Baishideng Publishing Group

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-223-8242
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

PEER-REVIEW REPORT

Reviewer's code: 03257773

SPECIFIC COMMENTS TO AUTHORS

1. **The modulator and non-modulator groups are genotypically different. The modulator group are all homozygous for F508del. In the non-modulator group, 9 out of 11 are heterozygous for F508del, only one homozygous, and one has no F508del mutation at all. Hence the difference in genotypes between the two groups may confound the result, rather than modulator. I think this at least should be discussed as a possible limitation/confounder more than is done. The one patient with F508del/F508del with high fat-level cannot be a guarantist for this effect.**

RESPONSE: Discussion paragraph 4, sentence 3 was edited to read "Although the single F508del/F508del homozygous subject not on lumacaftor/ivacaftor had significant hepatic steatosis, a single observation cannot exclude a genotype effect." Discussion paragraph 5, a sentence was added "Moreover, we are unable to exclude the possibility that the F508del/F508del genotype, rather than CFTR modulator use, is associated with reduced hepatic steatosis."

2. **I think the conclusions on no association between CFRD and fatty liver is presented too firm. This finding may be a type 2 error result. The patients are heterogeneous with regards to age and genotype and the number is small. As development of CFRD is multifactorial (loss of beta cells and increased insulin resistance), a non-significant result (that even is borderline) does not rule out such an association.**

Baishideng Publishing Group

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-223-8242
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

RESPONSE: The following sentence was added to the end of paragraph 1 of the discussion: "Given the small number of subjects not receiving lumacaftor/ivacaftor, we cannot exclude a relationship between CFRD and hepatic steatosis CF." The following sentences were added to the end of paragraph 5. "This study does not eliminate a possible association between CFRD and hepatic steatosis in CF. Further research is needed to understand how hepatic steatosis influences insulin sensitivity and risk for progression to CFRD."

Baishideng Publishing Group

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-223-8242
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

PEER-REVIEW REPORT

Reviewer's code: 00631989

SPECIFIC COMMENTS TO AUTHORS

Item that need minor revisions.

1. **The abstract summarizes and reflects the work described in the manuscript. However, the study of the effect of Lumacaftor/Ivacftor therapy is not listed within the aims. I suggest to add it as an additional point. On the other hand, one aim reported in the abstract would be to "identify predictors of hepatic steatosis in cystic fibrosis" but in the text this concept is not well focused.**

RESPONSE: The reason that aim 2 was left vague is because it was not our original hypothesis that lumacaftor/ivacaftor would reduce hepatic fat. Given the single case report (Hayes) we did think it was important to examine this. Since the major finding relates to lumacaftor/ivacaftor use, I edited aim 2 to read: "Explore the impact of lumacaftor/ivacaftor therapy on hepatic steatosis in cystic fibrosis."

2. **If the Authors intend to highlight predictors of hepatic steatosis, this should be better described in Results and Discussion. Otherwise, the point should be eliminated from the abstract.**

RESPONSE: Aim 2 was edited as above to be more specific. I agree that we did not identify other predictors of hepatic steatosis in CF so this aim was misleading.

3. **The key words reflect the focus of the manuscript. However, I suggest to add a keyword about the therapy and, possibly, about its effect on hepatic steatosis.**

RESPONSE: "lumacaftor/ivacaftor" and "CFTR modulator" were added to the key words. I am unsure what keyword would capture the impact of modulators on hepatic

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-223-8242
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

steatosis. Hopefully by adding the key words above, we will capture searches interested in the impact of modulator therapy on hepatic steatosis.

4. The manuscript adequately describes the background, present status and significance of the study. However:

a. it does not mention the genetics of CF. I suggest to add a short paragraph about CF genetics, mutational search in the CFTR gene and its influence on personalized therapy of CF in Introduction and to quote the following papers: Quotation 1: Cystic fibrosis genetics: from molecular understanding to clinical application. Nat Rev Genet. 2015 Jan;16(1):45-56. doi: 10.1038/nrg3849 Quotation 2: Targeted sequencing reveals complex, phenotype-correlated genotypes in cystic fibrosis. BMC Med Genomics. 2018 Feb 13;11(Suppl 1):13. doi: 10.1186/s12920-018-0328-z Quotation 3: A New Targeted CFTR Mutation Panel Based on Next-Generation Sequencing Technology. Journal of Molecular Diagnostics. 2017 19(5), 788-800. doi: 10.1016/j.jmoldx.2017.06.002 Quotation 4: The impact on genetic testing of mutational patterns of CFTR gene in different clinical macrocategories of cystic fibrosis. Journal of Molecular Diagnostics 2016, 18(4):554-565, doi: 10.1016/j.jmoldx.2016.02.007.

