nature portfolio

Peer Review File

Sex-specific transcriptome similarity networks elucidate comorbidity relationships

Corresponding Author: Dr Jon Sanchez-Valle

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)
1. Brief Summary of the Manuscript
This manuscript investigates sex differences in transcriptomic profiles across a wide range of diseases, focusing on their implications for comorbidity and potential sex-specific drug associations. The authors construct transcriptomic similarity networks separately for women and men. They demonstrate sex-specific patterns that are driven by different molecular mechanisms in men and women, particularly in the domains of immune response, metabolism, mitochondrial processes, and extracellular matrix signaling. Finally, the study explores how drug-disease associations may differ by sex and impact comorbidity patterns.

2. Overall Impression of the Work
This is a ambitious study that addresses a major gap in our understanding of sex differences in disease biology and comorbidity patterns. The use of large-scale transcriptomic data to build sex-specific disease similarity networks is valuable. The findings support the growing consensus that studying men and women separately is crucial for improving diagnostic accuracy and treatment strategies. The authors' integration of molecular data with epidemiological findings is particularly compelling. As a reviewer not specialized in computational biology or transcriptomic network construction, I found the overall concept and rationale clear. However, the manuscript would benefit from a more structured and concise presentation of the results.

3. Specific Comments and Recommendations
The current Results section includes rich information but often interleaves interpretation with results reporting. I recommend separating these more clearly or using structured subsections or tables to organize findings according to the three main analytical steps: transcriptomic-level disease similarities, biological pathways involved, sex-specific drug-disease associations. A tabular or matrix-based format summarizing the key sex-specific differences across diseases in each of these dimensions would enhance clarity and accessibility.

Moreover, while many examples are given, the manuscript would benefit from quantifying the strength of key associations. If available, reporting metrics such as effect sizes could add another analytical layer beyond the descriptive narrative and help prioritize findings.

The exploration of drug effects by sex is a strength of the paper. However, a stronger link between the drug-association findings and clinical implications (e.g. sex-specific side effects or repositioning opportunities) would strengthen the impact of this section.

Reviewer #2

(Remarks to the Author)
The research presented in the manuscript is innovative and well-structured, with a well-designed experiment that appears carefully implemented. The supplementary notes effectively elaborate on the findings, and the additional supplementary materials provide strong support for the hypotheses and reveal novel insights. However, several issues related to language, formatting, and clarity need to be addressed to enhance the manuscript's readability and impact.

Specific Feedback
1. Language and Grammar: The manuscript's English writing requires some improvement. Errors in sentence structure, word choice, and grammar hinder clarity and make it difficult to fully grasp the intended message. I recommend thorough editing, potentially by a professional editor, to refine the language and ensure the ideas are communicated effectively.
2. Figure 2: While Figure 2 is comprehensive and informative, its font size and resolution are suboptimal, reducing readability. Increasing the font size and improving the resolution would enhance its effectiveness.
3. Font Consistency: The text exhibits inconsistent font usage, particularly between Line 152–167 and Line 299–491. Standardizing the font throughout the manuscript would improve its professional presentation.
4. Figure Legends: The figure legends lack sufficient detail to stand alone. For example, in Figure 4, the color bars on the left are unclear without referencing the associated text. Legends should be self-contained, providing all necessary information to interpret the figures independently.
5. Dataset Specification: The manuscript would benefit from explicitly stating the datasets used in the study. Including this information in the methods section would improve transparency and reproducibility.
6. Tools and Packages: The authors should specify the tools and software packages used for statistical analysis, network construction, and figure generation. This addition would enhance the study's reproducibility and provide clarity on the analytical methods.
Conclusion
Overall, the manuscript presents a compelling and innovative study with well-designed experiments and informative figures. The supplementary materials are particularly strong, offering valuable context and support for the findings. Addressing the language issues, improving figure readability, standardizing fonts, enhancing figure legends, and specifying datasets and tools will significantly strengthen the manuscript. With these revisions, the research's impact and clarity will be greatly improved.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors addressed all raised concerns. I have no further comments.

