nature portfolio

Peer Review File

Epidemiological and Bioinformatics Analyses of Air Pollution and Genetic Susceptibility in Aortic Stenosis Risk

Corresponding Author: Professor Yaohua Tian

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This work has examined the association between long-term exposure to air pollution and aortic stenosis risk, and potential environment-gene interactions. The authors also integrate network toxicology and bioinformatics approaches to explore the underlying molecular mechanisms. The study reflects substantial effort, novel approaches, and rich research output. However, I have several concerns below:

1. In the abstract, the last several sentences are redundant with some information repeated: “Network toxicology and bioinformatics analyses identified … underlying air pollution-associated AS.” I recommend revising these sentences to remove redundancy and present the findings more clearly and succinctly.

2. The authors seemed to use the terms “developing AS” and “incident AS” interchangeably, which could be misleading. Please clarify the outcome definition. If the study focuses on incident AS, specify whether this refers to the first diagnosis of AS after cohort entry among participants without prior AS, and indicate whether hospital readmissions were included or excluded.

3. The authors have mentioned that “To investigate the dose-response relationships between long-term air pollutants exposure and the risk of AS incidence, this study utilized a restricted cubic spline model, with relevant parameter settings informed by previous study 45.” Could you clarify the rationale for choosing a restricted cubic spline model and whether you explored alternative modeling approaches? Additionally, the manuscript does not provide a concrete reference supporting the use of these parameter settings in a similar study context. Please provide appropriate justification or citations. Sensitivity analyses could also be added to enhance the robustness of the findings.

4. The authors utilized multiple imputation as part of the sensitivity analyses to address missing data; however, the manuscript does not provide sufficient detail on the imputation procedure, for example, the missingness mechanism assumed, and any diagnostics used to assess the quality of the imputations.

5. Line 319: The authors cited an "ambiguous dose-response relationship". This description is vague. Does “ambiguous” refer to a very small effect size, or a non-monotonic dose–response pattern?

6. One limitation of this study that requires further clarification concerns unmeasured individual-level exposure determinants, such as occupational exposures, commuting exposures, or indoor air pollution, as acknowledged by the authors in the discussion section. This study used residential outdoor air pollution as a proxy for personal exposure. These missing variables may lead to non-differential exposure misclassification and bias the estimates. The authors may more clearly discuss the potential bias and consider whether any sensitivity analyses or external data sources could help.

7. Lines 374-375: The authors stated that “the PRS for AS is constructed using data from individuals of European ancestry, limiting its generalizability to other populations.” Please expand the discussion to clarify how ancestry may impact PRS transferability and the interpretation of gene–environment interaction findings, e.g., through differences in gene expression, air-pollution exposures, and socioeconomic factors across populations. In addition, have existing studies reported ancestry-related differences in similar studies of air pollution and cardiovascular disease risk? Providing such context would strengthen the discussion.

8. Please explicitly specify the types of air pollution exposures assessed and clearly describe the target study population when summarizing the findings (e.g., in the Conclusion section). The current phrasing is general and may lead to ambiguity.

Reviewer #2

(Remarks to the Author)
The authors present an analysis of the association between air pollution particles of different sizes and development of aortic stenosis. The relationship between PM and cardiovascular disease has been increasingly studied, proven and accepted, and even though aortic stenosis may not have been studied before, other cardiovascular diseases of similar etiology have (e.g. coronary artery calcification), with similar results.
Most analyses are adequate and sensitivity analyses show no significant deviations. I have a few comments about the manuscript:
- Having over 3700 genes come from the CTD database seems overreach, which led to many non-specific and generic pathways. I suggest a subset of curated interactions, available on the website, which should shorten the list.
- "those with AS, valvular heart disease, and other cardiovascular conditions at baseline". It is essential to describe all cardiovascular conditions included in the analysis
- The authors describe a PRS consisting of 15 sequence variants. However, PRS is the term for a genome-wide genetic risk score, involving hundreds of thousands of variants. The authors should call it genetic risk score instead.
- Also regarding the genetic risk score, there is no explanation about how the variants were selected. Recent GWASs report over 30 variants associates with AS (https://pubmed.ncbi.nlm.nih.gov/38494474/, https://pubmed.ncbi.nlm.nih.gov/37038246/). What is the reason to not use all variants associated with AS?

Minor:
- There are multiple letters "s" forming plurals of words that should be singular in the standard English grammar, for example "Covariates assessments". It is only one of dozens of such cases.
- Conversely, "utilizing multiple imputation method" is missing an "s".
- "remained statistical significance" - either remained statistically significant or kept statistical significance.

Reviewer #3

(Remarks to the Author)
There is a strong body of evidence support a link between air pollution exposure and the risk of developing cardiovascular disease including atherosclerosis, coronary artery disease, aorta aneurysm, mitral annulus calcification etc. However, there is very few data on the association between air pollution exposure and the risk of incident aortic stenosis (AS). In the present study, the authors used the UK Biobank database to assess associations between air pollution exposure and AS risk. Genetic data were integrated to evaluate joint and interactive effects of genetic susceptibility and air pollutants. Network toxicology and bioinformatics analyses were used to identify shared target genes and potential biological pathways of air pollutants exposure and AS. Long-term exposure to air pollution was associated with an increased risk of AS. There were both joint and interactive effects of genetic susceptibility and air pollutants on AS risk. Network toxicology and bioinformatics analyses identified IL6 as a key molecular target underlying air pollution-induced AS. The authors conclude that Air pollution exposure is associated with an increased risk of AS, with a potential interaction between environmental exposure and genetic susceptibility. This is an interesting analysis reporting novel findings. I have the following comments and suggestions:

1- Although the investigators adjuster for multiple potentially confounding factors. However, it is difficult from the data presented in this paper to confirm the cause-effect relationship of air pollution on risk of incident AS.

2- The fact that genetic susceptibility may modulate the effect of air pollution on AS risk is plausible but again here the causal link between IL6, in particular, and exacerbation of air pollution effect on AS is difficult to establish with these data. Several studies reported an association between IL6 polymorphism and incident AS. So it is unclear whether IL6 is simply an additive contributor to AS besides Air Pollution or is truly a potentializing factor that exacerbates the effect of air pollution.

3- The investigators only used the data of UK Biobank. The results and impact of this study would be great reinforced if the investigators are able to replicate, at least in part, these findings in another independent cohort.

4- The present study examines the impact of air pollution on incident AS. As a future step, it would be interesting to assess the association between long-term exposure to air pollution and progression rate of AS or aortic valve calcification.

5- The review of literature regarding the genetic susceptibility to AS is incomplete and only focuses on LPA. Please consider citing and discussing these previously published studies including the following:
https://pubmed.ncbi.nlm.nih.gov/40329539/
https://pubmed.ncbi.nlm.nih.gov/39504041/

6- It is unclear how long-term exposure to ambient air pollution was defined and adjudicated in the UK Biobank and how robust is this data.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have satisfactorily addressed my concerns in the revised manuscript. I now consider the paper to be of good scientific quality.

Reviewer #2

(Remarks to the Author)
The authors have improved the manuscript according to my suggestions. No further comments.

Reviewer #3

(Remarks to the Author)
The authors have submitted a revised version and rebuttal that address very well and completely the comments that I made initially. The paper is further improved.

Point-by-point Responses to Comments of Editor and Reviewers

We sincerely appreciate the opportunities to revise our manuscript titled "**Air Pollution Exposure and Aortic Stenosis Risk: Integrating Epidemiology, Gene-environment Interactions, and Network Toxicology and Bioinformatics Analyses**" (**Manuscript #: NCOMMS-25-83850**). Your constructive comments are very valuable for improving the study to be suited for the standard of the top journal *Nature Communications*.

We have now revised our manuscript accordingly. Revisions and responses in the latest version of manuscript were marked in blue texts. Our study holds significant publication merit due to the following novel contributions:

(1) This study explored the relationships between air pollutants exposure and the risk of AS incidence. As the respected Reviewers' insightful suggestions, we implemented **a suite of advanced approaches, including inverse probability weighting, generalized propensity scores, E-values, and shape-constrained additive model**, to rigorously control for confounding and verify the stability of our results. These diverse methodologies **yielded remarkably consistent findings**, reinforcing the reliability of our primary conclusions.

(2) We performed **an independent external validation** using the Tianshan Community Cohort, a massive prospective study representing a distinct Northwest Chinese population. This cohort provides a distinct geographical and ethnic context compared to the UK Biobank. Consistent with our primary findings, long-term exposure to air pollutants was **significantly associated** with an increased risk of incident AS, with Hazard Ratios (HRs) per standard deviation (SD) increase of 1.30 (1.25 1.34) for $PM_{2.5}$, 1.22 (1.18, 1.27) for PM_{10}, and 1.15 (1.12, 1.18) for NO_2. This successful replication across diverse ethnic and geographical contexts robustly reinforces the robustness of our conclusions.

