Author's response to reviews

Title: Polymorphisms in the glutathione pathway modulate cystic fibrosis severity: a cross-sectional study

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Author's response to reviews: see over
Manuscript: Polymorphisms in the glutathione pathway modulate cystic fibrosis severity: a cross-sectional study

Authors: Fernando Augusto de Lima Marson, Carmen Sílvia Bertuzzo, Antônio Fernando Ribeiro and José Dirceu Ribeiro

The authors acknowledge the collaboration of the BMC group and the reviewers (Professor(s) Frauke Stank, Andreas Hector and Harriet Corvol), for their contribution with criticism, suggestions and corrections made on our paper entitled "Polymorphisms in the glutathione pathway modulate cystic fibrosis severity: a cross-sectional study".
Reviewer:

Frauke Stanke

Reviewer’s report:

Review for:

Revised version of MS: 1229360747911350; Manuscript Polymorphisms in the glutathione pathway modulate cystic fibrosis severity: a cross-sectional study. By Fernando AL Marson, Carmen S Bertuzzo, Antônio F Ribeiro and José D Ribeiro

The reviewer thanks the authors for the considerable effort that they have undertaken to improve their manuscript. However, a few points of criticism remain this might be due to the fact that neither the reviewer not the authors are native speakers and hence, information gets lost. Please clarify the following points, still open after last review:

A. for formal reasons:

Question to the authors, last version of this manuscript:

For clarification: The reviewer understands that this is a resubmission of the data previously submitted to The Journal in a manuscript entitled “Genetic interaction of GSH metabolic pathway genes in Cystic Fibrosis”. The authors have carefully explained how the previous manuscript differs from the present one. However, was the work formerly entitled “Genetic interaction of GSH metabolic pathway genes in Cystic Fibrosis” published. Based on the phrase. In the context of previously published article, we ..... it can be inferred that the genotyping data was evaluated twice and two manuscripts were derived. If so, is the work referenced in the present manuscript? — Answer: The authors thank by
the comments and the suggestions. The present article was send to the journal before the published article, then was not possible to cite it before, taking it into account, the new version of the manuscript show the reference about the published article as suggested by the reviewer. About this, the follow phrase was included: “For the same population, a first study taking into account the same polymorphisms and clinical variables was performed. The previous data analyzed the genetic interaction among GST and GCLC polymorphisms, CFTR mutations and clinical markers. The data showed an interaction of GSTM1 and GSTTI genes deletion, GSTP1*+313A>G, and CFTR mutations (p=0.008) and Bhalla clinical score by multifactor dimensionality reduction test. The Bhalla score is a computed tomography, which measures pulmonary involvement, therapeutic effects and selection of patients for transplantation, which detects anatomical changes of the lung parenchyma. The data published showed a first step to understand the complex mechanisms associated with the CF severity and modifier genes [55].”

B. for scientific reasons: On the topic of high prevalence of PS patients: apparently, reviewer and authors agree on the survivor bias in the patient sample see question and answer to.

1C. Please reconsider whether you want to keep this important conclusion hidden within the data (or maybe communicate this fact within the section that described the patient’s history). – Answer: This fact can be observed in the text: “An interesting aspect was the high frequency of PS patients. The presence of PS occurred at exactly 20% of the sample. However, there was no difference distribution between the groups of patients with CF taking into account CFTR mutations groups (p=0.621). Patients with two mutations identified in CFTR gene had 22.36% (19/85) of PS, values close to the other groups of patients [one identified mutation and no mutation identified with, respectively, 15.7% (8/51) and 20.5% (9/44)].” and in the conclusion we included
the phrase: We believe that both the prevalence as the comparison between patients with (PI) and without pancreatic insufficiency (PS) should be taken into account in future genetic studies.”

C. for scientific reasons: To detect the survivor bias reliably, the reviewer has proposed before to rank patients according to date of birth. If PS patients are more frequently among early-born individuals, this indicates a survivor effect in the population. Likewise, if patients with two unresolved CFTR mutations and/or patients with one resolved CFTR mutation and/or patients with two identified CFTR mutations cluster by birth cohort, this indicates a survivor effect in the population. The authors have answered that to understand the frequency for pancreatic sufficiency, having fixed the age of the patient as parameter was calculated, the age difference between the groups of patients with and without insufficiency (Table 13). In the case of groups with and no identified CFTR mutations, after exclusion of patients with pancreatic sufficiency no difference in age was observed. This is misleading the age of the patient was fixed. Was a patient born in 1970, recruited at age 18 in 1987 compared to a patient born in 1980, recruited at age 18 1989. If so, the parameter to be compared between the groups is the year of birth (1970, 1980, different) and not the age (18y, 18y, identical). In other words, if early-born patients are more likely to be PS than patients from later decades, the survivor effect is underlined. This also indicates that other risk alleles than CFTR PI mutations might be depleted from the population, and that in turn might lead weight to moderate P-values when comparing cases and controls for CF modifying genes. – Answer: The authors agree with the placement of the reviewer. This
study is transversal, in which patients were compared with different age, and possibly their age vary between the different groups analyzed considering the CFTR genotype and comorbidities. For the clinical variables associated with disease severity care was taken for the correct evaluation of medical records of patients, however, the effect of comorbidities and survival conditioned by the patient's genetic can hardly be controlled in the proposed study model, not being possible this approach in the present study. Thus, when we conducted analyzes for the genetic factor, we considered groups of mutations in the CFTR gene as a limiting factor, and in this context, the patients were grouped as proposed in article. It is clear that for analysis of gene modulation in CF, the group of patients with two mutations in the CFTR gene determined is the “main” group for association studies. However, considering the presence of IP as a marker of clinical severity of mutations associated with greater severity, better sample homogeneity can be achieved by reducing the population of patients with no or one mutation in the CFTR gene plus pancreatic insufficiency. Studies addressing the severity of CF and gene modulation have been performed by our group and always consider the duration of the disease in the patient, but the evaluation of survivor effect is complex. For future studies monitoring the patient should be assessed, including longitudinal clinical change. We apologize for the difficulty in answering the question in the previous version, however, the survival is really a limiting factor, and this factor is present in our study and other from literature.

Minor points:

1. The following names are misspelled in the manuscript: Karl Kunzelmann, Frauke Stanke. – Answer: We apologies about the missphelled in the manuscript (Karl Kunzelmann and Frauke Stanke). The names were corrected in new version.
2. Table 14: The designation of the patients subset in column 1 are missing; no mutation identified 44, One CFTR mutation identified 51, Two mutation identified 84, (this set: not accounting for PS status?), One CFTR mutation identified 35, Two mutation identified 43, (this set: PI only?) – Answer: The table 14 was corrected. The column one was provided.

3. Table 14: Heading says “Age’s” distribution and osteoporosis among CFTR mutation groups and the values range between 7 and 1274. These are not years, but? – Answer: The data were in months.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests: I declare that I have no competing interests

Reviewer: Harriet Corvol

Reviewer's report:

I thank the authors for their response and the modifications they have made in the revised version of their paper. It really improved the paper overall. As such, the paper is now acceptable as it is for publication and I do not have any further comment.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Reviewer:
Andreas Hector

Reviewer's report:
The authors have responded appropriately to the reviewer's points. This reviewer recommends to accept this paper.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: I declare that I have no competing interests