nature portfolio

Peer Review File

Proxied therapeutic inhibition on sclerostin and atrial fibrillation risk

Corresponding Author: Professor Hou-Feng Zheng

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)
In this manuscript the authors attempted to answer the following biological question: Does sclerostin (SOST) inhibition have cardiovascular effects using genetic evidence. The authors first show that circulating SOST influence both eBMD and CVDs. Next, they integrate genetic, transcriptomic, and proteomic data to explore the causality of SOST in both eBMD and CVDs and therefore assess the cardiovascular burden of using SOST inhibition in the treatment of osteoporosis.

Minor comments
a) Typographical:
- line 33: transcriptomic
- line 67: humans
- line 88: remove to from regarding to

b) Study:
- line 32: heel bone mineral density from the UK biobank is estimated therefore should be referred to as eBMD rather than BMD, this issue arises in other parts of the manuscript where authors use eBMD and BMD interchangeably
- line 424: code availability? I recommend adding all code to Github and all data files to Zenodo or a similar platform

Major comments
- eBMD GWAS: The summary statistics from the eBMD GWAS from Morris et al. 2018 is readily available, why did the authors rerun the GWAS study, while all the other CVD studies summary stats were used directly? Also, can you provide a complete comparison between your results and the Morris et al. results which uses 426,824 individuals. Additionally, the Morris et al. study reported 1103 GWS loci while it appears that this study reports nearly 30K loci. Could you please either report only lead SNP numbers or explain this discrepancy? How much of the variance did these loci explain compared to Morris et al. 20%?

- QTL Colocalization analysis: The current GTEx version is V10, with an increase of more than 200 donors used in eQTL analysis, could you please explain why use V7 rather than V8, or V10 was used? In addition, I understand the selection of the 6 cardiovascular related tissues, but are we considering whole blood a the only representative of "bone related gene expression"? if that's the case, then I suggest being more stringent with the H4PP as evidence of colocalization from 0.7 to at least 0.9. The same should happen with the pQTL colocalization analysis.

Reviewer #2

(Remarks to the Author)
The authors aim to shed light on the potential relationship between sclerostin and cardiovascular effects, presenting novel data derived from an interesting in silico approach. While the study offers potentially valuable insights, it lacks experimental

data to substantiate the proposed conclusions. Furthermore, the limited evidence linking the proxy gene with SOST and the complexity of the analysis required to establish these relationships raise concerns regarding the robustness of the associations.

Key concerns include:

The absence of experimental validation.

Limited evidence supporting the connection between the proxy gene and SOST.

The somewhat intricate analytical framework required to support the proposed relationships, which may cast doubt on the validity of the findings.

Specific issues:

Referring to papers published in 2021 as "very recent" (line 106) seems questionable.

It remains unclear whether these results are directly comparable to previous GWAS on bone mass conducted using the UK database. And what is the explanation for the differences.

The authors should clarify why no association was observed between the proxy gene and stroke/coronary heart disease, which are primary concerns regarding SOST inhibition in clinical settings. The mentioned association between atrial fibrillation (AF) and stroke/heart failure does not necessarily imply a shared genetic basis, as stroke and heart failure may arise as secondary consequences of AF.

A dedicated section discussing the key limitations of the study should be included in the Discussion.

Given these concerns, the authors should consider moderating some of their claims and conclusions.

Lastly, the manuscript would benefit from thorough language editing, with particular attention to revising the abstract for clarity and precision.

Version 1:

Reviewer comments:

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Reviewer #2

(Remarks to the Author)
Authros have addressed most of my concerns and I think the revised manuscript is a certainly improved version.

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

In this manuscript the authors attempted to answer the following biological question: Does sclerostin (SOST) inhibition have cardiovascular effects using genetic evidence. The authors first show that circulating SOST influence both eBMD and CVDs. Next, they integrate genetic, transcriptomic, and proteomic data to explore the causality of SOST in both eBMD and CVDs and therefore assess the cardiovascular burden of using SOST inhibition in the treatment of osteoporosis.

Minor comments
a) Typographical:
- line 33: transcriptomic
- line 67: humans
- line 88: remove to from regarding to

Response: Thank you. We have corrected these typos.

b) Study:
- line 32: heel bone mineral density from the UK biobank is estimated therefore should be referred to as eBMD rather than BMD, this issue arises in other parts of the manuscript where authors use eBMD and BMD interchangeably.

Response: Thank you for your suggestion. We have replaced eBMD with BMD throughout the main text.

- line 424: code availability? I recommend adding all code to Github and all data files to Zenodo or a similar platform

Response: We have uploaded all codes to Github (https://github.com/qianyu-west/sost).