(3) Following the Reviewers' constructive comments, we **updated** our GRS calculation by incorporating the suggested SNPs to better capture genetic susceptibility. Notably,

our results revealed **a significant additive interaction** between genetic risk and air pollution exposure on the risk of incident AS. These findings suggest that a potential gene-environment interaction plays a critical role in the development of AS.

We hope the updated manuscript will meet your expectation and satisfy all reviewers. Thank you very much again for reconsideration in advance.

The response details as following:

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

This work has examined the association between long-term exposure to air pollution and aortic stenosis risk, and potential environment-gene interactions. The authors also integrate network toxicology and bioinformatics approaches to explore the underlying molecular mechanisms. The study reflects substantial effort, novel approaches, and rich research output. However, I have several concerns below:

1. In the abstract, the last several sentences are redundant with some information repeated: "Network toxicology and bioinformatics analyses identified … underlying air pollution-associated AS." I recommend revising these sentences to remove redundancy and present the findings more clearly and succinctly.

Response:

We thank the Reviewer for this helpful suggestion. We have streamlined the conclusion of the abstract to eliminate redundancy while maintaining the clarity of our key findings.

Revised texts in the Abstract part (Lines 48-52)

"Network toxicology and bioinformatics analyses revealed key PM-AS target genes enriched in the lipid and atherosclerosis, fluid shear stress, and IL-17 signaling pathways. In conclusion, long-term exposure to $PM_{2.5}$, PM_{10}, NO_2, and NO_x was significantly associated with an increased AS risk, with a potential interaction between environmental exposure and genetic susceptibility."

2. The authors seemed to use the terms "developing AS" and "incident AS" interchangeably, which could be misleading. Please clarify the outcome definition. If the study focuses on incident AS, specify whether this refers to the first diagnosis of AS after cohort entry among participants without prior AS, and indicate whether hospital readmissions were included or excluded.

Response:

We thank the reviewer for this important clarification regarding our outcome definition.

As suggested, we have standardized the terminology to "incident AS" throughout the manuscript to avoid ambiguity. Specifically, incident AS is defined as the first diagnosis of AS during the follow-up period among participants who were free of AS at baseline. In our analysis, participants were followed from baseline until the date of the first diagnosis of AS, death, or the end of follow-up, whichever came first. Therefore, hospital readmissions for AS were not included as outcome events, as the observation period for a participant ended immediately upon the occurrence of the first incident event.

Revised texts in the Methods part (Lines 162-166)

"To focus on incident AS, we excluded participants with pre-existing AS at baseline. Incident AS was defined as the first occurrence of AS during the follow-up period, identified through linkage to hospital inpatient and death registry records. Participants were followed until the earliest of the following censoring events: the first diagnosis of incident AS, death, or the end of follow-up (September 22, 2021)."

3. The authors have mentioned that "To investigate the dose-response relationships between long-term air pollutants exposure and the risk of AS incidence, this study utilized a restricted cubic spline model, with relevant parameter settings informed by previous study." Could you clarify the rationale for choosing a restricted cubic spline model and whether you explored alternative modeling approaches? Additionally, the manuscript does not provide a concrete reference supporting the use of these parameter settings in a similar study context. Please provide appropriate justification or citations. Sensitivity analyses could also be added to enhance the robustness of the findings.

Response:

We sincerely thank the reviewer for the insightful comment.

(1) The Restricted Cubic Spline (RCS) model was chosen because it provides a superior balance between flexibility and interpretability in modeling non-linear associations (*N Engl J Med. 2023 Apr 13;388(15):1396-1404; BMJ. 2019 Dec 30:367:l6720; BMJ. 2024 Feb 21:384:e076939; Lancet Planet Health. 2021 Sep;5(9):e620-e632.*). Unlike

piecewise linear or simple polynomial models, RCS ensures that the fitted curve is smooth at the nodes (knots) and constrained to be linear in the tails, which prevents unstable oscillations at the extremes of air pollution exposure (*Stat Med. 1989 May;8(5):551-61; Springer-Verlag, New York.* http://dx.doi.org/10.1007/978-1-4757-3462-1). In addition to the RCS model, we analyzed air pollutant exposure as a trichotomous variable (T1 to T3). The consistent trend observed across tertiles (HR: T3 > T2 > T1, *P* for trend < 0.001, **Table 2**) aligns with the RCS findings, reinforcing the reliability of the observed associations.

(2) Following standard recommendations by Harrell (*Regression Modeling Strategies. Springer; 2012.*), we initially tested models with 3 or 4 knots. The model with three knots was selected based on the minimum Bayesian Information Criterion (BIC), representing the most parsimonious fit. We have now added these specific details in the latest version of the manuscript (**Table S3**) and revised the Methods section accordingly.

(3) As suggested, we further applied a shape constrained additive model (SCAM) to examine exposure-response relationship between air pollution exposure and the risk of AS (*Stat Comput 25, 543–559 (2015)*.). This method serves as an implementation of the shape constrained health impact function and is widely used in many previous papers (*Environ Int. 2021 Jan:146:106249; Ecotoxicol Environ Saf. 2024 Jan 15:270:115829.*). The results did not change. As depicted in **Fig. S7**, the dose-response curves generated by the SCAM were highly consistent with our primary RCS analysis. Such uniformity across models bolsters the reliability of the identified associations between air pollution exposure and AS risk.

Revised texts in the Methos part

"To characterize the exposure-response relationship between long-term air pollution exposure and AS risk, this study employed a restricted cubic spline (RCS) model. The optimal number and placement of knots were determined by minimizing the Bayesian Information Criterion (**Table S3**) [56]." **(Lines 234-237)**

"(7) To validate the functional form of the exposure-response curves, a shape-constrained additive model was implemented to complement the RCS analysis." **(Lines 268-269)**

Added Fig.S7

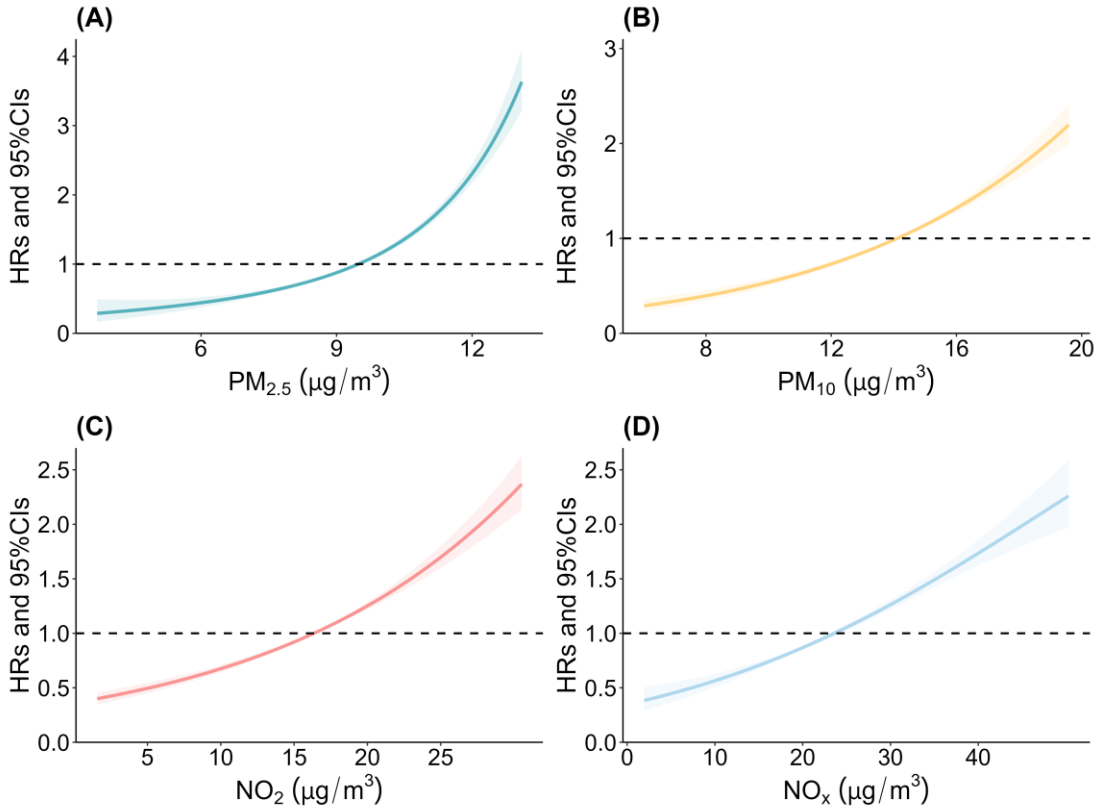


Fig. S7 Associations of long-term $PM_{2.5}$ (A), PM_{10} (B), NO_2 (C), and NO_x (D) exposure with the risk of AS events based on the shape constrained additive model.

