Peer Review File

Article information: https://dx.doi.org/10.21037/jtd-2025-1061

Reviewer A Comment 1: Lines 36-37: Please ensure consistent formatting. The text on these lines should use lowercase letters instead of uppercase.

Reply 1: Thank you for pointing this out. We have corrected the formatting and changed the text to lowercase for consistency.

Changes in the text:Revised lines 36–37 to use lowercase formatting (see Page 2, lines 36–37).

Reviewer A Comment 2: Rationale for WES: The background and justification for utilizing Whole Exome Sequencing (WES) are insufficiently described. Please elaborate on why this particular method was chosen for your study.

Reply 2: Thank you for the suggestion. We have expanded the Introduction to explain that WES was chosen for its ability to comprehensively detect coding-region mutations potentially related to postoperative cough, allowing exploration of novel genetic risk factors.

Changes in the text: Added rationale for WES in Introduction (see Page 3, lines 50–55).

Reviewer A Comment 3: Exclusion of Patient Selection Factors for Risk Factors: Several potential risk factors (young age, female sex, preoperative cough, right lung lobectomy, lobectomy, subcarinal lymph node dissection, mediastinal lymph node dissection, using a stapler to suture the bronchial stump, and postoperative gastroesophageal reflux) were identified but not incorporated into the patient selection criteria. This omission needs to be addressed.

Reply 3: Thank you for the comment. We clarify that these known risk factors were not used as inclusion criteria to avoid selection bias. Instead, they were assessed as variables in our statistical analysis. We have updated the Methods section to explicitly state this approach.

Changes in the text: Clarified rationale in Patient Selection and added explanation in Statistical Analysis (Page 4, lines 60–67 and 90–95).

Reviewer A Comment 4: Definition of Cough: The differentiation between patients with and without a cough is unclear. Please provide a clear definition of "cough present" to ensure accurate patient categorization.

Reply 4: We appreciate this important suggestion. We have now clarified that “persistent cough” was defined as a dry cough lasting ≥2 weeks after surgery, and was assessed using the Leicester Cough Questionnaire – Mandarin Chinese version (LCQ-MC) at 1, 3, and 6 months postoperatively.

Changes in the text: Revised Methods to clearly define "cough present" and added details on the timing and tool used for assessment (see Page 4, lines 66–68).

Reviewer A Comment 5: Focus on Segmentectomy: The study's exclusive focus on segmentectomy needs stronger justification. If this specific scope is indeed appropriate, then the title should be revised to explicitly include "segmentectomy" to accurately reflect the study's content.

Reply 5: Thank you for the insightful comment. While our cohort included both segmentectomy and lobectomy cases, the majority underwent segmentectomy. We have clarified this in the Methods section and revised the Title to accurately reflect the segmentectomy-predominant nature of the study.

Changes in the text:

1. Title revised to: “A Retrospective Study with Whole Exome Sequencing of Tissue Samples: Analysis of Risk Factors in Patients with Persistent Cough Following Lung Segmentectomy.”
2. Clarification added in Methods – Patient Selection (Page 4, lines 60–63).

The authors present a retrospective analysis combining clinical data with whole exome sequencing (WES) to investigate genetic risk factors associated with persistent cough (CAP) in non-small cell lung cancer (NSCLC) patients after lung resection.

Reviewer B Comment 1: The study includes only 20 patients, split into 9 with CAP and 11 without. This small sample size limits the statistical power and generalizability of the findings. The authors acknowledge this limitation, but they should more clearly qualify their conclusions as hypothesis-generating rather than definitive.

Reply 1: We fully agree with the reviewer. We have revised both the Abstract and Discussion sections to clearly state that our findings are exploratory and hypothesis-generating, intended to provide preliminary genetic insight into CAP, rather than draw definitive conclusions.

Changes in the text:

1. Added the phrase “hypothesis-generating” in the Abstract conclusion (Page 1).
2. Revised the Discussion conclusion to emphasize the need for further validation in larger studies (Page 15, lines 253–258).

1) **Reviewer B Comment 2:** The definition of persistent cough appears inconsistent across sections. The manuscript mentions "dry cough lasting no less than two weeks" but also refers to assessments at 1, 3, and 6 months. Clarify how CAP was defined and measured, and whether validated tools (e.g., LCQ-MC) were used longitudinally.

Reply 2: Thank you for this helpful comment. We have clarified that persistent cough (CAP) was defined as a dry cough lasting ≥2 weeks after surgery, and was assessed longitudinally using the LCQ-MC questionnaire at 1, 3, and 6 months postoperatively. This has been consistently updated in the Abstract, Methods, and Results sections.

Changes in the text:

1. Clarified CAP definition and timepoints in the Abstract (Page 1, lines 9–11).
2. Expanded measurement details in the Methods – Patient Selection (Page 4, lines 66–68).
3. Ensured consistency in Results section wording (Page 5, line 98).

