Professor Felipe Fregni, MD, PhD, MMSc, MPH
Editor-in-Chief, *Principles and Practice of Clinical Research (PPCR Journal)*

Dear Professor Felipe Fregni and reviewers,

We appreciate the comments from the October 3rd email regarding our manuscript entitled "Leukocyte Telomere Length and Vitamin D: A Systematic Review of Observational Research (2025 G8 - SR)". Following your recommendations, we have incorporated the requested changes.

Peer-Review comments and author responses

Reviewer 1:

> **Comment::** One concern I had with the study is that vitamin D levels in females can be influenced by hormonal factors, yet not all reviewed studies included comprehensive hormone panels.

Response: *The authors thank the reviewer for these thoughtful observations. With respect to sex and hormones, several included studies suggested that sex may influence 25(OH)D status and its association with leukocyte telomere length; however, not all studies reported comprehensive hormonal panels (e.g., estradiol, testosterone, SHBG), menopausal status, or hormone therapy. The revised manuscript now (i) explicitly acknowledges the absence of hormonal profiling as a limitation, (ii) highlights biological plausibility for sex-related effect modification, and (iii) recommends prespecified sex-stratified analyses with direct hormonal measurements in future work (Discussion section).*

> **Comment:** Additionally, seasonal variation was inconsistently reported, which could introduce bias due to differences between winter and summer.

Response: *Regarding seasonality, the authors agree this is a key potential confounder. Reporting of month/season of blood draw and statistical adjustment for season was inconsistent across studies, which precluded uniform harmonization. The revision clarifies which studies are adjusted for season (Results section on study characteristics: Richards, 2007; Normando, 2020) and emphasizes the risk of residual confounding by season in the interpretation of pooled evidence (Discussion section).*

> **Comment:** Another point worth considering is the geographic scope of the studies. I would have preferred a more region-specific approach to ensure that weather-related variables were balanced across participants.

Response: *On geography, we have added a short paragraph describing region-specific patterns. Three of the four studies reporting positive associations were conducted in the Northern Hemisphere (e.g., Jelmilla; Richards; Belfuss; Liu), whereas other studies from Europe (Germany, Finland), the United States, and South America did not detect differences. Taken together, these observations do not support a consistent global geographic effect; instead, they likely reflect unmeasured or inconsistently measured factors correlated with geography, particularly seasonality and latitude-related UV exposure. To avoid over-interpretation, the revised text frames geography as a proxy for these variables rather than as an independent causal determinant (Discussion section).*

Reviewer 2:

> **Comment:** Clearly describe your PICOS in the end of the introduction.

Response: *The authors thank the reviewer for this practical suggestion. The final paragraph of the Introduction has been revised to explicitly state the PICOS elements in a clear, standardized format. This addition aims to improve transparency and reader orientation at the outset of the manuscript.*

> **Comment:** Explain why RCT's were not included in your systematic review.

Response: *The authors thank the reviewer for this pertinent observation. The review was scoped a priori to synthesize observational evidence on the association between circulating 25-hydroxyvitamin D and leukocyte telomere length in adults, rather than the causal effect of supplementation. At the time the protocol was finalized and the searches were completed (last search: April, 2025), there was only one randomized controlled trial (RCT) reporting LTL outcomes that met the eligibility window. Prior syntheses either did not focus on LTL or examined telomerase activity in specific subpopulations, which differed from the present question. An RCT addressing related*

questions was published subsequently (July 2025), after the search cutoff and outside the registered scope. In response to the reviewer's suggestion, the revised manuscript now (i) makes this design choice explicit in the Methods (Eligibility Criteria) and (ii) acknowledges in the Discussion that emerging RCT evidence merits a separate, intervention-focused synthesis.

Comment: Explain why articles from before 2005 were not included.

Response: *Thank you for this helpful request for clarification. Leukocyte telomere length entered broader epidemiologic use after the introduction and diffusion of the quantitative PCR (qPCR) method in 2002, which markedly expanded study volume but also introduced platform-dependent variability. To enhance methodological comparability and reduce non-biological heterogeneity (e.g., differences in assay platforms, calibration, reporting units, and quality control practices) the eligibility window was restricted to studies published from 2005 onward, a period during which qPCR-based protocols and reporting conventions became more standardized and increasingly comparable with TRF/flow-FISH outputs. This decision is now stated explicitly in the Methods and briefly noted in the Discussion as a design choice to minimize era effects.*

Comment: Update table 1 including all extracted variables.