4. The authors utilized multiple imputation as part of the sensitivity analyses to address missing data; however, the manuscript does not provide sufficient detail on the imputation procedure, for example, the missingness mechanism assumed, and any diagnostics used to assess the quality of the imputations.

Response

We sincerely appreciate the reviewer's constructive suggestion regarding the multiple imputation (MI) procedure. We have significantly expanded the description in the Methods section and provided diagnostic plots in the Supplementary Materials to ensure the robustness of our findings.

We assumed that the data were Missing at Random, a standard and appropriate assumption for large cohort studies like the UK Biobank, where missingness can often

be explained by observed covariates (*Journal of Statistical Software. 2011;45(1):1-67*; *Statistics in Medicine. 2011;30(4):377-399.*). We generated five imputed datasets through Fully Conditional Specification. Specifically, Bayesian linear regression was applied to continuous variables, and polytomous regression was used for categorical variables.

To ensure the quality and validity of the imputations, two rigorous diagnostic steps were conducted: (1) **Convergence Check**: We generated **trace plots** (monitoring mean and standard deviation across 10 iterations) for all imputed variables. As shown in **Fig. S3**, the chains for each imputed dataset are well-mixed and exhibit no distinct trends or drifts, confirming the successful convergence of the Multiple Imputation by Chained Equations algorithm. (2) **Distributional Consistency**: We compared the distribution of categorical variables between the observed and imputed datasets. As illustrated in **Fig. S4**, the proportions of categories remained highly consistent after imputation, indicating that the procedure preserved the original data structure without introducing significant bias.

Revised texts in the Methos part (Lines 265-268)

"(6) addressing missing data via Multiple Imputation by Chained Equations under the Missing at Random assumption [59]. The specific details of the imputation procedure and its quality are detailed in **Text S1**, **Fig. S3** and **Fig. S4**."

Added Text S1:

"**Text S1**. Details of multiple imputation and data quality checks.

During the multiple imputation process, five imputed datasets were generated using Fully Conditional Specification. Specifically, linear and polytomous regression models were applied to continuous and categorical variables, respectively. Imputation quality and convergence were rigorously assessed using trace and density plots. Trace plots of the means and standard deviations confirmed that the imputation chains were well-mixed without distinct trends, indicating satisfactory convergence (**Fig. S3**). Additionally, distributional comparisons revealed that the proportions of categorical variables remained highly consistent between the observed and imputed datasets, confirming that the procedure preserved the original data structure

without introducing significant bias (**Fig. S4**).”

Added Fig. S3 and S4:

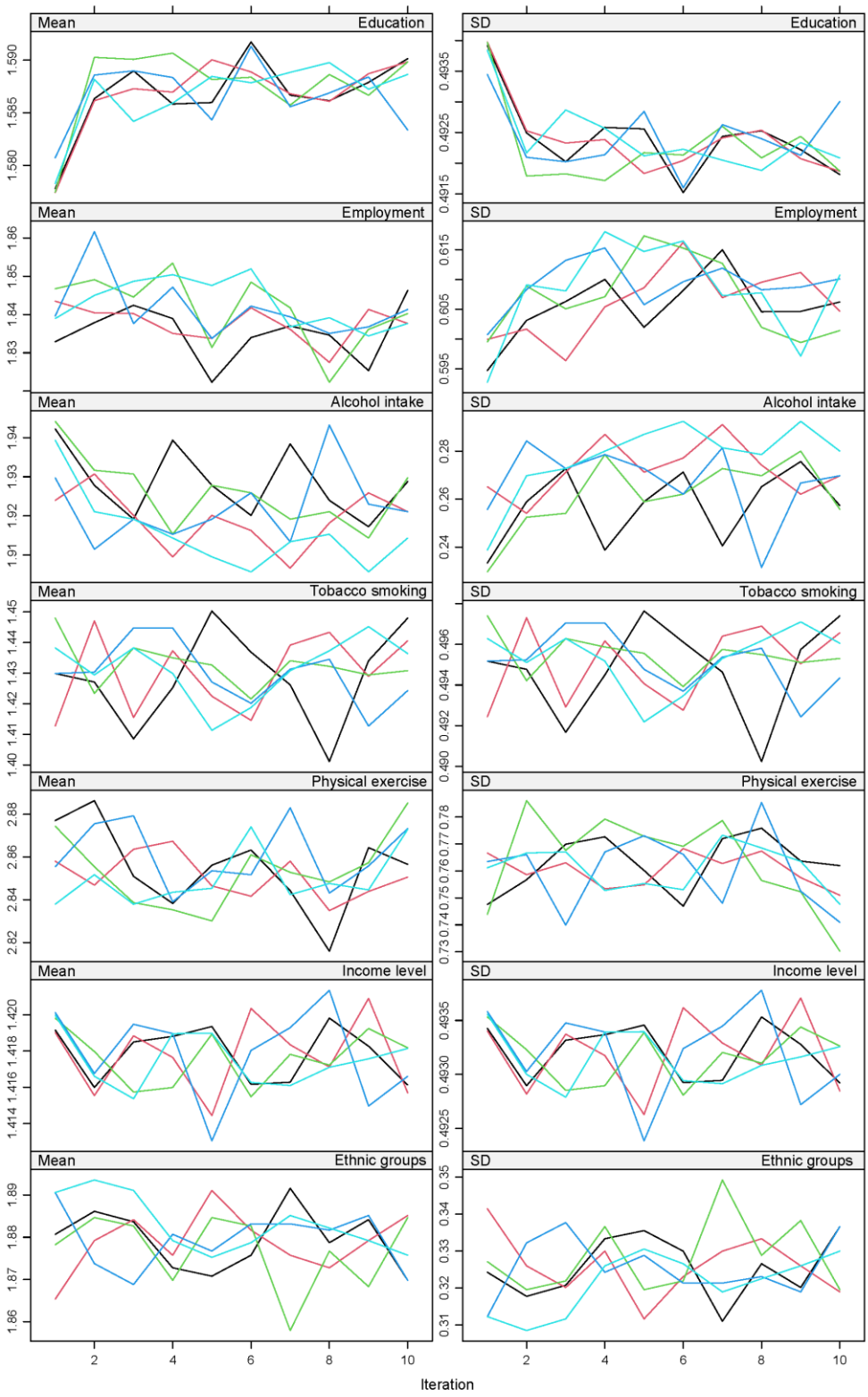


Fig. S3 Trace plots of mean and standard deviation for imputed covariates.

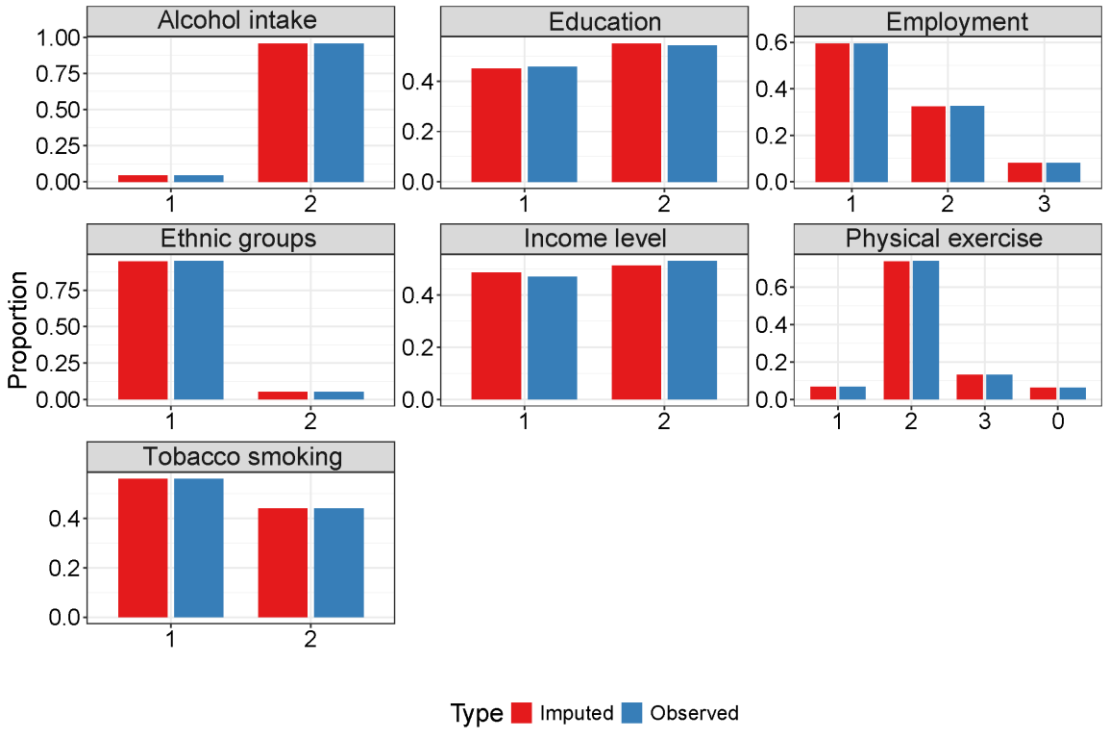


Fig. S4 Distributional balance of categorical covariates between observed and imputed datasets.