2) **Reviewer B Comment 3:** While the authors used chi-square tests and logistic regression, no adjustment for multiple testing appears to have been made despite numerous comparisons. This raises the risk of Type I error. Please clarify if and how multiple hypothesis testing corrections were applied.

Reply 3: Thank you for highlighting this important point. Due to the exploratory nature and small sample size of this study, we did not apply formal corrections for multiple testing. We have now

explicitly stated this limitation in the Statistical Analysis and Discussion sections and clarified that our findings should be interpreted cautiously.

Changes in the text:

1. Added explanation to Statistical Analysis section: “No correction for multiple comparisons was applied given the exploratory nature of the study.” (Page 4, line 95)

2. Added corresponding statement to Discussion: “We acknowledge the increased risk of Type I error due to multiple comparisons without adjustment.” (Page 15, lines 254–256)

3) **Reviewer B Comment 4:** The identification of TBC1D2 and SEPT12 as CAP-associated genes is novel, but the functional implications remain speculative. The discussion would benefit from a more critical appraisal of the biological plausibility, especially given the limited functional validation. Furthermore, it is unclear whether these were germline or somatic mutations, and how tumor-normal matching was handled.

Reply 4: We appreciate the reviewer’s insightful comment. We have revised the Discussion to more critically address the biological plausibility of TBC1D2 and SEPT12 in NSCLC and cough mechanisms, while emphasizing the speculative nature of our interpretation due to the lack of functional validation. Additionally, we clarified that the mutations identified were somatic, derived from tumor tissue, and that matched normal controls were not included in this study — which we now acknowledge as a limitation.

Changes in the text:

Added clarification on mutation type and matching in Methods – WES sequencing (Page 4, lines 80–81).

Revised Discussion to critically interpret functional implications and acknowledge limitations in validation (Page 15, lines 238–248).

4) **Reviewer B Comment 5:** There are multiple grammatical errors and instances of awkward phrasing throughout the manuscript, which occasionally impede understanding (e.g., “offering insights for cough diagnosis and treatment” is vague). Professional language editing is recommended to improve readability and clarity.

Reply 5: Thank you for this valuable suggestion. The manuscript has been thoroughly revised by a professional native English-speaking medical editor to improve grammar, clarity, and overall readability. We have also rephrased vague expressions as recommended.

Changes in the text:

1. Revised expressions such as "offering insights for cough diagnosis and treatment" for clarity (e.g., now reads "may assist in identifying potential diagnostic and therapeutic targets for postoperative cough"; Page 5, line 114).

2. Acknowledged editorial assistance in the Acknowledgments section.

5) **Reviewer B Comment 6:** Provide more detailed demographic and clinical data (e.g., exact staging, treatment regimens) in a supplementary table.

Reply 6: Thank you for this valuable suggestion. We have added a supplementary table (Supplementary Table S1) providing detailed demographic and clinical characteristics of all enrolled patients, including age, sex, smoking history, exact TNM staging, surgical approach, and postoperative treatment regimens.

Changes in the text:

1. Supplementary Table S1 added to the submission.

2. Reference to the supplementary table included in the Methods – Patient Selection and Results sections (Page 4 and Page 5).

7) **Reviewer B Comment 7:** Abbreviations such as CAP and WES should be defined once and used consistently.

Reply 7: Thank you for pointing this out. We have reviewed the manuscript to ensure that all abbreviations, including CAP (Cough After Pulmonary Resection) and WES (Whole Exome Sequencing), are defined upon first use and used consistently throughout the text, tables, and figure legends.

Changes in the text:

1. Definitions for CAP and WES added at first appearance in the Abstract and Introduction.

2. Checked and standardized abbreviation usage across the entire manuscript.

Reviewer C Comment 1: The paper aims to analyse the relationship between the occurrence of cough after pulmonary resection in patients with non-small-cell lung cancer and genetic mutations. Authors analyse a 20 patients' series, divided into two groups N=9 patients with cough and N=11 patients without.The series is very limited and retrospective. The statistical significance of differences cannot be reliable.

Reply 1: We thank the reviewer for this insightful and constructive comment. We fully acknowledge that the sample size of 20 patients in this study is limited and that this inevitably restricts the generalizability and statistical power of the findings. This study was designed as an exploratory pilot study intended to generate hypotheses rather than to draw definitive conclusions.

However, we believe that despite the limited sample size, our study provides novel insights through the application of whole-exome sequencing (WES) on matched tissue samples from NSCLC patients with and without postoperative persistent cough (CAP). The identification of TBC1D2 and SEPT12 as genes showing both INDEL and SNP mutations in patients with CAP adds a potentially valuable genetic dimension to the understanding of this underexplored postoperative complication. We also employed stringent inclusion/exclusion criteria and ensured rigorous sequencing and bioinformatic pipelines to minimize confounding.

To reflect the preliminary nature of our conclusions, we have revised the Discussion section to clarify that the results are exploratory and hypothesis-generating. We have also added a clear statement on the limitations due to sample size and encouraged further multi-center studies with larger populations to validate our observations (see Page 15, lines 253–258).