Response: *Thank you for the keen observation, the study period, conflict of interest, inclusion and exclusion criteria, health status and recruitment method were eliminated from the paragraph, because they were not informed on Table 1. Country of origin and study design for every study was added to Table 1.*

Comment: PRISMA flowchart says "references removed" should say "abstracts removed".

Response: *Thanks for the observation, the PRISMA flowchart was changed based on your recommendation*

Comment: The results section doesn't need subheadings for every paragraph.

Response: *We thank the reviewer for this stylistic recommendation. The Results section has been streamlined by removing paragraph-level subheadings. To preserve navigability, only two high-level subheadings are retained: Main Results and Risk of Bias and Sensitivity Analyses, which align with the manuscript's analytic hierarchy and the journal's style. Paragraphs have been merged where appropriate to improve flow, and all figure/table cross-references have been verified after these edits.*

Comment: Include how confounders were selected (forward, backwards method or by using DAG's.

Response: *Regarding confounders, we stated in the discussion section that authors selected covariates based on biological plausibility; there were no descriptions on any other selection method.*

Comment: The results section says that Jelmila et al found an increase of telomere length of 0.583 base pairs per 1 ng/ml of Vitamin D. When we look at the beta coefficient the increase is only 0.267. Is this mismatch a typo, if not explained why the increase of base pairs nearly doubles the beta coefficient.

Response: *The authors thank the reviewer for identifying this discrepancy. Upon re-auditing the original report, they confirmed that the value 0.583 bp per 1 ng/mL reflected an alternative specification/scale in the source (likely arising from a different model specification and/or a T/S-to-base-pair calibration), whereas the $\beta = 0.267$ bp per 1 ng/mL corresponds to the study's fully adjusted primary model on the reporting scale used in this review. To ensure consistency and avoid mixing scales across studies, the manuscript has been corrected to report $\beta = 0.267$ for Jelmila et al. per the prespecified extraction hierarchy (prioritizing fully adjusted estimates on the native reporting unit). The Results text and the corresponding table have been updated, and all study-level extractions were rechecked for scale, adjustment set, and rounding.*

Comment: Fonts and font size should be the same throughout the manuscript.

Response: *The authors thank the reviewer for pointing this out. Typography has been standardized across the entire submission in accordance with the journal's style template. The same font family and size now apply to all sections, including title page, abstract, main text, headings and references. The document was regenerated to ensure correct embedding and consistent rendering.*

Comment: In the discussion section it is stated that 6 studies showed no association between vitamin D and telomere length. There's no mention of power calculations done in these studies. So is there actually no association or the studies were underpowered?

Response: *The authors thank the reviewer for raising this important point. None of the included studies reported an a priori sample-size or power calculation for the vitamin D-telomere association, and several presented wide confidence intervals that encompassed effects of potential clinical relevance. Accordingly, the statement that "six studies showed no association" has been qualified in the revised Discussion to emphasize that these null results may reflect limited precision (type II error) rather than evidence of absence. To enhance interpretability, the Discussion now underscores that (i) most studies were not designed primarily for telomere outcomes; (ii) measurement variability in LTL assays further reduces effective power; and (iii) the heterogeneity of adjustment sets likely increased residual variance. Together, these factors may have biased results toward the null.*

> **Comment:** In the discussion it is stated that the positive association between vitamin D and telomere length can be attributable to the statistical analysis performed, stating that covariates like gender and age can have influenced the final outcome. In my opinion when you control for a covariate you actually remove the influence of the confounding variable in the relationship between outcome and explanatory variable. Not increase it's influence.

Response: *The authors thank the reviewer for this clarification and agree with the underlying principle: when a true confounder is correctly measured and appropriately modeled, adjusting for it should reduce bias, not increase it. The intent of the original statement was not to suggest that controlling for age or sex "increases their influence," but rather to acknowledge that between-study differences in adjustment sets, measurement quality, model specification, and potential effect modification can lead to divergent estimates. For example, (i) incomplete or mismeasured confounders may leave residual confounding; (ii) overadjustment (e.g., conditioning on mediators or colliders) can bias estimates toward or away from the null; (iii) model misspecification (e.g., omitting non-linear terms for age or season) may distort associations; and (iv) effect modification by age or sex would imply different true effects across strata, best addressed with interaction terms or stratified analyses rather than simple adjustment. To avoid ambiguity, the Discussion section has been revised to reflect this framing and to remove language implying that adjustment per se increases a covariate's influence. The text now emphasizes that appropriate adjustment reduces bias, while heterogeneity across studies may arise from residual confounding, overadjustment, model misspecification, or genuine effect modification (Discussion section).*

