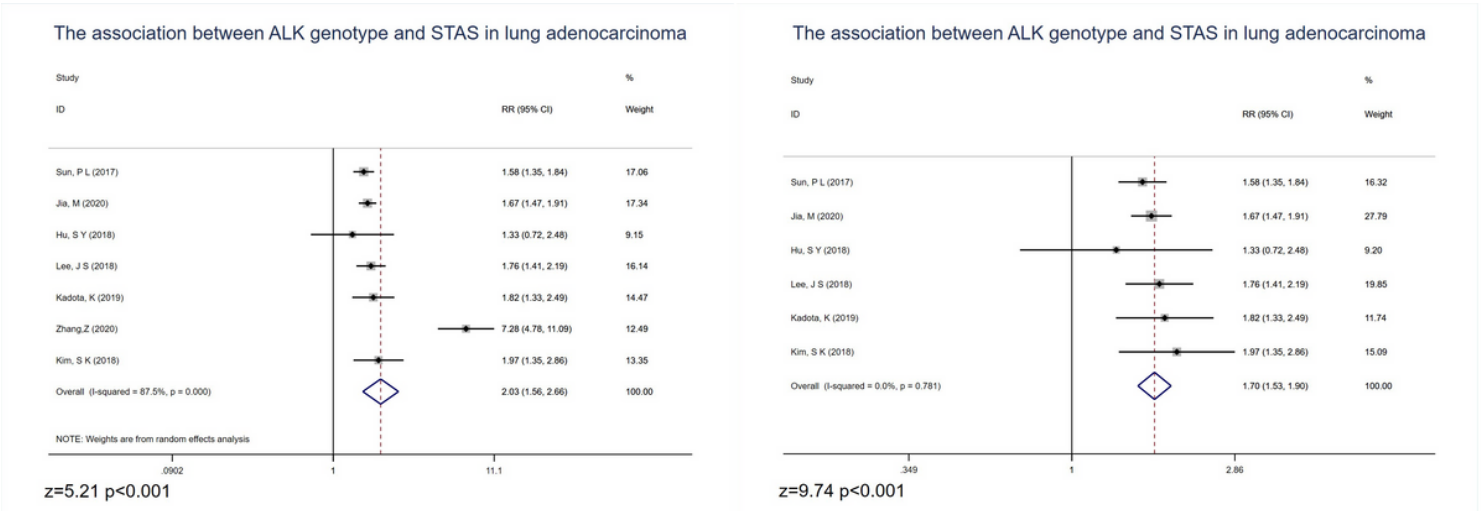

Figure 5

A total of 7 original studies were included 7, 11, 12, 14-16, 21. We combined these studies using random effects. Meta-analysis results showed that patients with ALK gene mutation had a higher incidence of STAS than patients with ALK wild type.RR=2.03 95%CI 1.56~2.66 z=5.21 p<0.001 Fig. 5.1However, during the merger process, we found that the heterogeneity could be eliminated only by eliminating the results of Zhang, Z. 16. After the exclusion of Zhang's study, we combined using fixed effect, and still concluded that patients with ALK gene mutation had a higher probability of STAS. RR=1.71 95%CI 1.53~1.90 z=9.74 p<0.001 Fig. 5.2)

