Author’s response to reviews

Title: Osteopontin promoter polymorphisms and risk of urolithiasis: a candidate gene association and meta-analysis study

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Responses to reviewers’ comments

We thank the editor, reviewers and associated editorial staff for their valuable time, efforts and inputs, which has certainly helped in improving the quality of this manuscript. Specific responses to the reviewers’ comments are listed below;

Reviewer 1 (Dian Nurputra)
Methodology and flow
Comment 1: Could you explain to us, what do you mean by "overlaps" in the identification phase of the meta-analyses?
Response: Potential studies for inclusion in the meta-analysis were identified from different online databases including Google Scholar, PubMed, ScienceDirect, Cochrane library and Embase, generating a list of studies from each database (n=217 total entries in all lists). Redundant entries that were common between two or more databases (same studies that were indexed in two or more of these databases) were referred to as overlaps (n=155) and only one entry was retained for such studies in the subsequent combined list of unique studies (n=62) for further screening. The same has been clarified and included in the legend of Figure 1 (page 31, lines 541-547).
Comment 2: Please provide a brief explanation about the adherence of PRISMA statement in conducting your meta-analysis study.
Response: PRISMA checklist for the current meta-analysis part of the study has been provided below at the end for reference and a brief description has been added in the methodology section under meta-analysis heading (page 7, lines 136-141).

Results and discussion
Comment 3: The basic characteristics of the subjects could be explained more by adding several variables or risk factors aside the genetics. The Authors mentioned about parental consanguinity factors in the table, however there was no explanation or discussion regarding the finding. Please explain.
Response: The basic characteristics of the subjects were provided in additional file 2 (supplementary data) and a brief description of which has been included in the results section (page 10, lines 201-207). We also did analyze the effect of additional risk factors (including gender, early age at presentation, multiple renal stones, recurrences, presence of familial history of urolithiasis and parental consanguinity) in modulation of SPP1 polymorphisms based genetic risk of urolithiasis (additional file 6 in supplementary data), the results of which were presented in results section (page 11, lines 226-229) and discussion of which has been added under discussion section (page 14, lines 295-300).

Comment 4: Many polymorphisms in SPP1 gene have been reported. Do the Authors ever consider about the possibility of having two polymorphisms of SPP1 gene in one individual. Among all subjects participated in the study, is there any individual found to harbor two polymorphisms?
Response: This is exactly what was presented in Table 3 and additional file 5 (supplementary data). First we analyzed which of the SPP1 polymorphisms are likely to be linked and inherited together through pair-wise linkage disequilibrium analysis. Then we analyzed the frequency of presence of 2 or more risk alleles of SPP1 polymorphisms in cases and controls and determined whether simultaneous presence of 2 or more risk alleles confers an increased risk of urolithiasis by haplotype association analysis which reflected that simultaneous presence of G-C-dG alleles of SPP1 rs2853744-rs11730582-rs11439060 polymorphisms, respectively, confers 1.68 times more risk of urolithiasis that is statistically significant. The detailed descriptions can be found in methodology (page 7, lines 126-128), results (page 11, lines 220-225) and discussion (updated) (pages 13-14, lines 286-289) sections.

Reviewer 2 (Surini Yusoff)
Typo errors
Comment 1: Page 12, Line 243: Figure to Figures
Response: Correction has been made accordingly (page 12, line 256).
Comment 2: Page 24, Table 1: In column Genotype/Allele, Row 2: T/G or G/T?
Response: Correction has been made accordingly (page 24, Table 1).
Comment 3: Page 29, Table 4: In column Samples: The word "Samples" is suggested to add as "Total Number of Samples" or "Samples (N)" where N should be added in the footnote as the Total Number of Samples
Response: Revised as Samples (N) where explanation of N has been given in the table footnotes as “Total number of samples” (page 29-30, Table 4 and line 537).
Abstract
Checklist item 2 (Structured summary): Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.
Response: Summary provided keeping in view that this is candidate gene association as well as meta-analysis study and word count limitations (Pages 2-3, lines 23-29, 32-35, 43-51).

Introduction
Checklist item 3 (Rationale): Describe the rationale for the review in the context of what is already known.
Response: A comprehensive background and rationale of the study provided (Pages 4-5).
Checklist item 4 (Objectives): Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).
Response: Provided and updated (Page 5, lines 92-98).

Methods
Checklist item 5 (Protocol and registration): Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.
Response: The protocol was not pre-registered (Page 7, line 144).
Checklist item 6 (Eligibility criteria): Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.
Response: Well-defined eligibility criteria provided (Pages 7-8, lines 144-152, 156-161).
Checklist item 7 (Information sources): Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.
Response: Details of information sources provided (Pages 7-8, lines 146-153).
Checklist item 8 (Search): Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.
Response: Search strategy provided (Pages 7-8, lines 146-153).
Checklist item 9 (Study selection): State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).
Response: Provided in sufficient detail (Pages 8-9, lines 161-177).
Checklist item 10 (Data collection process): Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.
Response: Provided (Page 8, lines 169-171).
Checklist item 11 (Data items): List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.
Response: Provided (Page 8, lines 165-169).
Checklist item 12 (Risk of bias in individual studies): Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level, and how this information is to be used in any data synthesis).
Response: Described (Page 9, lines 175-177).
Checklist item 13 (Summary measures): State the principal summary measures (e.g., risk ratio, difference in means).
Response: Provided (Page 9, lines 179-183).
Checklist item 14 (Synthesis of results): Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I²) for each meta-analysis.
Response: Provided (Page 9, lines 179-191).
Checklist item 15 (Risk of bias across studies): Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).
Response: Provided (Pages 9-10, lines 191-196).
Checklist item 16 (Additional analyses): Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.
Response: Sub-group analyses not done due to limited number of studies available (Page 10, lines 196-197).

Results
Checklist item 17 (Study selection): Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.
Response: Described with a flow diagram (Page 11, lines 231-239 and Figure 1).
Checklist item 18 (Study characteristics): For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.
Response: Described with table (Page 12, lines 241-247 and Table 4).
Checklist item 19 (Risk of bias within studies): Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).
Response: Updated (Page 11, lines 239-240).
Checklist item 20 (Results of individual studies): For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.
Response: Described with forest plots for each SPP1 polymorphism (Page 12, lines 248-263, and Figure 2 and additional files 7-8).
Checklist item 21 (Synthesis of results): Present results of each meta-analysis done, including confidence intervals and measures of consistency.
Response: Described with forest and funnel plots for each SPP1 polymorphism (Page 12, lines 248-263, and Figure 2 and additional files 7-8).
Checklist item 22 (Risk of bias across studies): Present results of any assessment of risk of bias across studies (see Item 15).
Response: Described in detail (Page 13, lines 264-270, and Figure 2 and additional files 7-8).
Checklist item 23 (Additional analysis): Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).
Response: Sub-group analyses not done due to limited number of studies available.

Discussion
Checklist item 24 (Summary of evidence): Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).
Checklist item 25 (Limitations): Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).
Response: Limitations also described (Page 16, lines 352-355).
Checklist item 26 (Conclusions): Provide a general interpretation of the results in the context of other evidence, and implications for future research.
Response: Conclusions provided (Page 17, lines 359-364).

Funding

Checklist item 27 (Funding): Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.

Response: Funding statement updated to include a statement specific for meta-analysis part of the study (Page 19, lines 404-405).