> **Comment:** In the discussion it's also stated that sex could attenuate any Vitamin D - Telomere length association when talking about the papers that reported no association between these variables. The problem with this statement is that 5 of the 6 papers that reported no association between Vitamin D - Telomere length were controlling for gender, meaning that gender is balanced. Eliminating any type of association gender can have in the model.

Response: *Thanks for this precise clarification. They agree that adjusting for sex as a covariate balances its main (confounding) effect and therefore should not, by itself, attenuate a true association between 25(OH)D and LTL. The intended point was different: several primary studies adjusted for sex but did not test for sex-by-25(OH)D interaction or report sex-stratified estimates, so any effect modification by sex could not be evaluated. In other words, controlling for sex removes confounding by sex but does not rule out that the association differs in magnitude or direction between males and females; detecting such heterogeneity requires interaction terms or stratified analyses, which were largely absent. To avoid ambiguity, the Discussion has been revised to: (i) state that confounding by sex is unlikely to explain the null findings, given that 5 of the 6 "null" studies adjusted for sex, (ii) distinguish clearly between confounding (addressed by adjustment) and effect modification (not assessable without interaction/stratification); and (iii) note that the lack of information on menopausal status/hormone therapy further limits assessment of sex-related modification.*

> **Comment:** 2 results from RCTs are included in the discussion. So why don't include RCTs in your systematic review.

Response: *Thanks again for the comment. This topic is already assessed on "reply 2".*

> **Comment:** It was stated that future research should aim to develop placebo controlled trials to test for an association between Vitamin D and Telomere length, but you already showed the results of 2 RCT's a couple of paragraphs above. If there are already RCT's published the next step will be to develop a metanalysis of this topic.

Response: *We agree that, given the existence of two published RCTs on vitamin D and leukocyte telomere length, the immediate next step for the intervention evidence is a pre-registered systematic review and meta-analysis of RCTs.*

The original phrasing ("future placebo-controlled trials are needed") has been revised to avoid implying that no RCTs exist. At the same time, the authors note that the available trials differ in population, dosing/duration, baseline 25(OH)D status, seasonality control, and LTL assay/reporting, which may limit quantitative pooling and interpretability at present. Accordingly, the revised Discussion now (i) recommends a focused meta-analysis of existing RCTs with transparent handling of heterogeneity, and (ii) outlines design standards for additional adequately powered RCTs to enable more definitive pooled estimates (assay harmonization, standardized outcome definitions and timing, season-of-draw control, prespecified sex-stratified analyses, and hormonal profiling where feasible).

Reviewer 3:

> **Comment:** The question addressed a relevant question. It essentially adheres to the PRISMA guidance. The tables and the flow diagram are helpful in guiding readers toward locating the pertinent information. The writing carries the essence, but some passages are rather not easy to follow due to the extent of the running of a few sentences. The placements of citations break the flow as well; grouping them or shifting them to the end of sentences would probably improve things. Readability would improve with a second run of editing.

Response: *Thank you for your note on the readability of the paper. The whole paper was adapted by grouping or shifting references to have more fluid sentences, without breaking the flow due to references.*

> **Comment:** Some aspects of the methods are not completely clear and do not conform to PRISMA 2020 clearly. The review does not indicate which effect measures were considered (item 12). The results section reports β coefficients, p-values, and base-pair changes for individual studies without predefined effect sizes intended to be extracted and units difference handling by the review itself. A short statement in the Methods could settle this, for example, by listing accepted effect measures (e.g., β coefficients, odds ratios, mean differences), explaining how units are harmonized (e.g., ng/mL vs nmol/L for vitamin D), and clarifying whether results are reported as adjusted or unadjusted. This would make findings much easier to interpret across studies also.

Response: *Appreciated your comment in item 12 of the PRISMA guidelines, as suggested we added a statement in the methods section (inclusion criteria) regarding the effect sizes, and units of vitamin D measurements.*

> **Comment:** Moreover, the manuscript did not include an assessment of reporting bias (item 14) or of certainty of evidence (item 15); both items are central in the evaluation of the strength of conclusion.