5. Line 319: The authors cited an "ambiguous dose-response relationship". This description is vague. Does “ambiguous” refer to a very small effect size, or a non-monotonic dose–response pattern?

Response

Thanks for your comment.

To make ensure a clearer presentation, we have rephrased this sentence to “Furthermore, in a study of US Medicare beneficiaries (n = 59,761,494), the exposure-response curve for valvular heart disease exhibited an initial increase followed by a subsequent decline as $PM_{2.5}$ levels rose [17].” **(Lines 378-380)**

6. One limitation of this study that requires further clarification concerns unmeasured individual-level exposure determinants, such as occupational exposures, commuting exposures, or indoor air pollution, as acknowledged by the authors in the discussion section. This study used residential outdoor air pollution as a proxy for personal

exposure. These missing variables may lead to non-differential exposure misclassification and bias the estimates. The authors may more clearly discuss the potential bias and consider whether any sensitivity analyses or external data sources could help.

Response:

We feel great thanks for your professional review work on our manuscript.

As suggested, we have conducted extensive sensitivity analyses to account for indoor, commuting, and occupational exposures, utilizing detailed information available in our dataset

Indoor air pollution: Ambient and indoor air pollution exposures are unique environmental factors affecting human health, and quantifying individual-level exposure precisely is challenging, especially for large-scale population-based studies. Therefore, almost all previous studies on ambient air pollution did not measure indoor air pollution levels (*N Engl J Med 2023;388:1396-1404; N Engl J Med 2017;376:2513-2522.*). Besides, previous studies have demonstrated that indoor concentration of PM was highly influenced by outdoor air quality, and the sources of indoor PM were strongly dependent on outdoor processes (*Sustain Cities Soc 2019;48; Indoor Air 2011;21:145-155.*). Therefore, exploring the health effects of outdoor air pollution holds significant public health implications. Although detailed information regarding indoor pollution levels was unavailable, in this revision, we have added **solid-fuel cooking or heating** (Field ID:6139, 6140) and **exposure to secondhand smoke at home** (Field ID:1269) as covariates in our models, which are two important sources of indoor air pollution. **Commuting exposures**: To account for exposure during transit, we have further incorporated **the frequency of commuting** and **mode of transport (**Field ID: 6143, 777**)** as covariates. These serve as robust proxies for commuting-related environmental insults. **Occupational exposure**: To mitigate the impact of workplace-related confounders, we added a comprehensive **occupational exposure variable (**Field ID: 22609-22615**)** to our model. This variable was defined based on the history of workplace exposure to dust, chemicals, fumes, passive smoke, asbestos, paint,

thinners, pesticides, and diesel exhaust.

In summary, additional sensitivity analyses were conducted by further adjusting for indoor air pollution (solid-fuel cooking or heating and exposure to secondhand smoke at home), commuting exposures (the frequency of commuting and mode of transport), and occupational exposures (the history of workplace exposure to dust, chemicals, fumes, passive smoke, asbestos, paint, thinners, pesticides, and diesel exhaust). Following these adjustments, the core association between ambient air pollution and incident AS **remained robust**, suggesting that the observed effects are not driven by these different exposure patterns.

Revised texts in the Methos part (Lines 263-265)

"(5) incorporating indoor, commuting, and occupational exposures into the models to control for their potential confounding influence on the associations".

Revised Table S11

Table S11. Sensitivity analyses of associations between air pollution exposure and the risk of AS incidence.

Air pollutants (per SD increase)	HRs (95% CIs)	*P* values
Sensitivity analysis I		
$PM_{2.5}$	1.60 (1.54, 1.66)	<0.001
PM_{10}	1.35 (1.30, 1.40)	<0.001
NO_2	1.37 (1.32, 1.42)	<0.001
NO_x	1.36 (1.31, 1.41)	<0.001
Sensitivity analysis II		
$PM_{2.5}$	1.62 (1.56, 1.68)	<0.001
PM_{10}	1.38 (1.33, 1.43)	<0.001
NO_2	1.39 (1.34, 1.44)	<0.001
NO_x	1.38 (1.33, 1.43)	<0.001
Sensitivity analysis III		
$PM_{2.5}$	1.62 (1.57, 1.68)	<0.001
PM_{10}	1.38 (1.34, 1.43)	<0.001
NO_2	1.38 (1.33, 1.43)	<0.001
NO_x	1.37 (1.32, 1.42)	<0.001
Sensitivity analysis IV		
$PM_{2.5}$	1.63 (1.56, 1.69)	<0.001
PM_{10}	1.38 (1.33, 1.43)	<0.001
NO_2	1.38 (1.33, 1.43)	<0.001

NO_x	1.37 (1.32, 1.42)	<0.001
Sensitivity analysis V		
$PM_{2.5}$	1.61 (1.56, 1.67)	<0.001
PM_{10}	1.37 (1.33, 1.42)	<0.001
NO_2	1.38 (1.33, 1.43)	<0.001
NO_x	1.37 (1.32, 1.42)	<0.001
Sensitivity analysis VI		
$PM_{2.5}$	1.59 (1.54, 1.65)	<0.001
PM_{10}	1.36 (1.32, 1.41)	<0.001
NO_2	1.37 (1.32, 1.42)	<0.001
NO_x	1.36 (1.31, 1.41)	<0.001

Sensitivity analysis I: Exclusion of participants with incident AS within the first two years of follow-up.

Sensitivity analysis II: Exclusion of participants with <5 years of residential stability.

Sensitivity analysis III: Employing age as the underlying time scale in Cox regression.

Sensitivity analysis IV: Using continuous blood pressure, glucose, and lipid levels instead of binary disease diagnosis.

Sensitivity analysis V: Additional adjustment for occupational exposure, commuting exposure, and indoor air pollution.

Sensitivity analysis VI: Analysis based on the multiple-imputed dataset.

7. Lines 374-375: The authors stated that "the PRS for AS is constructed using data from individuals of European ancestry, limiting its generalizability to other populations." Please expand the discussion to clarify how ancestry may impact PRS transferability and the interpretation of gene–environment interaction findings, e.g., through differences in gene expression, air-pollution exposures, and socioeconomic factors across populations. In addition, have existing studies reported ancestry-related differences in similar studies of air pollution and cardiovascular disease risk? Providing such context would strengthen the discussion.

Response:

We sincerely thank the reviewer for the insightful comment.

We have expanded the discussion according to your suggestion.

Revised texts in the Discussion part (Lines 453-468)

"Second, deriving the AS-GRS from European-ancestry data limits its broader generalizability. Such cross-population disparities in linkage disequilibrium architecture and allele frequencies often lead to biased risk estimates [70,71]. Furthermore, ancestral backgrounds can modify gene–environment pathways, as population-specific gene expressions may alter sensitivity to

pollution-induced oxidative stress and systemic inflammation [72,73]. Environmental inequality is another key factor resulting in disproportionate cumulative exposure. When coupled with heightened genetic sensitivity, these environmental disparities further amplify the overall health damage. For instance, several multi-ethnic studies have shown that Black populations have exhibited a higher risk of air pollution-associated incident CVD compared to White populations [74-76]. Moreover, a recent study found that the benefits of $PM_{2.5}$ reduction have been unevenly distributed, with non-Hispanic Black and Hispanic populations experiencing less pronounced decreases in cardiovascular mortality relative to their non-Hispanic white counterparts [77]. Thus, the integration of multi-ethnic genomic data is essential for optimizing GRS in future studies, enabling a more precise evaluation of AS risk within a gene-environment interaction framework".

8. Please explicitly specify the types of air pollution exposures assessed and clearly describe the target study population when summarizing the findings (e.g., in the Conclusion section). The current phrasing is general and may lead to ambiguity.

Response:

Thanks for pointing out the problem.

We have specified the types of air pollution exposures assessed and clearly described the target study population when summarizing the findings in the latest version of manuscript.

Revised texts in the Conclusion part (Lines 481-487)

"In conclusion, long-term exposure to ambient $PM_{2.5}$, PM_{10}, NO_2, and NO_x is positively associated with an elevated risk of AS incidence in the UK population. Furthermore, our findings underscore a significant gene-environment interplay, suggesting that genetic susceptibility may modify the impact of air pollutants on AS incidence. These findings bolster the existing epidemiological evidence regarding the role of air pollution in incident AS, potentially providing mechanistic insights into the broader health impacts of ambient pollutant exposure."