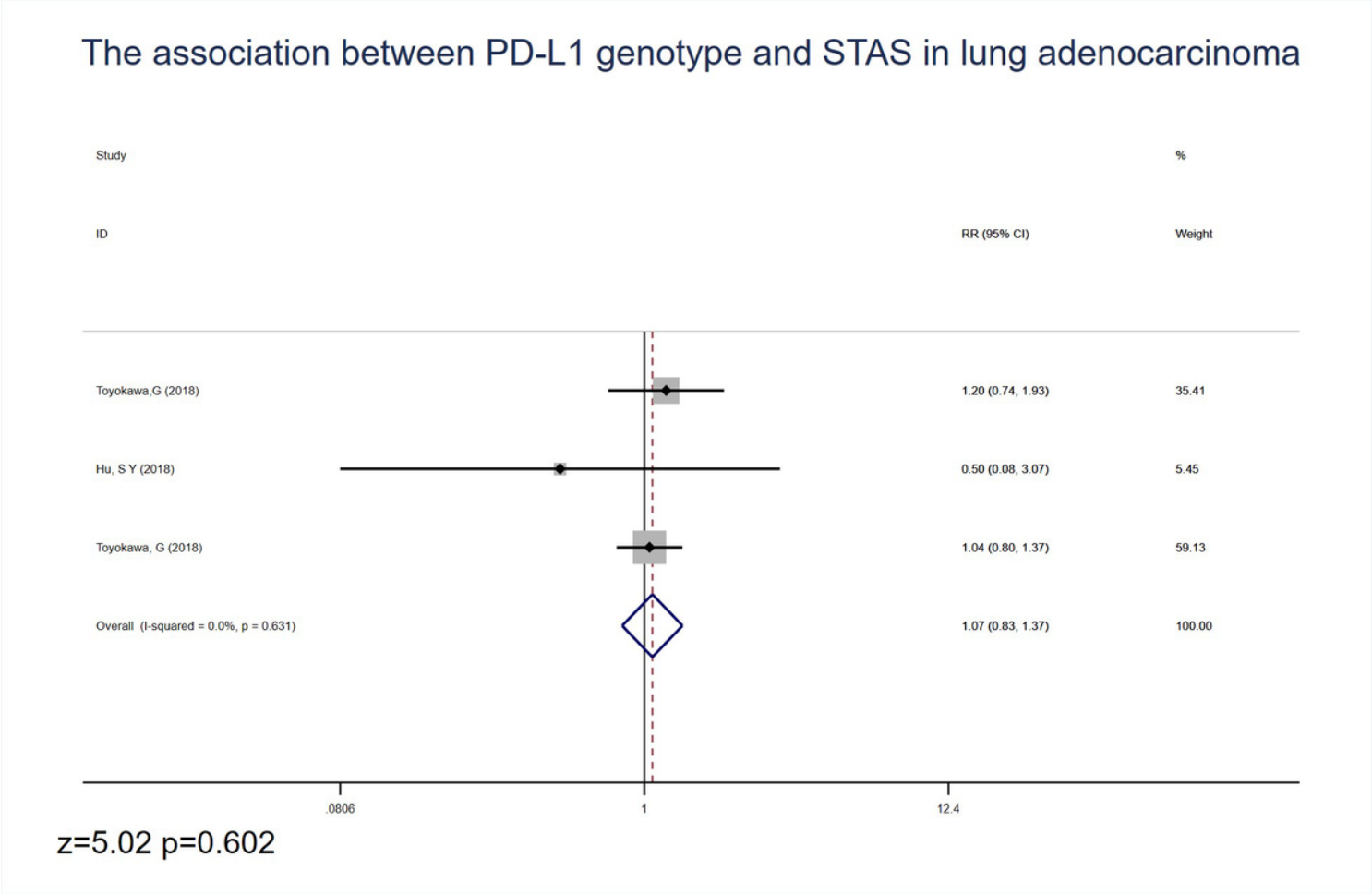

Figure 6

A total of 3 original studies were included 13, 14, 18. We combined these studies using fixed effects. In the original study, HU, S Y's study14 believed that among lung adenocarcinoma patients, the probability of PD-L1 mutation patients developing STAS was significantly lower than that of PD-L1 wild-type patients. However, the results of this meta-analysis indicated that there was no significant correlation between PD-L1 and the incidence of STAS in lung adenocarcinoma patients. RR=1.07 95%CI 0.83~1.37 z=0.52 p=0.602 Fig. 6

