Reviewer’s report

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

Version: 0 Date: 19 Feb 2019

Reviewer: Marco Lucarelli

Reviewer's report:

The manuscript points to the discovery of a novel / de novo variant of the CFTR gene, causing Cystic Fibrosis. The variant was discovered by a next generation sequencing approach. The proband had positive sweat test, clinical symptoms and respiratory tract infection. Paternity test was performed to clarify that the absence of the novel / de novo variant in the father and its presence in the proband is due to a de novo event. A theoretical analysis determined the onset of a stop codon. The Authors claim the novel / de novo variant as a CF-causing variant of the CFTR gene.

A very limited number of de novo CF-causing variants of CFTR gene have been found so far. This makes every novel finding interesting. The methods are appropriate and the conclusions drawn are correct. Although the Authors did not perform any experimental functional characterization of the novel /de novo variant, the expected onset of a stop codon, as deduced by a theoretical analysis, seems to be enough as a first step of characterization. Furthermore, the approach is comparable with that is usually done in similar papers dealing with predicted stop codons. The mutated genotype found well correlates with sweat test values and clinical findings.

I think this manuscript is suitable for the publication in BMC Medical Genomics as Case Report. The novel /de novo variant should be submitted to the CFTR1 database:
http://genet.sickkids.on.ca/Home.html

MINOR POINTS.

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 I suggest do not use "mutation", which is obsolete.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.

Background

Page 3, row 8. Please, delete ", with a frequency of 1/3000." and finish the sentence with "… Caucasians."
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." and quote this papers:


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:

1) Cystic fibrosis genetics: from molecular understanding to clinical application. Nat Rev Genet. 2015, 16(1):45-56. doi: 10.1038/nrg3849;

2) Genotype-phenotype correlation and functional studies in patients with cystic fibrosis bearing CFTR complex alleles Journal of Medical Genetics 54(4), 224-235. doi: 10.1136/jmedgenet-2016-103985;

then continue with "…such as the Latino …".

Page 3, row 19. Please, substitute "… almost 2,000 variants …" with "… over 2,000 variants …".

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.

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 …", although the list is very limited (5 mutations).

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.

Page 4, row 39. For "… W1089*)", which is the legacy name, see the note about nomenclature above.

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.

Discussion and conclusions

Page 5, rows 15-18. These kind of CFTR mutations can be easily found also by traditional mutational approaches such as, for example, classic Sanger sequencing. The Authors should better clarify what they intend with the sentence "… possibly we were not previously able to detect these variants using traditional technologies.". I suggest to clarify that the great advantage of NGS approach is the high-throughput and, consequently, the possibility to find also rare variants. For example, changing the following sentence as reported below.

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.", quoting:
then continue with: "By these approaches, also the possibility of identifying novel / de novo variants has dramatically increased".

Page 5, row 24-25. There is a formatting problem with "… in a Mexican …".

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. Suitable papers to quote may be:
1) Tobramycin is a suppressor of premature termination codons, J Cystic Fibrosis 2013, 12:806-811, doi: 10.1016/j.jcf.2013.02.007;

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.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.
Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.
Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.
Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.
Not relevant to this manuscript

**Quality of written English**
Please indicate the quality of language in the manuscript:
Needs some language corrections before being published

**Declaration of competing interests**
Please complete a declaration of competing interests, considering the following questions:
1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?
2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?
3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?
4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?
5. Do you have any other financial competing interests?
6. Do you have any non-financial competing interests in relation to this paper?
If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.
I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.
I agree to the open peer review policy of the journal.