Furthermore, in response to the reviewer's suggestion, we have rephrased several parts of the Conclusion to avoid overinterpretation, emphasizing the potential rather than confirmed predictive value of the identified mutations.

Changes in the text:

"These identified risk factors can be used to build a predictive model..." has been revised to "These identified candidate risk factors may inform the development of predictive models in future studies" (see Page 15, line 213).

A paragraph has been added in the Discussion explicitly acknowledging the sample size limitation and encouraging future large-scale validation studies (Page 15, lines 253–258).

The Abstract and Conclusion sections have been revised to frame the study as exploratory and hypothesis-generating rather than confirmatory (see Abstract, line 22; Page 15, line 259).

Reviewer C Comment 2: There are also several bias to be clarified, even in this short series:
Reply 2: We appreciate the reviewer's important observation regarding potential sources of bias. Given the retrospective nature and limited sample size of our study, we recognize that certain biases could influence the results. In response, we have carefully reviewed the study design and now explicitly addressed several potential sources of bias, both in the Methods and Discussion sections.
Specifically:
1.Selection Bias:All patients were enrolled consecutively from two large tertiary hospitals (Guangdong Provincial People's Hospital and Shenzhen People's Hospital) during a defined period, and strict inclusion/exclusion criteria were applied (see Page 4, "Patient Selection"), including exclusion of those with known chronic cough-related conditions (e.g., asthma, bronchiectasis). This minimized confounding due to pre-existing respiratory diseases.
2.Measurement Bias:Persistent cough was assessed using a validated instrument — the Leicester Cough Questionnaire—Mandarin-Chinese version (LCQ-MC) — at fixed postoperative timepoints (1, 3, and 6 months), ensuring standardized and reproducible measurement (see Page 4, lines 66–68).
3.Information Bias (recall or reporting):The clinical and genetic data were collected prospectively and analyzed retrospectively, and all sequencing was performed on archived tissue samples to ensure uniformity in specimen handling and data integrity.
4.Confounding Factors:We performed subgroup comparisons (e.g., age, BMI, smoking status, tumor size, lymph node removal, FVC%) and presented them in Tables 1 and 2 (see Pages 5–6). Most variables did not differ significantly between the cough and non-cough groups, suggesting that the observed differences (notably in TBC1D2 and SEPT12 mutation profiles) are less likely to be confounded.

To ensure transparency, we have updated the Discussion section to more thoroughly acknowledge these potential biases and emphasized the need for multi-center studies with broader demographic representation to validate our findings (see Page 15, lines 253–258).

Changes in the text:

Expanded Patient Selection section (Page 4) to clarify how selection bias was minimized.

Added statements in the Methods about use of validated assessment tools (LCQ-MC) to reduce measurement bias (Page 4, line 66).

Added a paragraph in the Discussion explicitly discussing sources of bias and how we attempted to mitigate them, while acknowledging their residual impact due to the study design (Page 15, lines 253–258).

Reviewer C Comment 3: Introduction and abstract refer to Segmentectomies. Inclusion criteria refer to anatomical resections (including lobectomy); Table 1 doesn't clarify this point.

Reply 3: We thank the reviewer for this valuable observation. We acknowledge the inconsistency in terminology. Although our cohort included both segmentectomy and lobectomy cases, the majority underwent segmentectomy. We have now revised the Abstract, Introduction, and Table 1 to reflect this accurately and consistently.

Changes in the text:

Revised the Abstract and Introduction to state "segmentectomy-predominant anatomical resections."

Updated Table 1 to indicate the specific type of surgery each patient received (see Page 5, Table 1).

Reviewer C Comment 4: Different segmentectomies should be reported

Reply 4: We agree with the reviewer's suggestion. We have now added details on the specific types of segmentectomies performed in the Methods section and included them in a revised Table 1 for clarity.

Changes in the text: Added specific segmentectomy types (e.g., S1+2, S6, S3) to the Methods (Page 4) and Table 1 (Page 5).

Reviewer C Comment 5: Gastroesophageal reflux is not mentioned/reported

Reply5: Thank you for this important comment. We have now included gastroesophageal reflux as a clinical variable in the Methods and Results, and discussed its potential role in the Discussion section.

Changes in the text:

1. Added reflux assessment using RDQ scale in Methods (Page 4).
2. Included reflux status in Table 1 (Page 5).
3. Discussed its relevance as a known CAP risk factor in the Discussion (Page 15, lines 207–209).

Reviewer C Comment 6: Chronic Bronchitis Asthma, Emphysema are not mentioned/reported

Reply6: We appreciate the reviewer's comment. These conditions were part of the exclusion criteria to reduce confounding. We have clarified this explicitly in the Methods section and added this information to Table 1.

Changes in the text:

1. Clarified exclusion of chronic bronchitis, asthma, and emphysema in Methods (Page 4, lines 73–75).
2. Added relevant data to Table 1 (Page 5).