Major comments
- eBMD GWAS: The summary statistics from the eBMD GWAS from Morris et al. 2018 is readily available, why did the authors rerun the GWAS study, while all the other CVD studies summary stats were used directly? Also, can you provide a complete comparison between your results and the Morris et al. results which uses 426,824 individuals. Additionally, theni reported 1103 GWS loci while it appears that this study reports nearly 30K loci. Could you please either report only lead SNP numbers or explain this discrepancy? How much of the variance did these loci explain compared to Morris et al. 20%?

Response: Sorry for the confusion, “the 29,060 genetic variants” we wrote before are not independent loci, after clumping, we identified 874 independently genome-wide significant genetic variants, which explained 16.6% variance of eBMD. The reason why we rerun the GWAS, because in Morris et al (2018), the regression model only controlled for age, sex, genotyping array, assessment center, and the first 20 ancestry-informative principal components. Several known eBMD-related covariates, such as body mass index (BMI), smoking, and alcohol, were not included in their model. To account for these potentially confounding factors and to improve the precision of the estimated genetic effects, we re-analyzed the eBMD GWAS using the same UK Biobank dataset but with additional abovementioned covariants. Therefore, the sample size in our analysis is smaller, and less independently loci were indentified. We directly compared the independently genetic variants from our results with the Morris et al 2018, in the 707 overlap SNPs, only 9 of them were not genome wide significant (*P* value > 5×10^{-8}) in Morris et al 2018, 7 of the 9 were still under suggestive significance (5×10^{-8} <P value < 5×10^{-6}).

For CVDs, the case numbers in the UK Biobank are relatively modest compared to those in large-scale published GWAS meta-analyses. Given that statistical power in GWAS is heavily influenced by sample size, we opted to use publicly available CVD summary statistics from well-powered, published GWAS meta-analyses to maximize detection power and reliability of associations.

We have added relevant descriptions on Page 13 Lines 272-274 in the revised manuscript.

- QTL Colocalization analysis: The current GTEx version is V10, with an increase of more than 200 donors used in eQTL analysis, could you please explain why use V7 rather than V8, or V10 was used? In addition, I understand the selection of the 6 cardiovascular related tissues, but are we considering whole blood a the only representative of "bone related gene expression"? if that's the case, then I suggest being more stringent with the H4PP as evidence of colocalization from 0.7 to at least 0.9. The same should happen with the pQTL colocalization analysis.

Response: Accordig to your suggestion, we have updated our results by using GTEx version 8. And we agree that we should consider the representative of "bone related gene expression". Due to the absence of direct osteoblast or trabecular bone tissue data in GTEx dataset, we analyzed expression quantitative trait loci (eQTL) data from three tissue types (Sun-exposed Lower Leg Skin, Tibial Nerve and Esophagus Mucosa) in the GTEx version 8 dataset, as the expression of these tissues is consistently associated with eBMD. We found that elevated *B4GALNT3* expression in these tissues was associated with reduced circulating SOST levels (Esophagus Mucosa: beta = -0.212, *P* = 2.75×10^{-6}; Nerve Tibial: beta = -0.198, *P* = 5.5×10^{-7}; Skin Sun Exposed Lower leg: beta = -0.398, *P* = 3.35×10^{-5}). Additionally, the increasing *B4GALNT3* expression in

these tissues was associated increased eBMD (Esophagus Mucosa: beta = 0.008, $P = 1.53\times10^{-5}$; Nerve Tibial: beta = 0.008, $P = 5.38\times10^{-6}$; Skin Sun Exposed Lower leg: beta = 0.016, $P = 1.00\times10^{-4}$) (Figure 4A), and was associated with increased risk of atrial fibrillation (Esophagus Mucosa: OR = 1.203, P = 0.018; Nerve Tibial: OR = 1.185, P = 0.019; Skin Sun Exposed Lower leg: OR = 1.418, P = 0.021). (Figure 4B). We also included pQTL data from whole blood tissue. In addition, we explicitly acknowledged the the absence of direct osteoblast or trabecular bone eQTL data in GTEx datasets in the limitations. We have updated the Methods, Results and Discussion accordingly (Page 16 Lines 344-352).

Additionally, we thank the reviewer for their valuable suggestion regarding colocalization stringency thresholds. Our analysis of shared genetic signals between circulating SOST and eBMD identified the *B4GALNT3* gene region as showing particularly strong evidence of colocalization, with a posterior probability of 0.987 for a shared causal variant - substantially exceeding the recommended 0.9 threshold. We have implemented a more stringent analytical approach throughout the study, now requiring all reported colocalizations to meet this higher confidence threshold (PP > 0.9). This change has been explicitly stated in Page 10 Lines 200-208 in the Methods section.

Reviewer #2 (Remarks to the Author):

The authors aim to shed light on the potential relationship between sclerostin and cardiovascular effects, presenting novel data derived from an interesting in silico approach. While the study offers potentially valuable insights, it lacks experimental data to substantiate the proposed conclusions. Furthermore, the limited evidence linking the proxy gene with SOST and the complexity of the analysis required to establish these relationships raise concerns regarding the robustness of the associations.