Reviewer #2

(Remarks to the Author)
The authors have addressed all of my concerns. Overall, I find this study innovative, solid, and well organized.

Reviewer #1 (Remarks to the Author):

1. Brief Summary of the Manuscript

This manuscript investigates sex differences in transcriptomic profiles across a wide range of diseases, focusing on their implications for comorbidity and potential sex-specific drug associations. The authors construct transcriptomic similarity networks separately for women and men. They demonstrate sex-specific patterns that are driven by different molecular mechanisms in men and women, particularly in the domains of immune response, metabolism, mitochondrial processes, and extracellular matrix signaling. Finally, the study explores how drug-disease associations may differ by sex and impact comorbidity patterns.

2. Overall Impression of the Work

This is a ambitious study that addresses a major gap in our understanding of sex differences in disease biology and comorbidity patterns. The use of large-scale transcriptomic data to build sex-specific disease similarity networks is valuable. The findings support the growing consensus that studying men and women separately is crucial for improving diagnostic accuracy and treatment strategies. The authors' integration of molecular data with epidemiological findings is particularly compelling. As a reviewer not specialized in computational biology or transcriptomic network construction, I found the overall concept and rationale clear. However, the manuscript would benefit from a more structured and concise presentation of the results.

3. Specific Comments and Recommendations

The current Results section includes rich information but often interleaves interpretation with results reporting. I recommend separating these more clearly or using structured subsections or tables to organize findings according to the three main analytical steps: transcriptomic-level disease similarities, biological pathways involved, sex-specific drug-disease associations. A tabular or matrix-based format summarizing the key sex-specific differences across diseases in each of these dimensions would enhance clarity and accessibility.

We thank the reviewer for this constructive suggestion to improve the readability of the Results section. In response, we have revised the text throughout this section (lines 291–506) to clearly separate the reporting of findings from their interpretation. Specifically, we now introduce the main results first, followed by their corresponding interpretations or supporting references. We opted not to include summary tables within the main text, as these would be excessively large. Instead, we have provided them as Supplementary Data 2–4.

Moreover, while many examples are given, the manuscript would benefit from quantifying the strength of key associations. If available, reporting metrics such as effect sizes could add another analytical layer beyond the descriptive narrative and help prioritize findings.

We thank the reviewer for this helpful comment. In the revised manuscript, we have included three new tables (Supplementary Data 2–4) to present the results more clearly and quantify the associations.

- Supplementary Data 2 lists the Reactome pathways that are significantly enriched in pairs of diseases that co-occur significantly only in women or only in men. The table includes pathways that are significantly altered in the same direction in both diseases within the corresponding sex, along with the Normalized Enrichment Score (NES) for each disease.
- Supplementary Data 3 summarizes, for disease pairs that co-occur more frequently than expected by chance in both sexes, the pathways that are significantly altered in the same direction in both diseases in only one of the sexes, together with their respective NES values.
- Supplementary Data 4 presents all drugs indicated for one disease that are enriched in another disease, specifying the sex in which this relationship is observed, as well as the corresponding NES.

We believe that this information makes the paper stronger and provides evidence fo the significant to the data reported. These tables complement the one added to the main document, where only those associations cited in the results are highlighted in order to clarify the previous point.

The exploration of drug effects by sex is a strength of the paper. However, a stronger link between the drug-association findings and clinical implications (e.g. sex-specific side effects or repositioning opportunities) would strengthen the impact of this section.

We thank the reviewer for their comment. It is indeed essential to include results and discuss the relevance of discoveries in drug repositioning. Therefore, we have added a fourth scenario to the results section describing drug-driven disease associations in which the drug indicated to treat one disease

could be used to treat a disease in which it is significantly enriched, discussing the possible impact of patient sex on a different response to the candidate drug to be repurposed. The added text can be found between lines 4 and 8.