RESPONSE: I added a paragraph in the introduction outlining functional mutations in CF and the types of modulators available. Many of the suggested references suggest a more sophisticated method for defining CFTR dysfunction aside from the tradition 5 or 6 class system. I felt that a discussion to this depth was beyond the scope of this manuscript, so limited the paragraph to the more traditional classification system.

b) The abbreviations have to be checked (also in the abstract). Some terms are not

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-223-8242
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

abbreviated the first time they appears and, on the contrary, terms already abbreviated are used in full.

RESPONSE: CF, CFRD, CFLD, CFTR, PDFF are now defined the first time they are used in both the abstract and the first time they are used in the manuscript. The only abbreviations defined and used in the core tip are CF and CFTR. Per recommendations, I left everything in the core tip defined in case this is the only section that a reader views. There were a few instances where the full term was used for a word that had already been abbreviated and defined. In these instances, I removed the full term and replaced it with the abbreviation.

c) The sentence which refers to hepatic steatosis as "… a benign finding" (Introduction, page 6) should be better explained.

RESPONSE: I added to this sentence to make the meaning more clear. "Historically, hepatic steatosis in CF patients was attributed to malnutrition and considered a benign finding that did not increase risk for hepatic cirrhosis.[14]" I hope that this addresses the reviewer's concern.

d) In Materials and Methods, Patients characteristics (page 7), the sentence "… with genetically confirmed cystic fibrosis …" is unclear. The Authors should affirm that the diagnosis of CF has been performed accordingly to guidelines and quote: Quotation 5: Farrell PM, White TB, Ren CL, Hempstead SE, Accurso F, Derichs N, et al. Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation. J Pediatr. 2017;181S:S4-S15 e1, doi: 10.1016/j.jpeds.2016.09.064.

RESPONSE: I added a sentence affirming that all patients were diagnosed with CF in accordance with the suggested guideline to paragraph 1 of materials and methods under patient characteristics. The sentence reads "All subjects were diagnosed with cystic

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-223-8242
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

fibrosis based on sweat chloride and genetic testing according to established guidelines." I appreciate the reference.

e) All the clinical and biochemical characteristics described in Table S2 and statistically significant should be described and commented, even in separate paragraphs. On the contrary, in Results the Authors included only two paragraphs about "Hepatic steatosis" and "Diabetes" almost neglecting the other statistically significant aspects.

RESPONSE: I expanded the results section to make note of all statistically significant results. Specifically, under *Hepatic Steatosis,* I now discuss PDFF, alk phos, alk phos SD, LDL, and HDL results. Under *CFTR modulator*, I now discuss PDFF, BMI, total bilirubin, and alkaline phosphatase. Under *Diabetes*, I now discuss PDFF, age, BMI, FEV1, A1C, alk phos. Since table S2 is now part of the main manuscript, I did not feel it was necessary to list all of these results. I list the most important results and refer to the tables for most of the others.

f) the sentence "… standard deviation …" (Discussion, page 10) is unclear; I think it is better to reformulate it.

RESPONSE: I re-worded this to read "CFTR modulator use was also associated with lower total bilirubin and a trend toward lower age-adjusted alkaline phosphatase levels (z-score)." I hope that this is clearer to the reader.

5) The Methods section should be expanded to include the description of the methods used for each variable/data reported in Results section and in Figure and Tables (also supplemental).

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-223-8242
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

a) For example, it is not sufficient to affirm that: " Pancreatic insufficiency was defined by a need for pancreatic enzyme replacement." (page 7). Was fecal elastase dosage performed? By which method?

RESPONSE: Unfortunately we did not perform fecal elastase testing for this study. Participants may have had fecal elastase testing done in the past; however many of the patients are in their 20s and 30s and that documentation is no longer readily available. I clarified this point by updating the paragraph. "Pancreatic insufficiency was defined by a clinical need for pancreatic enzyme replacement. No fecal elastase testing was performed as part of this study."

b) "Only subjects with two class 1-3 mutations were included in the final analysis.". (page 7) Which was the mutational search method used, allowing the genetic findings reported in Table S1?

RESPONSE: A sentence was added to the first paragraph of the materials and methods section stating that "CFTR variants were classified into classes 1-5 using the CFTR 2 database and existing guidelines for functional classification."

c) How was assessed the glucose dosage, glucose tolerance and diabetes?

RESPONSE: I added a more detailed description of the glucose tolerance test to the materials and methods section stating that "Glucose tolerance testing was performed according to standard guidelines. After an eight hour fast, subjects ingested 1.75g/kg (maximum 75g) of glucose dissolved in water. Plasma glucose was evaluated at baseline and 2 hours post glucose ingestion.[39]"

d) How was assessed the HbA1C, Alk Phos, Total bilirubin, AST, ALT, GGT, Triglyceride (so, all data reported in Table 2 and Table S2)? Indeed, the only method

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-223-8242
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

referred is the MRI proton density but also the other laboratory methods should be synthetically reported or, at very least, paper(s) for method(s) should be quoted.

RESPONSE: I added a paragraph describing the technique for blood collection and the analyzer used to perform chemistries. The procedures for these chemistries are standard, so I did not feel that I needed to list the specific chemistry technique for each lab.

