CIC bioGUNE
CENTER FOR COOPERATIVE RESEARCH IN BIOSCIENCES
CIC biomaGUNE
CENTER FOR COOPERATIVE RESEARCH IN BIOMATERIALS

José M. Mato, Ph.D.
Director General de CIC bioGUNE
Director General de CIC biomaGUNE
Profesor de Investigación del Consejo
Superior de Investigaciones Científicas (CSIC)

May 3, 2019

Dear Dr. Lian-Sheng Ma,

Please, find enclosed the revised version of the review manuscript, with number ID 03387815 and authored by Mato JM, Alonso CA, Noureddin M, and Lu SC, entitled “Biomarkers and subtypes of non-alcoholic fatty liver disease”, we have written in response to your kind invitation to Dr. Shelly C Lu for its publication in the *World J Gastroenterol*. In the revised manuscript we have addressed all the questions and comments raised by the reviewers. Changes in the manuscript are in red.

Below is our answers to the reviewers.

Reviewer #1

1. The background of MAT1A-based phenotype and intricate details of associated changes in lipid metabolism are perhaps overwhelming in a primarily translational paper. Most these issues have been laid out already in the original Gastro publication and this review could streamline this part and refer to those earlier discussions.

Response: Although the background of MAT1A-based phenotype and the details of its connection with lipid metabolism may seem intricate, we think it would be important for the general reader of *World J Gastroenterol* to understand why SAMe is one of the key molecules that power cell metabolism. In this respect, we have included a new reference in page 11 of the revised manuscript (Walsh et al. *Chem Rev* 2018; 118:1460-1469) that may facilitate readers to understand better the function of SAMe in lipid metabolism.

2. The original paper used an 'indeterminate' M subtype, which is missing in the current discussion. Since it was not negligible (19%), it would be reasonable to discuss this issue here, unless interim advances better clarified the status of these patients.
3. It is somewhat disappointing that the M phenotype is equally distributed among patients with steatosis and NASH according to the original paper, indicating that it may have little or no impact on the natural history of NAFLD. Of course this would not take away the importance of administering drugs that appropriately exploit the underlying metabolic deficiency, but the fact that this particular constellation has little to do with progression should be more clearly pointed out.
4. Authors showed in the earlier report that the non-M subtype in humans differs in several ways such as age, ALT, and 1-carbon metabolism, and one wonders if there are additional clinical/laboratory parameters that may help distinguish and explain the impact of this subtype in human NAFLD. Authors could also address the problematics of lean NAFLD in this context.

Response: As suggested by the reviewer in remarks 2-4, we have improved the discussion section in the revised version of the manuscript, addressing the relevance and/or limitations of biomarkers related to the NAFLD subtypes and disease heterogeneity. As the reviewer

CIC bioGUNE
Parque Científico y Tecnológico de Bizkaia, Edificio 801-A
48160 Derio (Bizkaia)
Tel.: +34 944 061 300 · Fax: +34 944 061 301
Email: info@cicbiogune.es · www.cicbiogune.es

José M. Mato, Ph.D.
Director General de CIC bioGUNE
Director General de CIC biomaGUNE
Profesor de Investigación del Consejo
Superior de Investigaciones Científicas (CSIC)

pointed out, classification based on this approach is not indicative of disease progression. In our opinion, the lack of classification of all the patients, previously named as indeterminate, can be inherently linked to the unsupervised classification methodology and validation procedure. Therefore, we agree that the classification can be improved by the inclusion of additional clinical parameters, multiomics data, or further characterization of the patients in longitudinal studies in order to confirm the impact on the NAFLD history of NAFLD.

5. There are many different metabolic functions in NAFLD based on which we hope to find subtypes for targeted prognosis and therapy. A review on biomarkers and subtypes could presumably embrace these efforts. Deficiency in 1-carbon metabolism is probably just one of these phenotypic differences and - as mentioned above - regrettably it may not distinguish less from more advanced forms (i.e., steatosis vs. NASH) very well. It may be therefore appropriate to consider a more specific title for this review manuscript, just to reflect that it will not discuss any other efforts in this area (.e.g., Biomarkers and subtypes of deranged lipid metabolism in NAFLD' or 'Biomarkers and subtypes of NAFLD based on hepatocellular one-carbon metabolism' etc.)

Response: Following the reviewer's suggestion, we have changed the title of the manuscript to "*Biomarkers and subtypes of deranged lipid metabolism in NAFLD*".

6. It may be counterintuitive to discount the importance on de novo lipid synthesis in NAFLD as authors do here, since increased DNL rates may just as well overwhelm a deficient VLDL exporting system as it is presumably caused by increased hepatocellular lipid uptake. In this regard, authors may consider mentioning Vidal- Puig's lipoexpediency concept. Also, the pathophysiologic importance of increased vs. deficient FA oxidation as mentioned on page 8 may need a bit more clarification for the average reader.

Response: As suggested by the referee, we indicate in the revised version of the manuscript that the importance of DNL in NASH development should not be minimized, since increased DNL may just overwhelm a deficient VLDL-TG exporting system which, presumably, is already saturated due to increased hepatocellular lipid uptake. We have also included the concept of lipoexpediency and mentioned the work of Virtue and Vidal-Puig (2010) and Lodhi et al. (2011). The pathophysiologic importance of increased FA vs. deficient FA oxidation has been clarified and the publication of Chakravarthy et al, showing that fatty acid synthase activation increases the synthesis of PC(16:0/18:1) which in its turn accelerates FA oxidation via activation of PPARα, is also included.