Response: *The authors appreciate this essential point. Because a quantitative synthesis was not performed, formal small-study assessments (such as funnel plots or Egger's test) were not applied. To reduce the risk of bias due to missing results, the search strategy was broadened beyond standard databases to include conference abstracts and Google Scholar, and backward/forward citation chasing was undertaken. Gray literature beyond these sources (for example, theses, institutional reports, or trial registries) was not systematically searched, which may limit completeness. Notably, several included studies reported negative or non-significant findings, mitigating concerns that only positive results were captured. Regarding certainty of evidence (PRISMA item 15), the manuscript now provides a concise, narrative appraisal using GRADE domains adapted for observational evidence. Certainty was judged low to moderate overall, reflecting study-level risk of bias, heterogeneity in populations and laboratory methods, imprecision, and unclear risk of publication bias in the absence of meta-analytic tests.*

> **Comment:** No statement is provided about review registration (item 24a) or about protocol availability (item 24b). Even if no PROSPERO registration or protocol were available, PRISMA expects a direct statement from authors to this effect.

Response: *The authors thank the reviewer for this important clarification. They agree that PRISMA requires an explicit statement on both registration (24a) and protocol availability (24b). This review was not prospectively registered (PROSPERO). Registration was considered only after data extraction had begun; therefore, the authors did not pursue retrospective registration to avoid the appearance of post-hoc protocol modification. To enhance transparency and reproducibility, the revised manuscript now includes a direct PRISMA-conforming statement, we provide supplementary materials, including the full search strategies and a list of excluded studies with reasons. A note has been added to the discussion section as part of its limitations.*

Comment: Another key issue is that two relevant randomized controlled trials (Rahman et al., 2023; Zhu et al., 2025) are cited in the Discussion but were neither included in the review and nor Zhu et al. 2025) is listed among the excluded studies. The eligibility criteria appear to restrict inclusion to observational studies, but the rationale for doing so is not explained. To improve transparency, the authors should either justify why RCTs were excluded or list them among the excluded papers with reasons provided.

Response: *The authors thank the reviewer for this pertinent observation. The review was scoped a priori to synthesize observational evidence on the association between circulating 25-hydroxyvitamin D and leukocyte telomere length in adults, rather than the causal effect of supplementation. At the time the protocol was finalized and the searches were completed (last search: April, 2025), there was only one randomized controlled trial (RCT) reporting LTL outcomes that met the eligibility window. Prior syntheses either did not focus on LTL or examined telomerase activity in specific subpopulations, which differed from the present question. An RCT addressing related questions was published subsequently (July 2025), after the search cutoff and outside the registered scope. In response to the reviewer's suggestion, the revised manuscript now (i) makes this design choice explicit in the Methods (Eligibility Criteria) and (ii) acknowledges in the Discussion that emerging RCT evidence merits a separate, intervention-focused synthesis.*

Comment: Last but not least, in the summary section of Jelmila et al. (2020) (around lines 213-216), the positive β is juxtaposed with a "reduction in telomere shortening." That sounds conflicting. It would help to justify whether to have a rate of shorter shortening, fitting a positive β, or whether the effect direction is misconstrued.

Response: *The authors thank the reviewer for this precise observation. They agree that juxtaposing a positive β with the phrase "reduction in telomere shortening" is potentially confusing, as the latter implies a longitudinal rate (change over time), while Jelmila et al. reported a cross-sectional association on a level scale (leukocyte telomere length in base pairs). To avoid mixing level and rate language, the manuscript has been revised to report the effect only on the native cross-sectional scale. The summary for Jelmila et al. now reads that higher 25(OH)D is associated with longer telomere length (positive β), without referring to "shortening" or rates. The ambiguous wording has been removed wherever it appeared, and the coefficient reported is the fully adjusted β = 0.267 bp per 1 ng/mL used consistently across the text and table. These edits standardize interpretation across studies and prevent conflation of cross-sectional levels with longitudinal shortening.*

Comment: The PRISMA flow chart works well and is easy to follow. Tables 1 and 2 give much detail on the topics under study, but there could be some streamlining to make it easier to compare results. For example, units could be standardized, effect sizes reported in the same form, and the difference between adjusted and unadjusted results made more explicit.