8) The figures, diagrams and tables are sufficient, good quality and appropriately illustrative of the paper contents. They do not require labeling with arrows, asterisks, etc., nor better legends. However:

a) table S2 is a key table of the manuscript. I suggest to move it from supplementary to main body

RESPONSE: Table S2 was moved to the main body of the article and re-named Table 3.

b) also Table S1 (along with Table 1) should be cited in "Participant characteristics"

RESPONSE: A citation for Table 1 and Table S1 was added after sentence 2 under participant characteristics as suggested.

c) in table S1 the legacy name of "1898 1G>A" is "1898+1G>A", with "+" and the legacy name of "1717 1G>A" is "1717-1G>A" with "-".

RESPONSE: The legacy names for 1898+1G>A and 1717-1G>A were fixed to reflect the correct format in table S1.

10) The manuscript meets the requirements of use of SI units. However:

a) sometimes micromole is written as "umol" (with "u"). For example in Table 2 (legend) and Table S2 (footnote), but please check throughout the manuscript. The "u" should be changed to the greek letter "m" (micro)

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-223-8242
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

RESPONSE: The erroneous umol has been changed to μmol in all places in the document.

b) the name of each CFTR mutation should be reported in HGVS 19.01 nomenclature, both nucleotidic and proteic, as well as in legacy name. For example, for F508del as follows: c.1521_1523delCTT p.Phe508del (HGVS 19.01 name), F508del (legacy name). The HGVS name of each mutation can be easily found in the CFTR2 database and could be added to the Table S1 and within the text.

RESPONSE: The HGVS 19.01 name was added to the legend of the table to avoid the table becoming overly crowded. If the editor feels that it is imperative that the HGVS names be in the table, I will happily revise again. I also added the HGVS nomenclature to paragraph 5 of the introduction.

c) the term "mutation" is obsolete. It is generally recognized that it is better to use "pathogenic variant" (instead of "mutation") and "non-pathogenic variant" (instead of "polymorphism"); also "CF-causing variant" or "non CF-causing variant" may be used. If there is no indication about the ability of a variant to cause CF, just "variant" can be used.

RESPONSE: The term "mutation" was replaced with the term "CF-causing variant" or "pathogenic variant" in the following locations: (1) paragraph 1 under materials and methods; (2) first paragraph in results (2 instances); (3) table S1.

11) The manuscript cites appropriately the latest, important and authoritative references in the introduction and discussion sections. The Author do not over-, self-, omit or incorrectly cite. The 5 papers reported in point 4 could be usefully added to quotations in the Introduction.

RESPONSE: I utilized the paper by Ivanov et al. to further support the occurrence of CFRD

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-223-8242
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

in patients with class 1-3 variants.

14) The Authors uploaded the authorization to publish of their ethical committee. However, the expiration date for the study was 09/12/2017. I think the Authors should submit to the Journal an explanation for this.

RESPONSE: I will upload the most recent ongoing review approval from our local IRB.

Baishideng Publishing Group

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-223-8242
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

PEER-REVIEW REPORT

Reviewer's code: 00002080

SPECIFIC COMMENTS TO AUTHORS

1. **Unfortunately the study design is not adequate to address the aims of the study as there is no baseline MRI to demonstrate the level of hepatic steatosis prior to commencement of Orkambi. While MRI is not standard practice this is understandable, however prior liver ultrasound data, even with the limitations of ultrasound would be helpful to the reader in determining if participants had any evidence of hepatic statosis prior to commencing modulator therapy.**

RESPONSE: I agree with the reviewer that the data would be stronger if we had pre-treatment data regarding hepatic steatosis. Unfortunately, we do not have that data available for this cohort. I reviewed the patient records to evaluate whether any qualitative data on the presence of steatosis before starting Orkambi is available. Only 2/9 subjects had abdominal imaging before starting Orkambi which may have captured steatosis. None of these studies were MRI. Given the limited data of a qualitative nature, I do not feel it is helpful to add to the paper. While acknowledging this important limitation, I believe that this initial cross-sectional data is important to share with the scientific community as it highlights a previously undescribed impact of CFTR modulator therapy. In the discussion, we acknowledge that future longitudinal studies will be needed to determine whether CFTR modulator therapy, in fact, causes reduced hepatic steatosis.

2. **In addition this study also has many other study design issues which are very difficult to overcome but do bias the results including a very high proportion of males, (80%) those who are older have CFRD and coincidently are likely to take**

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-223-8242
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

Orkambi.

RESPONSE: I acknowledge the potential for confounding given the relatively small sample size in this initial cross-sectional study. As CFRD is more common with increasing age, this confounder is particularly difficult to overcome. We attempted to remove the confounding effect of CFTR modulator use by re-analyzing the CFRD and NGT groups after removing these subjects. Unfortunately, the sample size becomes quite small after removing these subjects. In follow-up, longitudinal studies we plan to include a larger number of patients to further investigate the impact of these variables.