Reviewer’s report

Title: ACE gene D/I polymorphism as a modulator of severity of cystic fibrosis

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Reviewer: Frauke Stanke

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Marson and colleagues report on their findings on the ACE gene D/I polymorphism which they have analysed in a group of cystic fibrosis patients. The preliminary manuscript submitted by the authors is encouraging as it is likely to contain a valid association. However, the known major confounder, i.e. the role of the CFTR mutation genotype, has not been addressed adequately in the manuscript in its present form. The manuscript will benefit if the authors recalculate their data, taking care not to pool severe and mild CFTR mutation genotypes. Moreover, the present narrative is rather cumbersome to read and might only serve as a valuable template for a revised manuscript, devoid of grammatical and typographical errors, that presents the main findings and guides the reader through the analyses undertaken step-by-step.

Major Compulsory Revisions of General Impact for the Manuscript:

1. Stratification of patient subgroups according to CFTR mutation genotype must ensure that mild PS CFTR genotypes and severe PI CFTR genotypes are not pooled. In this respect, patient with one identified class I, II and III mutation cannot be pooled with patients who carry two identified class I, II and III mutations. The former might be severe/unidentified_mild_mutation while the latter are obligatory PI. The analysis must be repeated to circumvent this bias. At least one subset of analyses must be restricted to patients with known and reasonably homogeneous CFTR mutation genotype, of which those with two class I, II and III mutations will be the only sufficiently large subpopulation.

2. The authors’ conclusions that ACE is a modifier of CF rely to a large amount on the parameter BS, which is not explained in sufficient detail within the manuscript. The following points must be clarified before the work can be appreciated: What is BS and how does it differ from other, more commonly used assessments of CF disease severity? Can the authors explain why BS detects ACE as a modifier while lung function values, BMI and various other clinical parameters do not?

3. The data presentation is very detailed. While a comprehensive presentation of all statistical analyses is suitable for an online supplement, the reader will profit from a carefully explained selection within the core manuscript.

4. The manuscript is hard to read. This is partially due to various mistakes in grammar and wording (see below), but also due to very technical sentences with numbers and abbreviations that have little or no explanatory bylines. Examples are listed below (e.g. Major point #9, #13).
Major Compulsory Revisions in point-by-point format:

1. Background, page 3: “Pro-inflammatory activity is activated by the TGF-β1 enzyme and perhaps, for this property is related to the development of severe lung damage.” Please clarify the implied relationship of TGFβ1 and ACE and its relevance for the author's findings (as TGFβ1 was not investigated).

2. Material and methods: heading should be changed to Patients and methods.

3. Patients and methods: “Some mutations in adult patients with CF was obtained by …” Please specify which mutations were detected. Also check grammar: (mutations WERE obtained).

4. Patients and methods: “Clinical scores of Kanga, Shwachman-Kulczycki and BS were performed …” Please specify BS. Explicitly, as BS is not a standard phenotype and the authors conclusion rely largely on BS, this parameter must be explained in more detail.

5. Same paragraph: BMI, although a commonly used abbreviation, needs to be spelled out when first mentioned.

6. Statistical analysis: “Variables described for the onset of illness (age at diagnosis and onset symptom pulmonary, digestive and first isolated P. aeruginosa) were categorized into two groups according to the median of the data.” Please reword to make it easier to understand that two subgroups of equal size were analyzed in all quantitative parameters.

7. Statistical analysis: “the level of significance #, was adjusted using Bonferroni correction”. Please specify how many independent tests were considered by the authors for correction.

8. Statistical analysis: “All mutations observed in our study were included in classes I to III, thus, patients with two identified mutations, presented no influence of CFTR gene in analysis.” Please reword – the authors state in their abstract that conclusions were obtained “in the different groups of mutations in the CFTR gene.” How were the patients grouped to exclude an influence of the CFTR mutation genotype on the outcome?