Reviewer #2 (Remarks to the Author):

The authors present an analysis of the association between air pollution particles of different sizes and development of aortic stenosis. The relationship between PM and cardiovascular disease has been increasingly studied, proven and accepted, and even though aortic stenosis may not have been studied before, other cardiovascular diseases of similar etiology have (e.g. coronary artery calcification), with similar results.

Response:

We thank the reviewer for this insightful comment regarding this point. However, a closer review of the literature reveals that the relationship between ambient air pollution and coronary artery calcification is far from settled. Although some studies have identified positive associations (*Eur Heart J Cardiovasc Imaging. 2021 Jul 20;22(8):922-929; Lancet. 2016 Aug 13;388(10045):696-704.*), other large-scale investigations (including the MESA and SCAPIS studies) have **reported null or equivocal findings** (*Environ Health. 2017 Dec 21;16(1):133; Environ Res. 2022 Nov;214(Pt 2):113926; Eur J Prev Cardiol. 2025 Oct 27:zwaf688.*). This evidentiary **heterogeneity** in the literature underscores the critical importance of our study. By providing robust and replicated evidence specifically for incident AS, our findings address a significant knowledge gap in environmental cardiology, even when considering shared etiologies of CVDs. In response to the Reviewer's insightful suggestion, we have incorporated this discussion into the Introduction of the revised manuscript to further underscore the necessity and novelty of our study.

Added texts in the Introduction part (Lines 75-81)

"Although some studies have identified positive associations between air pollution and cardiovascular diseases (CVDs), such as coronary artery calcification, other large-scale investigations (including the MESA and SCAPIS studies) have provided inconclusive evidence regarding this association [13-15]. Moreover, to our knowledge, no study has yet investigated the impact of long-term air pollution exposure on the risk of incident AS, particularly within large-scale prospective cohorts."

Most analyses are adequate and sensitivity analyses show no significant deviations. I have a few comments about the manuscript:

1. Having over 3700 genes come from the CTD database seems overreach, which led to many non-specific and generic pathways. I suggest a subset of curated interactions, available on the website, which should shorten the list.

Response:

We feel great thanks for your professional review work on our manuscript.

Following your valuable suggestion, we refined our analysis by restricting gene selection to a subset of curated chemical–gene interactions from the CTD Chemical–Gene Interactions module, limited to human-specific evidence (*BMC Bioinformatics. 2022;23(1):51; Nucleic acids research 53, D1328-d1334*). After refinement, 1,308 particulate matter–related genes were retained, substantially fewer than the initial dataset.

Revised texts in the Methods part (Lines 183-187)

"Air pollution–related genes were retrieved from the Chemical–Gene Interactions module of the Comparative Toxicogenomics Database (CTD; https://ctdbase.org) [40,41], with results restricted to human-specific interactions identified using the keywords "Particulate Matter" or "Nitrogen Dioxide"."

Revised texts in the Results part (Lines 331-334)

"A total of 1308 candidate genes associated with PM were retrieved from the CTD database. Additionally, 598 genes highly relevant to AS were extracted from the GeneCards and OMIM databases. A Venn diagram identified 118 target genes linking PM exposure to AS (**Fig. 3A**)."

2. "those with AS, valvular heart disease, and other cardiovascular conditions at baseline". It is essential to describe all cardiovascular conditions included in the analysis.

Response:

We thank the reviewer for this constructive suggestion. Accordingly, we have explicitly listed all the excluded cardiovascular conditions in the Methods section.

Revised texts in the Methods part (Lines 120-122)

"From the initial pool of 502,480 subjects, individuals were excluded based on the following criteria: (1) participants with a prior history of AS, ischemic heart disease, stroke, aortic valve disease, or diseases of the arteries (n = 32,192)".

3. The authors describe a PRS consisting of 15 sequence variants. However, PRS is the term for a genome-wide genetic risk score, involving hundreds of thousands of variants. The authors should call it genetic risk score instead.

Response:

We appreciate the reviewer's attention to terminological precision.

We have replaced "PRS" with "GRS" (or "genetic risk score") throughout the entire manuscript, including the Abstract, Methods, Results, Discussion, as well as in all Tables and Figures.

4. Also regarding the genetic risk score, there is no explanation about how the variants were selected. Recent GWASs report over 30 variants associates with AS (https://pubmed.ncbi.nlm.nih.gov/38494474/, https://pubmed.ncbi.nlm.nih.gov/37038246/). What is the reason to not use all variants associated with AS?

Response:

We thank the reviewer for this insightful comment. We agree that incorporating the most recent and comprehensive GWAS findings is crucial for the robustness of the GRS. Following your suggestion and based on the recent literature (*Nat Commun. 2024 Mar 18;15(1):2407; Eur Heart J. 2023 Jun 1;44(21):1927-1939.*), we have incorporated 40 SNPs and updated the GRS construction. We have re-performed all analyses using this updated GRS. The new results are **highly consistent** with our previous findings (Fig 2, **Table S9** and **S10**).

Revised texts in the Methods part (Lines 146-148)

"A total of 40 independent (r^2<0.05) AS-related SNPs (minor allele frequency > 0.05) were incorporated into the process of genetic risk score (GRS) construction (**Table S1**)."

Revised Table S9 and S10:

Table S9. Associations of GRS with the risk of incident AS.

GRS	HRs (95% CIs)	*P* values	*P* for trend
GRS categories			
Low genetic risk	1.00 (Ref.)	--	
Intermediate genetic risk	1.42 (1.30, 1.56)	<0.001	<0.001
High genetic risk	2.11 (1.94, 2.30)	<0.001	
GRS, per SD[a] increase	1.41 (1.36, 1.45)	<0.001	--

Table S10. Additive interactions between air pollutants and GRS on the risk of incident AS.

Variables	RERIs (95% CIs)	APs (95% CIs)
$PM_{2.5}$		
Medium genetic risk		
$PM_{2.5}$-T2	0.27 (-0.07, 0.61)	0.14 (-0.03, 0.31)
$PM_{2.5}$-T3	0.83 (0.36, 1.30)	0.21 (0.10, 0.32)
High genetic risk		
$PM_{2.5}$-T2	0.49 (0.10, 0.87)	0.17 (0.04, 0.30)
$PM_{2.5}$-T3	2.03 (1.47, 2.59)	0.35 (0.27, 0.42)
PM_{10}		
Medium genetic risk		
PM_{10}-T2	0.29 (-0.04, 0.63)	0.14 (-0.02, 0.29)
PM_{10}-T3	0.36 (-0.05, 0.76)	0.12 (-0.01, 0.25)
High genetic risk		
PM_{10}-T2	0.65 (0.27, 1.02)	0.21 (0.09, 0.32)
PM_{10}-T3	1.30 (0.84, 1.77)	0.29 (0.20, 0.38)
NO_2		
Medium genetic risk		

NO_2-T2	0.31 (0.02, 0.61)	0.18 (0.01, 0.35)
NO_2-T3	0.53 (0.17, 0.90)	0.19 (0.06, 0.31)
High genetic risk		
NO_2-T2	0.29 (-0.05, 0.63)	0.11 (-0.02, 0.25)
NO_2-T3	0.97 (0.55, 1.39)	0.24 (0.14, 0.33)
NO_x		
Medium genetic risk		
NO_x-T2	0.31 (0.01, 0.61)	0.17 (0.01, 0.34)
NO_x-T3	0.53 (0.15, 0.90)	0.18 (0.06, 0.31)
High genetic risk		
NO_x-T2	0.33 (-0.02, 0.67)	0.13 (-0.01, 0.26)
NO_x-T3	1.02 (0.59, 1.45)	0.25 (0.15, 0.34)

Minor:

5. There are multiple letters "s" forming plurals of words that should be singular in the standard English grammar, for example "Covariates assessments". It is only one of dozens of such cases.

Response:

Thank you for your suggestion.

As suggested, we have conducted a comprehensive, word-by-word proofreading of the entire manuscript. We have corrected all instances where plural nouns were incorrectly used as modifiers.

6. Conversely, "utilizing multiple imputation method" is missing an "s".

Response:

We appreciate the comment and have revised the text in the specific sections to improve clarity and grammar.

7. "remained statistical significance" - either remained statistically significant or kept statistical significance.

Response:

We appreciate the reviewer's careful reading and the helpful suggestion. We have revised the phrase to "remained statistically significant"

Revised texts in the Results part (Lines 303-306)

"Mirroring the trends observed in the exposure–response curves, the associations between air pollutant exposure and the risk of incident AS remained statistically significant even at levels below the WHO Air Quality Guidelines (**Table S7**)."