Reviewer #2

1. Please improve the aim of this study in the abstract and in the introduction section to help better readers understanding.
Response: We have done as suggested.

2. Please add the following recent and interesting references to improve the NONALCOHOLIC FATTY LIVER DISEASE paragraph: Fatty liver disease and lifestyle in youngsters. Diet, food intake Frequency, exercise, sleep shortage and fashion. Liver International. 2016 Mar;36(3):427-33. Early effects of high-fat diet, extra- virgin olive oil and vitamin D in a sedentary rat model of non- alcoholic fatty liver disease. Histology and Histopathology. 2018, 33(11), 1201-1213 Echocardiography and NAFLD (non-alcoholic fatty liver disease). Int J Cardiol. 2016 Oct 15;221:275-9.

José M. Mato, Ph.D.
Director General de CIC bioGUNE
Director General de CIC biomaGUNE
Profesor de Investigación del Consejo
Superior de Investigaciones Científicas (CSIC)

Response: As suggested by the reviewer, we have included the reference by Trovato et al. in *Liver Int.* related to NAFLD and life style in youngsters. The reference by Trovato et al. on the effect of a high fat diet, extra-virgin oil and vitamin D in rats was however not added since, although interesting, the NAFLD paragraph in the manuscript refers only to human NAFLD. The work by Trovato et al. in the *Int. J. Cardiol* was also not included as echocardiography and NAFLD was not the subject of this review.

3. Please strengthen and improve the conclusion, adding the clinical relevance of your work and some important suggestions for the scientific community. Please refresh and update the reference list section.

Response: As suggested by the reviewer, we have improved the introduction and conclusions, and included new references.

Reviewer #3

It is an interesting review regarding the possible role of lipid metabolism and lipidomic signatures allowing identifying different subtypes of NAFLD. Lipidomic may be helpful to identify severity, risk of progression and possible response to treatment. I suggest the authors down tone the isolated importance of these lipidomic signatures. Based on currently knowledge, although there are recent advances in the field of genomics, transcriptomics, proteomics, and metabolomics that may contribute to the diagnosis and risk prediction of NAFLD progression and response to therapy. However, there are still no uniform metabolites which could be used as the diagnostic markers of NAFLD. Some studies showed that metabolomic patterns are different in patients with NAFLD, compared to healthy controls. (Gitto, S et al; Metabolites 2018, 8, 17) However, the discrimination between NAFL and NASH remains a true challenge. (Carulli L; Metabolites 2019, 9, 25; doi: 10.3390/metabo9020025) Further, data derived from single-omics analysis are not enough to explain the complexity of liver diseases. Integration of multiomics data with biological network models may allow advances in our understanding of the complex biochemical processes and pathophysiological responses in liver diseases. (Mardinoglu A et al; Nat. Rev. Gastroenterol. Hepatol. 2016, 13, 439–440; Mardinoglu A et al Nat. Rev. Gastroenterol. Hepatol. 2018, 15, 365–377) Moreover, it is also important to integrate gene products, mRNA, proteins, and metabolites, as well as their molecular interactions with the environmental factors (such as diet) (Maldonado EM et al; NPJ Syst. Biol. Appl. 2018, 4, 33 ; Mardinoglu A et al . Cell Metab. 2018, 27, 559 –571.e5).

Response: As suggested by the reviewer, we mention in the manuscript that numerous studies have been published aiming to the identification of circulating biomarkers, using genomics, transcriptomics, proteomics, and metabolomics, for the diagnosis of steatosis, NASH and fibrosis, as well as for the risk prediction of NAFLD progression and response to therapy. Two recent reviews in this subject, Pirola and Sookian *World J Gastroenterol* 2018; and Iruarrizaga-Lejarreta et al. Ref. 44 of the original manuscript, are included.
As suggested by the reviewer, we also indicate in the revised version of the manuscript that some studies showed that metabolomic patterns are different in normal liver and NAFLD (Barr et al. *J Prot Res* 2012, and Gitto et al. *Metabolites* 2018); and indicate that the discrimination between simple steatosis and NASH is challenging (Mayo et al. *Hepatol Commun* 2018, and Caussy et al. *Gut* 2018). The paper suggested by the reviewer by Carilli et al. published in 2019 in *Metabolites* is not mentioned as was recently retracted.
Following the reviewer's suggestion, we now refer in the Conclusion of the revised version of the manuscript to the integration of multiomics data with biological network models to obtain

CIC bioGUNE
CENTER FOR COOPERATIVE RESEARCH IN BIOSCIENCES
CIC biomaGUNE
CENTER FOR COOPERATIVE RESEARCH IN BIOMATERIALS

José M. Mato, Ph.D.
Director General de CIC bioGUNE
Director General de CIC biomaGUNE
Profesor de Investigación del Consejo
Superior de Investigaciones Científicas (CSIC)

a comprehensive landscape of the main NASH drivers and the 2 references of Mardinoglu et al. included. We also refer to the importance to integrate multiomics with environmental factors.

Figures 1 and 3 have been slightly modified in the revised version.

We hope the editor and reviewers will be satisfied with the revised version.

Sincerely,

José M. Mato