9. Results and discussion, second paragraph: “Bacteria in secretion: 76 (42.2%) with P. aeruginosa mucoid and 101 (56.1%) non mucoid, 141 (78.3%) S. aureus, 25 (13.9%) B. cepacia and 18 (10%) A. xylosoxidans. Comorbidities were; 143 (79.4%) with pancreatic insufficiency, 33 (18.3%), nasal polyps, 33 (18.3%) diabetes mellitus, 29 (16.1%) osteoporosis and 27 (15%) meconium ileus. For variables with numerical distribution; see data listed in Table 1.” Firstly, the sentence is incomplete (no verb). Secondly, a repetition of the data that is presented in tabular format is unnecessary unacceptable. Thirdly, no conclusion is drawn from the accumulated number set– is the data as expected for the CF population? Are some comorbidities more frequent that expected?

10. Results and discussion, third paragraph: “ACE gene D/I polymorphism was associated with severity of CF.” This sentence need to be backed up with data – the main conclusion should not precede the findings.

11. Results and discussion: “…. when only one mutation in the CFTR gene was
identified.” Are these carriers confirmed CF patients by other techniques? See also major point #8 above, the population structure should be better explained in the methods section.

12. Results and discussion: “The D allele in the ACE gene is associated with a higher gene expression and, consequently, promotes a greater inflammatory response in the body, leading to early symptoms. The earliest onset of signs and symptoms are accompanied by early onset of inflammation and deterioration of lung and pancreatic function. These symptoms characterize severe patients.” This paragraph is better suited for an introduction. This is not a result by the authors and unlinked to the surrounding information.

13. Results and discussion: “Association of the infection/colonization by B. cepacia with ACE gene D/I polymorphism was identified only for patients without distribution according to the CFTR gene mutation, OR: 3.309 (1.476 to 6.256) to D/D genotype (Table 4).” Please reword: what was observed in an elevated frequency in which subgroup? Which covariates were considered to mask/unmask the association?

14. Results and discussion: “There was no difference between BS and the age of patients after categorization.” BS not a standard in CF phenotyping - is this an age-independent description of LF in a progressive disease? Does BS employ CF-specific centiles?

15. Results and discussion: “Evolution of CF is secondary to the type of mutation and environment. Few studies have correlated mutations, polymorphisms and clinical variables in CF.” This is not correct, there are numerous publications on genotype-phenotype relations in CF. Please review the literature.

16. Results and discussion: “The main environmental factor in the clinical variability of CF is the access of patients to treatment. At our center, treatment is guaranteed by the public health system, which allows equal access for all patients included in the study and it is not an additional factor in the analysis of data.” In the US, there is a considerable disadvantage for patients treated under the public health system (Schechter MS. Non-genetic influences on CF lung disease: the role of sociodemographic characteristics, environmental exposures and healthcare interventions. Pediatr Pulmonol Suppl. 2004;26: 82-85.). Can the authors rule out a similar effect for Brazil?

17. Results and discussion: “On the best of our knowledge, only the study by Arkwright et al. (2003) [8] had characterized the ACE gene as a potential factor in the clinical CF severity.” ACE was also investigated by Drumm et al (Reference 25) and others.

18. page 12, table 1: CFTR mutation spectrum is missing.

19. page 14, table 3: “Association of ACE gene D/I polymorphism, in patients with one identified mutation (class I, II or III) in the CFTR gene, with onset of clinical symptoms of patients in months.” Please also give data for the other patient subgroup. Stratification should ensure that mild PS CFTR genotypes and severe PI CFTR genotypes are not pooled.

20. Page 15, table 4: “….. without CFTR genotype distribution …..” please
specify (without talking CFTR mutation genotype into account?)

Minor Essential Revisions:
1. Abstract: “….and Bhalla score (SB) (p= 0.015) ….” Abbreviation BS ?
3. Patients and Methods: “Comorbidities (nasal polyposis, osteoporosis, diabetes mellitus, pancreatic insufficiency and meconium ileus) were analysis.” Check grammar (were analysed).
4. Sometimes, the font changes, e.g. page 5 “and the R program version 2.12 (Comprehensive R Archive Network, 2011).” and page 6: “derived and 15 (7,4 %) were African-derived individuals.”
5. Page 15, table 4: “Ausence” typo: absence

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

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