Reviewer #3 (Remarks to the Author):

There is a strong body of evidence support a link between air pollution exposure and the risk of developing cardiovascular disease including atherosclerosis, coronary artery disease, aorta aneurysm, mitral annulus calcification etc. However, there is very few data on the association between air pollution exposure and the risk of incident aortic stenosis (AS). In the present study, the authors used the UK Biobank database to assess associations between air pollution exposure and AS risk. Genetic data were integrated to evaluate joint and interactive effects of genetic susceptibility and air pollutants. Network toxicology and bioinformatics analyses were used to identify shared target genes and potential biological pathways of air pollutants exposure and AS. Long-term exposure to air pollution was associated with an increased risk of AS. There were both joint and interactive effects of genetic susceptibility and air pollutants on AS risk. Network toxicology and bioinformatics analyses identified IL6 as a key molecular target underlying air pollution-induced AS. The authors conclude that Air pollution exposure is associated with an increased risk of AS, with a potential interaction between environmental exposure and genetic susceptibility. This is an interesting analysis reporting novel finding. I have the following comments and suggestions:

1. Although the investigators adjuster for multiple potentially confounding factors. However, it is difficult from the data presented in this paper to confirm the cause-effect relationship of air pollution on risk of incident AS.

Response:

We appreciate the reviewer's insightful comment regarding the causal interpretation of our results. We fully agree that while observational studies cannot definitively establish causality, the application of advance causal inference methodologies can significantly strengthen the evidence for a potential causal link.

(1) To further address the concern of residual confounding and strengthen the basis for potential association inference, **we have performed additional analyses using a**

causal inference framework. Specifically, we implemented Cox proportional hazards models weighted by Inverse-Probability Weights (IPW) and adjusted for Generalized Propensity Scores (GPS). GPS is an extension of the propensity score approach, which represents the probability of being exposed to the observed exposure level given the covariates (*Sci Adv. 2020; 17;6(29):eaba5692; Environ Int. 2023; 174:107872; J Hazard Mater. 2024; 5:469:133944.*). IPW is an advanced method aimed at achieving well balancing of confounders and simulates a randomized assignment of exposure. By assigning weights to participants, IPW adjusts the sample distribution to construct a pseudo-population that approximates the conditions of a randomized trial, ensuring that individuals across different exposure levels are balanced in terms of their covariates (*BMJ. 2016; 15:352:i189; Stat Methods Med Res. 2019;28(5):1365-1377.*). These models have been extensively applied in our previous research and numerous large-scale cohort studies to investigate the relationship between air pollution exposure and health outcomes (*Nat Cardiovasc Res. 2025 Oct;4(10):1397-1408. Science advances. Jul 2020;6(29):eaba5692; Environment international. Apr 2023;174:107872; Journal of hazardous materials. May 5 2024;469:133944.*).

The results remained robust across the Cox proportional hazards model, IPW, and GPS methods (**Table S6**), with hazard ratios (HRs) estimated from the IPW and GPS methods being **highly consistent**.

(2) To further evaluate the potential impact of unmeasured confounding, **we calculated the E-value** (*Ann Intern Med. 2017 Aug 15;167(4):268-274.*). The E-values for $PM_{2.5}$, PM_{10}, NO_2, and NO_x (per SD increase) were 2.58, 2.07, 2.08, and 2.06, respectively (**Table 2**). These E values suggest that an unmeasured confounder would need a very strong association with both the exposure and the outcome to nullify our observed risk. Such a strong unmeasured confounder is biologically unlikely given our extensive adjustment for known risk factors.

(3) Considering concern from the respected reviewer, we have added this point in the limitation part in the latest version of manuscript.

Revised texts in the Methods part (Lines 224-234)

"To achieve a balanced distribution of confounding variables, this study utilized inverse probability weighting (IPW) and generalized propensity scores (GPS) as robust measures to minimize residual confounding and strengthen the basis for causal interpretation of the observed associations [52,53]. GPS is an extension of the propensity score approach, representing the probability of an individual being exposed to the observed level given the covariates [28]. IPW is an advanced method aimed at achieving optimal covariate balance and simulating a randomized assignment of exposure [54]. In implementing IPW, we fulfilled the core criteria of consistency, positivity, and exchangeability (**Fig. S1** and **S2**). In addition, E-values were utilized to estimate the minimum association required for an unmeasured confounder to nullify the observed effects [55]."

Revised texts in the Discussion part (Lines 446-453)

"Firstly, while causal inference methodologies including IPW and GPS were employed to balance measured covariates, the observational design of this study limits our ability to confirm a definitive causal effect. The possibility of residual confounding from unmeasured variables cannot be entirely excluded. Nevertheless, results remained robust across multiple modeling approaches, and the temporal precedence inherent in our prospective cohort design further bolsters the inference of a potential association between exposure and outcome."

Revised Table 2:

Table 2. Associations between air pollutants and the risk of incident AS.

Air pollutants	HRs (95% CIs)	E values	*P* values	*P* for trend
$PM_{2.5}$				
$PM_{2.5}$-T1	1.00 (Ref.)	--	--	
$PM_{2.5}$-T2	1.34 (1.22, 1.47)	2.01	<0.001	<0.001
$PM_{2.5}$-T3	2.58 (2.37, 2.81)	4.60	<0.001	
$PM_{2.5,}$ per SD[a] increase	1.60 (1.55, 1.66)	2.58	<0.001	--
PM_{10}				

PM_{10}-T1	1.00 (Ref.)	--	--	
PM_{10}-T2	1.42 (1.30, 1.55)	2.19	<0.001	<0.001
PM_{10}-T3	2.02 (1.86, 2.20)	3.46	<0.001	
PM_{10}, per SD[b] increase	1.37 (1.32, 1.41)	2.07	<0.001	--
NO_2				
NO_2-T1	1.00 (Ref.)	--	--	
NO_2-T2	1.23 (1.13, 1.34)	1.76	<0.001	<0.001
NO_2-T3	1.98 (1.82, 2.15)	3.37	<0.001	
NO_2, per SD[c] increase	1.37 (1.32, 1.42)	2.08	<0.001	--
NO_x				
NO_x-T1	1.00 (Ref.)	--	--	
NO_x-T2	1.25 (1.15, 1.37)	1.82	<0.001	<0.001
NO_x-T3	2.01 (1.85, 2.19)	3.43	<0.001	
NO_x, per SD[d] increase	1.36 (1.31, 1.41)	2.06	<0.001	--

Added Table S6:

Table S6. Associations between air pollutants exposure and the risk of incident AS using the causal inference models.

Air pollutants (per SD increase)	HRs (95% CIs)	*P* values
Weighted Cox proportional hazards model (by IPW)		
$PM_{2.5}$	1.57 (1.50, 1.64)	<0.001
PM_{10}	1.31 (1.26, 1.36)	<0.001
NO_2	1.32 (1.27, 1.38)	<0.001
NO_x	1.30 (1.24, 1.37)	<0.001
Weighted Cox proportional hazards model (by trimmed IPW)		
$PM_{2.5}$	1.55 (1.49, 1.61)	<0.001
PM_{10}	1.31 (1.27, 1.36)	<0.001
NO_2	1.32 (1.27, 1.38)	<0.001
NO_x	1.30 (1.25, 1.36)	<0.001
Cox proportional hazards model adjusted for GPS (linear)		
$PM_{2.5}$	1.41 (1.37, 1.46)	<0.001
PM_{10}	1.27 (1.23, 1.32)	<0.001
NO_2	1.23 (1.20, 1.27)	<0.001
NO_x	1.22 (1.18, 1.26)	<0.001
Cox proportional hazards model adjusted for GPS (quintiles)		
$PM_{2.5}$	1.42 (1.38, 1.47)	<0.001
PM_{10}	1.28 (1.24, 1.32)	<0.001
NO_2	1.24 (1.20, 1.28)	<0.001
NO_x	1.23 (1.19, 1.27)	<0.001

2. The fact that genetic susceptibility may modulate the effect of air pollution on AS risk is plausible but again here the causal link between IL6, in particular, and exacerbation of air pollution effect on AS is difficult to establish with these data. Several studies reported an association between IL6 polymorphism and incident AS. So it is unclear whether IL6 is simply an additive contributor to AS besides Air Pollution or is truly a potentializing factor that exacerbates the effect of air pollution.

Response

We appreciate the reviewer's rigorous assessment.

We fully agree that our current data, derived from observational epidemiology and bioinformatics, are insufficient to definitively classify IL6 as a 'potentializing factor' or as an independent 'additive contributor' to the observed association between air pollution exposure and AS risk. Accordingly, we have adopted a more conservative interpretation of the role of IL6 in our study. First, we have removed the statement "IL6 serves as a key molecular target underlying air pollution-induced AS" from both the Abstract and the Conclusion section. Instead, we now frame our findings as a hypothesis-generating exploration that necessitates further mechanistic validation. Furthermore, we refined the Discussion section to acknowledge that the precise role of IL-6 in this association remains speculative and necessitates future experimental research to elucidate the underlying biological mechanisms.

