Author’s response to reviews

Title: NEXT-GENERATION SEQUENCING FOR IDENTIFYING A NOVEL/DE NOVO PATHOGENIC VARIANT IN A MEXICAN PATIENT WITH CYSTIC FIBROSIS

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Author’s response to reviews:

Matteo Pasini, Ph.D.
Editor in Chief
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Dear Pasini

Please find enclosed the revised version of our manuscript “Next-generation sequencing for identifying a novel/de novo pathogenic variant in a Mexican patient with cystic fibrosis”. It is important to mention that the pedigree was modified because more family agreed to participate in the study.

We appreciate the comments and suggestions as they improved the quality of the paper.

Sincerely,
Reviewer 1.
1) The use of the NGS is presented to have a key role in this case report, but any references are directly related to the use of NGS in Cystic Fibrosis molecular diagnosis, although there are several examples in the literature. It would serve to reinforce the role of NGS technologies in CF.
Answer: You are right, the suggested papers were included in the Discussion and conclusion section. We added the phrase “The high-throughput of NGS technologies enormously have enhanced the possibility of identifying rare CFTR variants and, consequently, to better clarify genetic heterogeneity of CF and we quote the suggested papers.

2) The use of NGS is very important, but equally important is the functional characterization of the new variants identified. Also, this concept, treated in part by the authors with the use of in silico predictions, should be treated also citing some examples from the literature.
Answer: The suggested papers were cited as you recommended. At the end of the paragraph 2, in the Discussion and conclusions section, we added the phrase ”although further functional characterization of the novel variant is necessary.

Reviewer 3.
3) The novel /de novo variant should be submitted to the CFTR1 database.
Answer: Thank you very much for your suggestion, the variant c.1762G>T has already been submitted of the CFTR1 database and is being revised.

4) Within the text, the Authors use both terms "mutation" and "variant", which is misleading. I suggest do not use "mutation", which is obsolete.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. I also evidence that, when the Authors refer to the novel / de novo variant without specifically referring to its pathogenicity, the general term of "variant" can be used.
Answer: You are right, these changes were made through the text.

5) Page 3, row 8. Please, delete ", with a frequency of 1/3000." and finish the sentence with "… Caucasians."

6) Page 3, row 10. At completion of the previous sentence, I suggest to change this sentence to "… among different populations, spanning from 1 / 900 to 1 / 25 000, or even very lower in Eastern populations."
Answer: The suggested changes were made.

7) Page 3, rows 15-16. Please, change the sentence "… is a challenge among countries with high genetic heterogeneity …" to "… is a challenge for the complex genotype - phenotype relationship in CF and its high genetic heterogeneity" and quote the following papers:
Answer: Background section, paragraph 2: the phrase was changed and the papers cited.

8) Page 3, row 19. Please, substitute "… almost 2,000 variants …" with "… over 2,000 variants …".
Answer: Background section, row 9: the suggested change was made.

9) Page 3, row 21. Substitute "… or…" with a comma. It seems appropriate that in this manuscript the CFTR variants are reported only in HGVS 15.11 nomenclature (I kindly ask the Authors to check throughout the text) and that they are reported, whenever possible, in both nucleotidic and protein name separated by a comma. For example: c.1521_1523delCTT, p.Phe508del. Alternatively, also the legacy name could be reported (in addition to HGVS name). Please standardize throughout the text.
Answer: We standardized the nomenclature throughout the text, according to Den Dunnen JT. et al. HGVS recommendation for the description of sequence variants: 2016 Update. Hum Mutat 2016, 37:564-569. doi: 10.1002/humu.22981 and https://cftr2.org/.

10) Page 4, rows 13-14. The Authors affirm the molecular analysis was done by "directed mutagenesis" and quote a very old paper (1991). The correct definition of that technique is "PCR-mediated site-directed mutagenesis" and not simply "directed mutagenesis" which usually indicate another kind of tool not related to mutational search. In addition, it appears singular that in a laboratory where a NGS facility is available, an NGS-based I level analysis by a panel of frequent variants was not initially performed. The Authors should better clarify this choice.
Answers:
a) The "directed mutagenesis" was exchange by "PCR-mediated site-directed mutagenesis" in molecular analysis section, rows 6-7.
b) The five variants more common in Mexican population is done according to the recommendations by other authors, where they suggest a first screening with the most common mutations depending on the population (Bergougnoux A, 2018). Additionally, we are following the European guidelines established for the molecular diagnosis of CF, since for Latin populations it does not exist. ( Dequeker E, et al. Best practice guidelines for molecular genetic diagnosis of cystic fibrosis and CFTR-related disorders – updated European recommendations. 2009. Eur J Hum Genet 17:51-65).

11) Page 4, rows 17-19. The sentence "… none of the previously variants were identified …" could be better reworded as "… none of the above-listed variants were identified".
Answer: The phrase was reworded in the Molecular analysis section, row 9

12) Page 4, row 37. Please, change "… trans variants …" to "… variants in trans …". As the father is negative and one of the variant is novel / de novo, there is no experimental simple way to demonstrate, at molecular level, that the two variants are in trans. The fact that the trans status is deduced, and the reasons of this deduction (for example clinical outcome), should be disclosed by the Authors.
Answer: Molecular analysis section, row 19:
a) The suggested change was made.
b) We deduced that variants are in trans because: 1) the patient has a recessive disease, like CF, and 2) because both mutations were screened in both parents, and the p.Trp1089* variant was found in the mother, but not the p.Glu588* variant.

13) Page 4, row 39. For "… W1089*)", which is the legacy name, see the note about nomenclature above.
14) Page 4, rows 48-50. I suggest to modify as follows: "… screened for both variants. The analysis revealed that the mother carried only the p.Trp1089* …", to reinforce the concept that both variants were searched in both parents and that the mother did not have the novel / de novo variant in cis with the other variant.
Answer: We included the word “only” to reinforce the concept in the Molecular analysis section, row 27.

15) Page 5, rows 17-21. I suggest to change the sentence "Since the emergence of NGS technologies …" to "The high-throughput of NGS technologies enormously enhanced the possibility of identifying rare CFTR mutations and, consequently, to better clarify genetic heterogeneity of CF."
Answer: Discussion and conclusions section, rows 8-12: You are right, the phrase was added for the better understanding.

16) Page 5, row 24-25. There is a formatting problem with "… in a Mexican …".
Answer: Discussion and conclusions section, row 16: we rephrased the sentence for better understanding.

17) Page 5, rows 35-40. As the genotype is made of two nonsense pathogenic variants, a comment about a future possibility of a personalized therapy based on the readthrough approach may be appropriate.
Answer: Discussion and conclusions section, row 30-32: we added the sentence “Since our patient is harboring two nonsense pathogenic variants, a future possibility of a personalized therapy based on the readthrough approach may be appropriate.”

18) Page 6, rows 7-12. An additional conclusion about the usefulness of high-throughput NGS approaches in the finding of very rare variants, such as those novel / de novo, appears to be suitable.
Answer: You are right, the phrase “including the identification of rare or exclusive variants of the populations” was added to reinforce the utility of the NGS in the last paragraph.