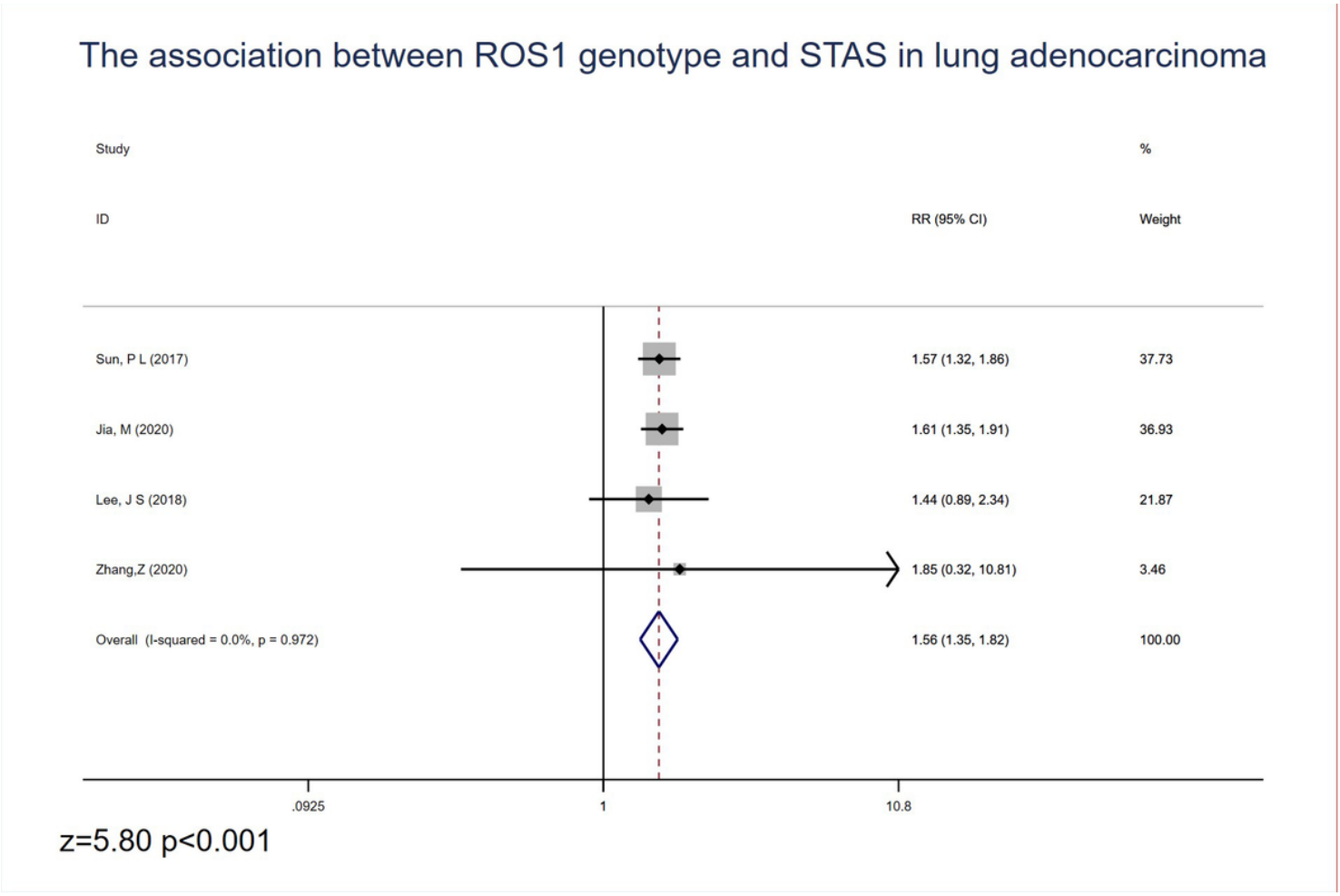

Figure 7

A total of 4 original studies were included 11, 12, 15, 16. We combined these studies using fixed effects. In the original study, three studies suggested that patients with ROS1 gene mutation had a higher incidence of STAS11, 12, 15. The combined results of this meta-analysis showed that patients with ROS1 gene mutation had a higher incidence of STAS□RR=1.57 95%CI 1.35~1.82 z=5.80 p<0.001 Fig. 7□