Response: *The authors thank the reviewer for this valuable suggestion. We agree that ease of comparison is important. At the same time, we chose not to standardize units or re-scale effect sizes to preserve fidelity to the primary studies and avoid mixing calibration-dependent transformations across heterogeneous assay platforms. Instead, the tables and accompanying text have been streamlined for comparability without altering original scales. Now, (i) Clear columns now specify each study's exposure scale and outcome unit. (ii) Abbreviations and assay acronyms are harmonized, and concise footnotes explain any non-standard reporting used by the source. (iii) We will add to as a footnote on table 1: "All reported coefficients and confidence intervals reflect the adjusted model. Unadjusted estimates are not displayed", so that we remain fully transparent.*

Comment: As a very minor optional improvement, the authors may also consider adding a simple visual-a schematic or bubble plot, for example-to provide a quick summary of study features or effect directions. In any event, though, the tables perform well in communicating this information.

Response: *Taking notice of your suggestion, we created a bubble-scatter plot of the included studies, which we will be adding to the supplementary material. This is informed in the results section.*

Reviewer 4:

Comment: Depth of Critical Analysis: While the review accurately summarizes current observational evidence, it could benefit from a more in-depth critical appraisal. Expanding the discussion to address potential sources of heterogeneity including sex- and age-specific differences, cohort characteristics, laboratory methods, and statistical adjustments would strengthen the manuscript. Consider also addressing

possible publication or language biases, as well as the influence of sample size and measurement sensitivity.

Response:. *The authors thank the reviewer for this constructive suggestion. To address it, the Discussion has been lightly expanded with a concise paragraph synthesizing potential sources of heterogeneity (sex- and age-specific differences, cohort characteristics, laboratory methods, and statistical adjustments) and a brief note on publication/language biases, sample size, and measurement sensitivity. These additions clarify interpretability while preserving the manuscript's scope and flow.*

Comment: Novelty and Contribution: This review is original as the first systematic review focused exclusively on observational studies of VD and LTL. Emphasizing how these findings differ from previous reviews or specifying the research gaps identified for future studies would further enhance the manuscript's contribution.

Response: *The authors thank the reviewer for recognizing the manuscript's originality. To make the contribution more explicit, brief language has been added to the Introduction and Discussion highlighting how this review differs from prior syntheses and what research gaps it identifies for future work.*

Comment: Integration of Evidence: The discussion of heterogeneity is strong; however, linking findings more explicitly to potential clinical implications or underlying biological mechanisms (e.g., hormonal, inflammatory, or genetic modulators) would enhance relevance. Including a summary table connecting individual study results with population characteristics and outcomes could improve clarity.

Response: *The authors thank the reviewer for this valuable suggestion. We agree that explicitly linking heterogeneity to biological pathways and clinical relevance can strengthen the manuscript. The Discussion has been lightly revised to (i) synthesize plausible mechanistic modulators, hormonal milieu (e.g., sex hormones/menopausal status), inflammatory-immune tone, oxidative stress, and genetic variation in vitamin D metabolism/signaling, and (ii) clarify clinical implications: current observational evidence does not justify vitamin D supplementation solely for telomere preservation, while underscoring the importance of addressing vitamin D deficiency for established indications. Regarding the request for a summary table, the authors note that Table 1 already functions as an integrative map of population characteristics, assay methods, adjustment sets (including sex), and study findings. To enhance clarity, Table 1 has been updated to clarify the narrative.*

Comment: Figures and Tables: The figures and tables are clear and informative. While the PRISMA flowchart and summary tables are appropriate, a concise visual summarizing associations by sex, age, and study quality could further facilitate comprehension.

Response: *On this note, the authors of the paper created a bubble-scatter plot of the results regarding publication date, sex, sample size number and main results. This figure will be added to the supplementary material, as well as informing this in the results section.*

Comment: Review the text for minor grammar and syntax issues, avoid repetition in certain sections and aim for concise statements, particularly in the discussion, ensure consistency in citation style and verify that all references are current and relevant.

Response: *Thank you for your suggestion. Regarding your minor comments, the text was reviewed to check for grammar and syntax issues. Also, re-writing the discussion section allowed us to avoid repetition and use concise statements. All references were checked and cited as indicated.*

Reviewer 5:

Comment: Although there seems to have been a comprehensive search of the literature, by use of four key databases (PubMed, Cochrane Library, EMBASE, and Scopus), I would further stress the possibility of language bias in the discussion (limitations) section, given that only English language papers were considered in this analysis.