Revised texts in the Discussion part (Lines 432-437)

"Nevertheless, it is essential to distinguish between statistical interaction and biological synergism. While our analysis revealed a significant additive interaction between genetic predisposition and air pollutants, and network toxicology identified *IL6, TNF,* and *IL1B* as hub genes, these findings remain exploratory in nature. They provide a hypothesis-generating framework that necessitates future experimental research to elucidate the precise biological mechanisms of these associations."

3. The investigators only used the data of UK Biobank. The results and impact of this study would be great reinforced if the investigators are able to replicate, at least in part,

these findings in another independent cohort.

Response:

We sincerely thank the reviewer for the insightful comment.

As suggested, we have expanded our study by performing an independent external validation using the Tianshan Community Cohort, a massive prospective study in Northwest China (https://clinicaltrials.gov/study/NCT07207863). This cohort provides a distinct geographical and ethnic context compared to the UK Biobank. Despite these differences, our primary findings were successfully replicated, with long-term air pollution exposure showing consistent associations with incident AS risk. Furthermore, we have integrated a comprehensive discussion to account for the observed discrepancies in effect magnitudes between the two cohorts.

Added texts in the Method part (Lines 203-213)

"**External validation**

External validation was performed using the Tianshan Community Cohort (https://clinicaltrials.gov/study/NCT07207863), a large-scale prospective study tracking longitudinal health in Northwest China. Data were integrated from a multi-tier network of 7,523 primary care centers and 226 hospitals, encompassing both health examinations and clinical records. Detailed cohort characteristics have been documented in prior literature [49-51]. From an initial pool of 9,254,018 screened individuals aged > 18 years who underwent baseline examinations in 2017, we applied exclusion criteria identical to those utilized in the UK Biobank analysis. The final validation subset comprised 8,329,679 participants, among whom 6,256 incident AS cases were identified during the follow-up period (2017–2024)."

Added texts in the Results part (Lines 306-313)

"**Table S8** presents the findings of the external validation conducted within the Tianshan Community Cohort. Consistent with the primary analysis, long-term exposure to air pollutants was significantly associated with an increased risk of incident AS in the Chinese population. The HRs and 95% CIs per SD increase were 1.30 (1.25 1.34)

for $PM_{2.5}$ (per 21.83 μg/m^3 increase), 1.22 (1.18, 1.27) for PM_{10} (per 90.88 μg/m^3 increase), and 1.15 (1.12, 1.18) for NO_2 (per 7.77 μg/m^3 increase), respectively. Furthermore, the exposure-response curves in this validation cohort closely mirrored those observed in the UK Biobank (**Fig. S5**)."

Added texts in the Discussion part (Lines 383-395)

"Findings from our external validation demonstrated a consistent association between air pollution exposure and incident AS risk, albeit with a discernible discrepancy in effect magnitudes observed between the two cohorts. Specifically, the UK Biobank benefits from relatively mature electronic medical record linkage and a decade-long follow-up, which effectively captures AS cases in a predominantly middle-aged and older population at peak risk. In contrast, the Tianshan Community Cohort includes a larger proportion of younger adults (over 18 years) with a shorter follow-up; the inherent cardiovascular resilience in these individuals may partially offset the adverse effects of air pollutants. Furthermore, whereas air pollution in UK is primarily traffic-derived with high biological toxicity, the air pollution in the Tianshan Community Cohort (Northwest China) may consist of a higher fraction of crustal mineral components, which typically exhibit lower per-unit toxicity despite higher mass concentrations."

Added Table S8 and Fig. S5

Table S8. Associations between air pollutants and the risk of incident AS: external validation in the Tianshan Community Cohort.

Air pollutants	HRs (95% CIs)	*P* values	*P* for trend
$PM_{2.5}$			
$PM_{2.5}$-T1	1.00 (Ref.)	--	
$PM_{2.5}$-T2	1.14 (1.06, 1.23)	0.001	<0.001
$PM_{2.5}$-T3	1.60 (1.47, 1.75)	<0.001	
$PM_{2.5}$, per SD increase	1.30 (1.254 1.34)	<0.001	--
PM_{10}			
PM_{10}-T1	1.00 (Ref.)	--	<0.001

PM_{10}-T2	1.14 (1.06, 1.23)	0.001	
PM_{10}-T3	1.45 (1.33, 1.58)	<0.001	
PM_{10}, per SD increase	1.22 (1.18, 1.27)	<0.001	--
NO_2			
NO_2-T1	1.00 (Ref.)	--	
NO_2-T2	1.23 (1.15, 1.31)	<0.001	<0.001
NO_2-T3	1.41 (1.32, 1.51)	<0.001	
NO_2, per SD increase	1.15 (1.12, 1.18)	<0.001	--

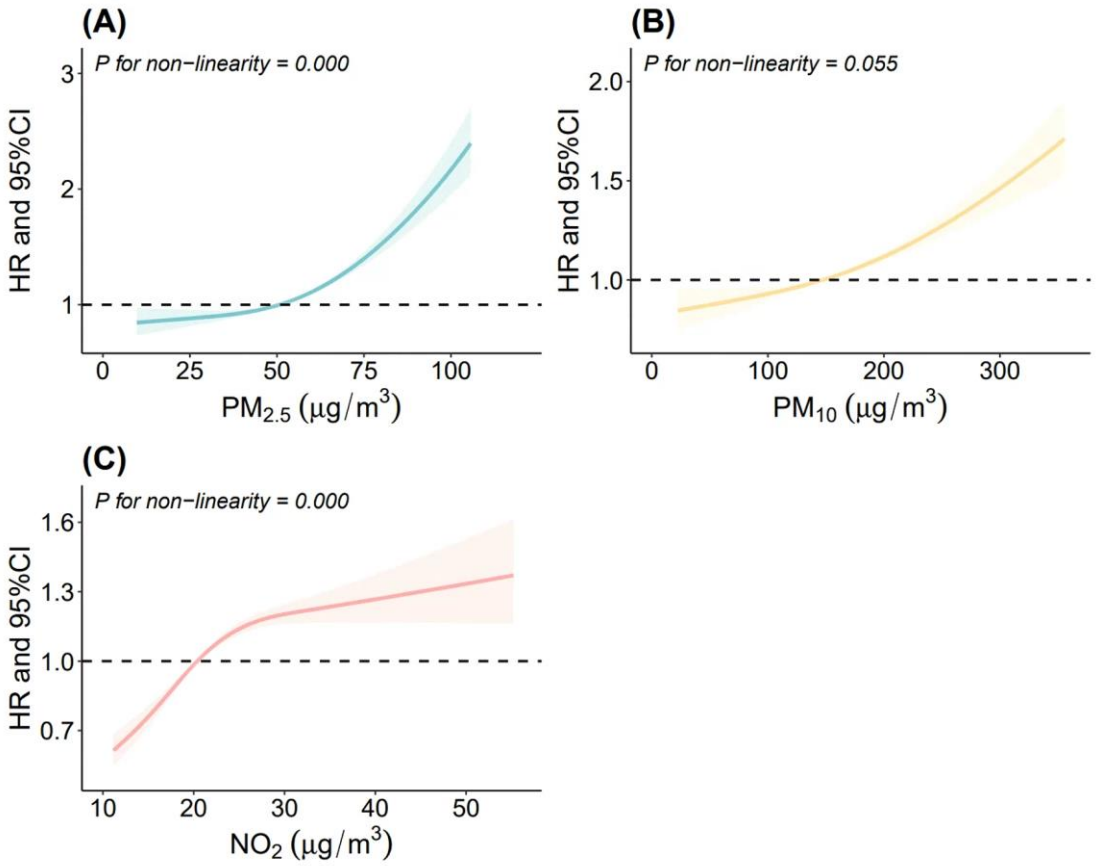


Fig. S5 Associations of long-term $PM_{2.5}$ (A), PM_{10} (B), and NO_2 (C) exposure with the risk of AS events based on the Tianshan Community Cohort.

4. The present study examines the impact of air pollution on incident AS. As a future step, it would be interesting to assess the association between long-term exposure to air pollution and progression rate of AS or aortic valve calcification.