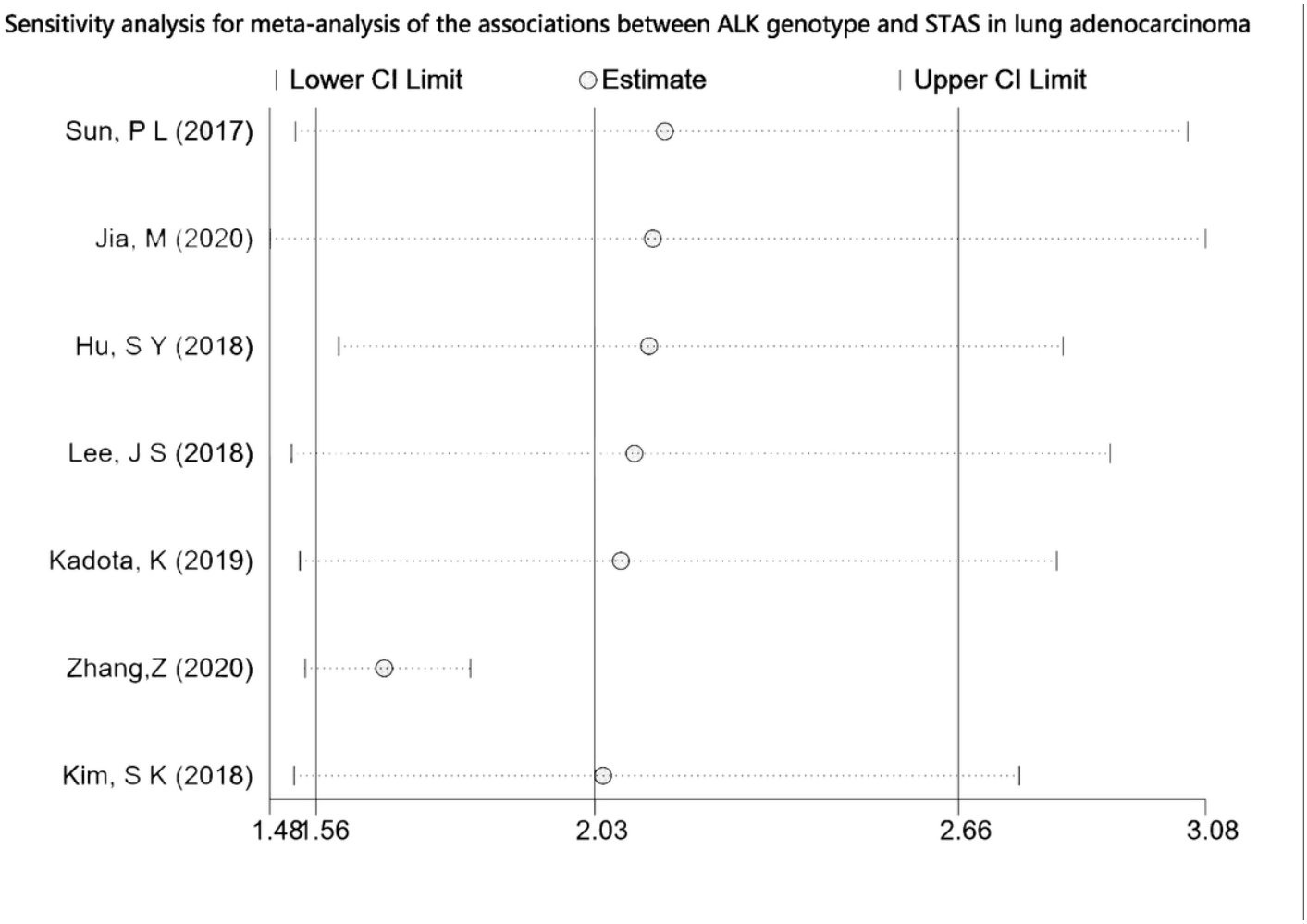

Figure 8

Begg's funnel plot and Egger's linear regression test were used to study the publication offset of the included studies with the combined number of literatures greater than or equal to 4. All content within a confidence interval of P>0.1 can be considered to have no publication offset (Table. 2). In this analysis, it was found that the research conclusions of Zhang, Z. 16 on the relationship between ALK and STAS occurrence were greatly deviated from other studies, which had a great impact on the results of data merging.(Fig .8) Therefore, the heterogeneity could be eliminated if this paper was excluded. However, whether this study is excluded or not, it can be concluded that patients with ALK gene mutation have a higher incidence of STAS. We believe that the heterogeneity may be related to the selection of reagents