In the fourth scenario, patients with type 2 diabetes have been observed to be at greater risk of developing liver cancer, with the risk being higher in men 88. One hypothesis to explain this sex difference involves higher circulating levels of adiponectin in women 89. Notably, both diseases are linked through metformin, which is indicated for the treatment of type 2 diabetes and whose targets are significantly up-regulated in liver cancer. Interestingly, metformin has been described as exerting a protective effect against the development of liver cancer, with this effect being stronger in men 90–92. A possible explanation for the sex difference in metformin's protective effect could lie in hormonal context: higher baseline adiponectin levels in women may already confer protection, whereas in men, metformin partly compensates for their lower adiponectin levels and higher baseline risk. These observations underscore the importance of considering hormonal context when evaluating potential drug repositioning strategies.

Reviewer #2 (Remarks to the Author):

The research presented in the manuscript is innovative and well-structured, with a well-designed experiment that appears carefully implemented. The supplementary notes effectively elaborate on the findings, and the additional supplementary materials provide strong support for the hypotheses and reveal novel insights. However, several issues related to language, formatting, and clarity need to be addressed to enhance the manuscript's readability and impact.

Specific Feedback

1. and Grammar: The manuscript's English writing requires some improvement. Errors in sentence structure, word choice, and grammar hinder clarity and make it difficult to fully grasp the intended message. I recommend thorough editing, potentially by a professional editor, to refine the and ensure the ideas are communicated effectively.

We thank the reviewer for this comment. We have thoroughly reviewed the text to improve the grammar and style.

2. Figure 2: While Figure 2 is comprehensive and informative, its font size and resolution are suboptimal, reducing readability. Increasing the font size and improving the resolution would enhance its effectiveness.

We thank the reviewer for their comment. As suggested, we have increased the text size by making the figure longer on the vertical axis. We have also ensured that all figures are of high quality, which could be reduced when integrated into the Word document (but which is preserved in the attached files).

3. Font Consistency: The text exhibits inconsistent font usage, particularly between Line 152–167 and Line 299–491. Standardizing the font throughout the manuscript would improve its professional presentation.

We thank the reviewer for their comment. we have now corrected this issue and standardize the font throughout the manuscript.

4. Figure Legends: The figure legends lack sufficient detail to stand alone. For example, in Figure 4, the color bars on the left are unclear without referencing the associated text. Legends should be self-contained, providing all necessary information to interpret the figures independently.

We thank the reviewer for their comment. In this new version, we have included a legend indicating the meaning of the colours of the side bars (the category of diseases to which they belong).

5. Dataset Specification: The manuscript would benefit from explicitly stating the datasets used in the study. Including this information in the methods section would improve transparency and reproducibility.

We thank the reviewer for their comment. Information on the datasets used in the study was available at the GitHub link provided in the Data availability section. To facilitate access and reading, this table long has now been added to the supplementary material as Supplementary Table 1.

6. Tools and Packages: The authors should specify the tools and software packages used for statistical analysis, network construction, and figure generation. This addition would enhance the study's reproducibility and provide clarity on the analytical methods.

We thank the reviewer for their comments. We have now added to the text the tools and software packages used for statistical analysis, network construction, and figure generation. Specifically, in those figures showing networks, we have indicated the software used to draw them. Furthermore, with regard to statistical methods and visualisations, we have indicated in the methodology section that all the packages (R scripts) used in the work can be found on the Github page, as usually done in the field, not to collapse the text with the specific package names. The specific text added to the methods section is as follows:

All R packages used for analysis and visualization are listed at https://github.com/jonsv89/SHDC.

Conclusion

Overall, the manuscript presents a compelling and innovative study with well-designed experiments and informative figures. The supplementary materials are particularly strong, offering valuable context and support for the findings. Addressing the issues, improving figure readability, standardizing fonts, enhancing figure legends, and specifying datasets and tools will significantly strengthen the manuscript. With these revisions, the research's impact and clarity will be greatly improved.

We thank the reviewer for this comment and hope to have satisfied adequatly all the comments and suggestions.