Key concerns include:

The absence of experimental validation. Limited evidence supporting the connection between the proxy gene and SOST. The somewhat intricate analytical framework required to support the proposed relationships, which may cast doubt on the validity of the findings.

Response: Thanks for pointing this out. In revision, we have performed addtional experiments in vitro to validate the relationship between *B4GALNT3* expression and SOST protein. We determined secreted SOST levels in cell culture medium following *B4GALNT3* knockdown and overexpression (Figure 3B-3H). Transfection of Saos-2 cells with three designed siRNAs targeting *B4GALNT3* identified two effective siRNAs that reduced B4GALNT3 expression at both mRNA and protein levels; these were selected for subsequent assays (Figure 3C-3F). Consistent with our MR findings,

B4GALNT3 knockdown significantly elevated SOST protein secretion from Saos-2 cells, as demonstrated by western blot analysis (Figure 3D and 3F). Furthermore, forced overexpression achieved through lenti-B4GALNT3 infection of Saos-2 cells revealed that B4GALNT3 overexpression significantly decreased SOST protein secretion into the culture medium (Figure 3G and 3H). The MR analyses with both transcriptomic data (eQTL) and proteomic data (pQTL) supported the relationship between SOST inhibition and AF, and the results were replicated in different MR approaches. We have upedated these results in Methods, Results, and Discussions (Pages 12-13 Lines 247-261, Page 16 Lines 330-340). And we added a new Figure 3 to visualize these results.

Specific issues:

Referring to papers published in 2021 as "very recent" (line 106) seems questionable.

Response: Thank you, we have removed these words.

It remains unclear whether these results are directly comparable to previous GWAS on bone mass conducted using the UK database. And what is the explanation for the differences.

Response: Thanks for this question, which is also asked by Reviewer #1. The reason why we rerun the GWAS, because in Morris et al (2018)., the regression model only controlled for age, sex, genotyping array, assessment center, and the first 20 ancestry-informative principal components. Several known eBMD-related covariates, such as body mass index (BMI), smoking, and alcohol, were not included in their model. To account for these potentially confounding factors and to improve the precision of the estimated genetic effects, we re-analyzed the eBMD GWAS using the same UK Biobank dataset but with additional abovementioned covariants. Therefore, the sample size in our analysis is smaller, and less independently loci were indentified. We identified 874 independently genome-wide significant genetic variants, which explained 16.6% variance of eBMD. We directly compared the independently genetic variants from our results with the Morris et al 2018, in the 707 overlap SNPs, only 9 of them were not genome wide significant (*P* value > 5×10^{-8}) in Morris et al 2018, 7 of the 9 were still under suggestive significance (5×10^{-8} <P value < 5×10^{-6}). We have added relevant descriptions on Page 13 Lines 272-274 in the revised manuscript.

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Response: We agree that, although other CVDs did not show significant results in our

MR analyses, we could not rule out their association with SOST inhibition, since the patients with atrial fibrillation were more likely to develop CAD, stroke, and heart failure. These null results may reflect limited power in stroke/CHD GWAS (explaining only 0.6-1.8% and 8.5% heritability, respectively) compared to AF (11.2%). This substantial difference in explained heritability suggests that the stroke/CHD GWAS cohorts used in our analysis may have been underpowered to detect modest effects. We have added relevant descriptions on Pages 22, Lines 474-477 in the revised manuscript.

A dedicated section discussing the key limitations of the study should be included in the Discussion.

Response: Thank you for your suggestion. We have acknowledged three main limiations in the Discussion section (Page 21-22 , Lines 471-481).

Given these concerns, the authors should consider moderating some of their claims and conclusions.

Response: Thank you for your suggestion. We have moderated the conclusion that "This multi-omics study identifies a potential association between reduced SOST and an increased risk of AF, which may warrant consideration in patients undergoing SOST inhibition treatment."

Lastly, the manuscript would benefit from thorough language editing, with particular attention to revising the abstract for clarity and precision.

Response: Thank you, we have revised the Abstract.

Thank you for the opportunity to revise our manuscript to *Communications Medicine* for consideration. For ease of reading, we have directly pasted the reviewers' concerns below, which are in Verdana font.

Our responses are in Times New Roman font, preceded by "Response:"

REVIEWER COMMENTS

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Reviewer #1 (Remarks to the Author):
Satisfied.

Response: Thanks a lot, we are pleased that the revised manuscript has addressed your concerns and improved as expected.

Reviewer #2 (Remarks to the Author):

Authors have addressed most of my concerns and I think the revised manuscript is a certainly improved version

Response: We sincerely appreciate your thoughtful review and are glad to hear that the revised manuscript has addressed most of your concerns and shows improvement. Thank you for your valuable suggestions.

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