Peer Review File

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Reviewer A

Comment #1: Summarizing the data related to NAC in non-APAP ALF was a herculean task. And this is quite clearly laid out in the manuscript. I still don't see the point of the PheWAS as it relates to NAC. Do those identified with the variant have increased susceptibility to liver injury or increased benefit from NAC for some reason. None of this seems to be clear as you move right on to do the literature review. To me, these are apples and oranges. Happy to look at a revised version.

Reply #1: We have edited the rationale in the Introduction regarding our selection of this methodologic approach. In the Methods and Results, we have also included further information regarding the variant as well as inferences regarding its effects and the cluster of liver-related phenotypes that we identified. We have further elucidated our thought process and evolution of thinking as we explored the literature, particularly in the Results section in our review of glutathione's involvement in liver injury.

Reviewer B

The authors describe the results of a phenome-wide association study (PheWAS) on glutathione synthetase (GSS) variant R418Q suggesting that N-acetylcysteine (NAC) could be repurposed for the management of non-acetaminophen-induced acute liver failure.

Comment #1- The rationale behind choosing the GSS R418Q (p.Arg418Gln) variant rather than other common GSS variants in particular is not clear in the study.

Reply #1 – We have added our rationale for selection of this variant to the Methods section, clarifying that this SNP was the only GSS variant available in our existing Exomechip dataset.

Comment #2 - It is not clear how some of the phenotypes studied (liver abscess, Vitamin B-complex deficiencies) link to the indications for treatment.

Reply # 2 – we have added further narrative to clarify that we viewed the set of liver related phenotypic manifestations as a cluster of diseases, conditions, and findings concordant with hepatic dysfunction, as well as the way in which we oriented on these effects to determine the directionality of the SNP's *in vivo* effects in relationship to NAC.

Comment #3- The phenotype "Abnormal results of function study of liver" in table 1 is not clear. Does this indicate biochemical abnormalities such as transaminitis, or synthetic functional abnormalities?.

Reply #3 – We have clarified in the Results text that this phenotype is most

commonly found in the medical record when laboratory testing indicates elevated liver enzymes.

Comment #4- The authors note in table 1 the phenotype "Esophageal bleeding (varices/hemorrhage)". This is a complication of chronic liver disease, cirrhosis and portal hypertension, which are listed as separate phenotypes. It is not clear whether the cases of esophageal bleeding are included as well under the other phenotypes or listed as separate phenotypic entity.
Reply #4 – We have added text in the Results describing our inference that the combination of liver related phenotypes we found indicates the presence of both acute and chronic liver failure in the underlying dataset.

Reviewer C

This is an interesting report informing on the potential therapeutic effects, especially its repurposing, of N-acetylcysteine against non-acetaminophen induced acute liver failure. The study is well written and informs on evidence scan from a global health perspective, especially considering that NAC is considered safe and part of The World Health Organization's Essential Medicines List. Only a few/minor comments to further enhance the quality of the manuscript.

Specific comments:
Comment #1 - A schematic diagram to enrich the report and also attract readership is necessary to be added.
Reply #1 – We have added a schematic overview at the beginning of the Methods section, and have aligned the titling of subsections with this overview to ease reader understanding and interpretation of our approach and findings. We have also added a new figure to accompany this new text.

Comment #2 - Considerations of a broad spectrum of literature/a clear indication of the progressive analysis of literature that has been published over the years is necessary
Reply #2 – We have further elucidated our general sense of findings at the beginning of the NAC in ALF subsection of the results, including temporal trends in this literature.

Comment #3 - What are the required steps that are needed for the potential use of NAC for acute liver failure, this should be clearly stated/articulated….
Reply #3 – We have retitled the section summarizing dosing and administration approach used for NAC in the literature we identified, and we have also added further discussion of the need for future research, the limitations of the identified evidence, and the role that clinician judgment plays in determining whether NAC may be a reasonable option for a given patient.

Comment #4 - What are the currently limitations with the use of NAC for acute 1

liver failure.

A progressive buildup of literature is necessary, especially citing recent reviews that have been conducted on the potential use of NAC for liver diseases is necessary:

https://pubmed.ncbi.nlm.nih.gov/34221501/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8668310/

https://pubmed.ncbi.nlm.nih.gov/35063860/

Reply #4 – In the process of revising the manuscript, we have reviewed the more recent literature and included substantive updates inclusive of the most relevant literature. We have also noted the trend toward increasing publication on this topic. We also appreciate the reference suggestions and have worked these in, along with relevant thoughts, to various places throughout the manuscript.