Response: *The authors are grateful for the reviewer's thorough and encouraging assessment. We appreciate the recognition of the manuscript's novelty, methodological rigor, and balanced interpretation. All edits are tracked in the annotated version submitted with this response. According to your suggestions, we will respond point by point that were marked in alphabetic order. Thank you for your note, language bias is being addressed in the discussion (limitation paragraph).*

Comment: There was no mention of prior registration of this systematic review in freely-available registries, such as PROSPERO. Do you think this might limit the reliability, transparency and potential reproducibility of the protocol? Even though the usefulness of such registries might actually be questioned, please consider adding a note on this to the Discussion section.

Response: *The authors thank the reviewer for this important point. The review was not prospectively registered in PROSPERO. After drafting the protocol, the authors only considered registration at a stage subsequent to data extraction, at which point retrospective registration would not have been transparent. To address the reviewer's concern, the revised manuscript now explicitly acknowledges the absence of prospective registration and discusses its implications for reliability, transparency, and reproducibility. A note has been added to the discussion section as part of its limitations.*

Comment: The authors highlight that there was substantial heterogeneity across studies, with outcomes mainly varying according to the age range, presence (or inclusion/exclusion) of comorbidities, and gender. They further stress that placebo-controlled randomized trials might be useful to test if there is, in fact, an association between vitamin D supplementation and telomere preservation. One should, however, consider the feasibility of such a study design, given the expected long-term nature for any causal effect to take place when it comes to such an intervention. Would you, instead, consider narrowing the target population for the sake of greater homogeneity and for further exploring the diverse results seen in the specific strata mentioned? Could this, perhaps, make the data meta-analyzable? Still, would a subgroup analysis possibly play a role here?

Response: *The authors agree that long-term causal effects make placebo-controlled RCTs challenging. The revised Discussion now notes that enriched, more homogeneous target populations would improve feasibility and signal detection. It also states that pre-specified subgroup analyses are essential in both trials and observational syntheses. Narrowing inclusion criteria and standardizing outcomes/exposures could render future datasets meta-analyzable with meaningful between-study comparability. A brief sentence was added highlighting these design considerations and their implications for future RCT and meta-analytic work.*

Comment: Again in the language arena, I would carefully revise the manuscript regarding a few typos worth correcting.

Response: *In line with the reviewer's suggestion, the revised manuscript has undergone a careful, line-by-line proofreading to correct typographical errors and minor language issues. Terminology has been standardized throughout, abbreviations have been harmonized, and style inconsistencies have been addressed.*

Comment: In "Main results", "The results across the 11 included studies were heterogeneous (…), "the others did not showed a statistically significant (…)", please rephrase as: "the others did not show a statistically significant (…)";

Response: *Thanks for your correction regarding grammar. We have included that recommendation in our main results section.*

Comment: In the Discussion section, please rephrase "population with mean age 60 years" as "population with a mean age of 60 years".

Response: *We appreciate you for noting and suggesting grammar corrections. The phrase was corrected in the discussion section.*

Comment: As for the main study sections (Title and Abstract, Introduction, Methods, Results, Discussion/Conclusion), the study is well structured, with a nice overall presentation and good quality tables and figures (PRISMA flowchart), as well as correctly formatted references, for which I have no extra remarks.

Response: *We appreciate you for noting and suggesting grammar corrections. The phrase was corrected in the discussion section. The authors thank the reviewer for the positive assessment of the manuscript's structure, presentation, figures (including the PRISMA flowchart), and reference formatting. We are grateful for the confirmation that no further changes are required in these sections. Minor copyediting has nonetheless been performed to maintain consistency in style and terminology across all sections and tables, without altering content.*

On behalf of all authors, we thank the Editor and Reviewers for their careful evaluation and constructive feedback. We have implemented the requested revisions, clarified our methodological choices, standardized tables/figures and reporting, and added the supplementary material noted in our responses (including the updated PRISMA flowchart and the bubble-scatter figure). We believe these changes improve transparency, interpretability, and alignment with PRISMA 2020. We hope the revised manuscript now meets the journal's standards, and we remain at your disposal for any additional clarifications or further edits needed for publication.

Sincerely,

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