Response:

We appreciate the reviewer's forward-looking suggestion. We fully agree that

584 investigating the association between air pollution and the progression rate of AS or
585 aortic valve calcification is a crucial next step to clarify the clinical trajectory and
586 underlying pathophysiology. As noted by the reviewer, our current study focuses on
587 incident AS. However, the UK Biobank currently lacks the serial, high-resolution
588 imaging data required to quantify the rate of valve narrowing or the accumulation of
589 calcium over time.
590 We have embraced this excellent suggestion by incorporating it into our Discussion
591 section as a priority for future research.
592 **The revised texts in the Discussion part (lines 474-478):**
593 "Finally, the UK Biobank currently lacks the longitudinal imaging data necessary to
594 quantify the rate of stenotic progression or calcium accumulation. Future studies
595 employing serial imaging are warranted to elucidate the impact of air pollution on the
596 clinical trajectory of AS, thereby identifying specific windows of vulnerability along
597 the disease course."
598
599 5. The review of literature regarding the genetic susceptibility to AS is incomplete and
600 only focuses on LPA. Please consider citing and discussing these previously published
601 studies including the following:
602 https://pubmed.ncbi.nlm.nih.gov/40329539/
603 https://pubmed.ncbi.nlm.nih.gov/39504041/
604 **Response:**
605 We sincerely thank the reviewer for this constructive suggestion and for pointing us
606 toward these seminal studies. Accordingly, we have significantly expanded the
607 Introduction sections to incorporate these findings. We believe these additions provide
608 a much stronger and more up-to-date scientific foundation for our study.
609 **The revised texts in the Introduction part (lines 84-93):**
610 "Genetic determinants represent another primary driver of AS incidence. Recent large-scale
611 genomic evidence has identified shared genetic features between AS and coronary artery
612 disease, primarily related to inflammation, lipid metabolism, smooth muscle cell proliferation,

and calcification. However, AS also possesses a distinct genetic risk profile involving cell differentiation and development, DNA-damage response and apoptosis, and shear stress, among others [19-21]. Furthermore, tissue-specific transcriptomic analyses have revealed that a significant portion of genes dysregulated in AS are uniquely expressed within the aortic valve, highlighting localized pathological processes [22]."

6. It is unclear how long-term exposure to ambient air pollution was defined and adjudicated in the UK Biobank and how robust is this data.

Response:

We sincerely appreciate the valuable insights you have provided on this issue and the deep reflections you have prompted. We have addressed the definition and robustness of the air pollution data as follows:

(1) "Long-term exposure" was defined as the time-weighted average concentration of ambient air pollutants for each participant during follow-up. To account for temporal variations, we calculated the average concentration from the baseline recruitment date up to the date of the incident AS diagnosis or censoring. This approach effectively captures the cumulative exposure burden and is a well-established method in large-scale cohort studies (*N Engl J Med. 2023 Apr 13;388(15):1396-1404.*).

(2) The primary exposure data were sourced from the UK Department for Environment, Food & Rural Affairs (DEFRA). DEFRA utilizes sophisticated atmospheric dispersion models integrated with the National Atmospheric Emissions Inventory, secondary inorganic aerosol measurements, and dust resuspension models. These models are rigorously calibrated against national monitoring network data to ensure accuracy. The model performance is excellent (For instance, the cross-validated R^2 for $PM_{2.5}$ reaching up to 0.85). This dataset has been extensively validated and utilized in high-impact environmental studies (***J Hepatol**. 2024; 28:S0168-8278(24)02573-X; **Nature Cities** (2024): 1-12; **Nature Mental Health**, 2024, 2(5): 525-534; **Environ Health Perspect**. 2024;132(10):107002.*).

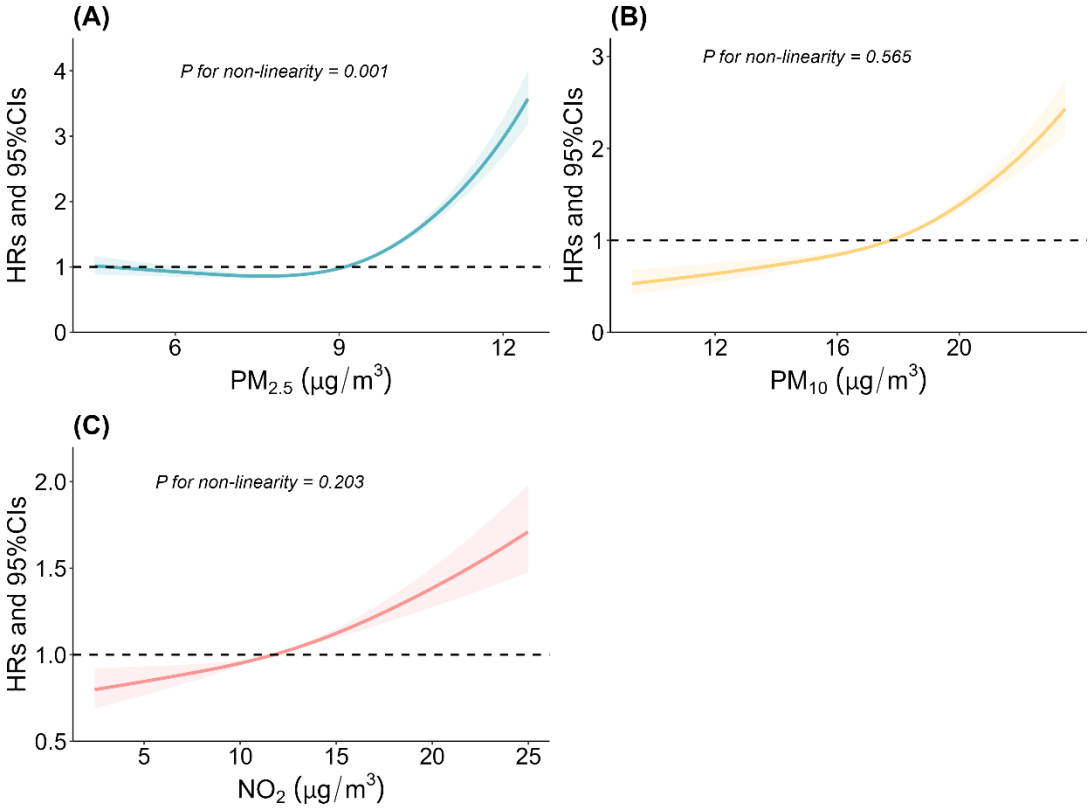
(3) To further valid our findings on the relationships between air pollution exposure and

the risk of AS, we also captured air pollution data from the Environmental Information Data Centre (EIDC). In this platform, $PM_{2.5}$, PM_{10}, and NO_2 concentrations at 3 × 3 km^2 grid in the United Kingdom (UK) (https://eidc.ac.uk/) were estimated using the European Monitoring and Evaluation Programme (EMEP) model applied to the UK (EMEP4UK) driven by Weather and Research Forecast model meteorology (WRF), which was developed by the Natural Environment Research Council as part of the UK Status, Change and Projections of the Environment (UK-SCAPE) (*NERC EDSEnvironmental Information Data Centre; 2022. https://doi.org/10.5285/ca302d30-7b8b-46ec-90b6-67b79df00c92*). EMEP4UK-WRF model has undergone extensive evaluation in the UK, demonstrating good performance when compared against measurement data (***Environ Int** 2023;171:107676; **Atmos Chem Phys** 2014;14:8435–8447.*). We utilized Cox proportional hazards models and restricted cubic splines to assess the robustness of the association between air pollution exposure—estimated via the EMEP4UK model—and incident AS risk. The findings remained consistent across various air pollution sources (**Table A1** and **Fig. A1**). Since the EMEP4UK does not estimate NO_x concentrations, we have only presented the results for $PM_{2.5}$, PM_{10}, and NO_2, **demonstrating the robustness of our findings**. Considering that the above analysis is only for validation purposes, the results will not be presented in the main text.

Table A1 and Fig A1:

Table A1. Associations between air pollutants and the risk of incident AS based on data from the EMEP4UK.

Air pollutants (per SD increase)	HRs (95% CIs)	E values	*P* values
$PM_{2.5}$	1.33 (1.29, 1.38)	2.00	<0.001
PM_{10}	1.34 (1.29, 1.38)	2.01	<0.001
NO_2	1.17 (1.14, 1.21)	1.62	<0.001



666

667 **Fig. A1** Associations of long-term $PM_{2.5}$ (A), PM_{10} (B), and NO_2 (C) exposure with

668 the risk of AS events based on data from the EMEP4UK.

669

Point-by-point Responses to Comments of Editor and Reviewers

Reviewer #1 (Remarks to the Author):

The authors have satisfactorily addressed my concerns in the revised manuscript. I now consider the paper to be of good scientific quality.

Reviewer #2 (Remarks to the Author):

The authors have improved the manuscript according to my suggestions. No further comments.

Reviewer #3 (Remarks to the Author):

The authors have submitted a revised version and rebuttal that address very well and completely the comments that I made initially. The paper is further improved.

Response:

We would like to express our most sincere gratitude to the Editor and all the Reviewers for their time, expertise, and highly constructive feedback throughout the review process. We are delighted that our revisions have satisfactorily addressed all the concerns raised. The reviewers' insightful suggestions have undoubtedly strengthened the scientific rigor, clarity, and overall quality of our manuscript.