Reviewer’s report

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

Version: 2 Date: 21 May 2012

Reviewer: Frauke Stanke

Reviewer’s report:

MS by Marson & colleagues on the role of the ACE D/I polymorphisms in cystic fibrosis; revised version

The reviewer thanks the authors for the effort undertaken to improve the presentation of their data. Most of the reviewer’s concerns raised by the previous version of the manuscript are resolved by the revised version as the data show consistently that the D allele is associated with a severe course of disease in the population studied. However, a few points remain to be clarified. The reviewer apologizes for not raising these concerns in her last review, but the previous data presentation did not yet reveal these issues.

Major compulsory revisions:

1. The observed effect, i.e. the association of severe disease with the D allele, manifests in different subpopulations. Phenotype B. cepacia – one CFTR mutation resolved (manifests also in entire cohort); phenotype onset – one CFTR mutation resolved (not observed in entire cohort); phenotype BS – one or two mutations resolved (manifests also in entire cohort). The data is consistent with respect to the assignment of the severe allele, which is D in all cases. The data is inconsistent with respect to the patient subgroup that shows the association. This needs to be discussed, and if possible, resolved. A likely explanation that may be offered to the reader could be that the patient subgroups that were defined on the basis of CFTR mutation analysis also differ in comorbidities which may unmask the role of the modifier ACE that is studied here. Example: onset different for patients with one resolved CFTR mutation in comparison to the other two subgroups? Colonisation with B. cepacia different in subgroup with one CFTR mutation resolved in comparison to the other two subgroups? This data is not given within the manuscript and the information might be displayed in table 1.

2. Patients and methods – page 6: text reads “was adjusted using the Bonferroni correction for four tests.” Many different clinical variables were tested. The reviewer assumes that the Bonferroni correction was intended to correct for the four evaluations for which the 180 patients were divided into three subgroups? Please specify. If that is the case (correction for four patient subgroups), a sentence like “to err on the side of caution, we have chosen to correct for four independent analyses” will tell the reader that the correction for multiple testing is very conservative, as a Bonferroni correction assumes that the analyses are independent and this is not true for the 180-patients-group in relation to their subgroups.
3. Patient and methods – page 6: text reads “For comparison between genotypes and the variables with numerical distribution, T-student test was used, when LRT was positive.” Minor: abbreviation LRT is not explained; major: the boxplots in figure 1 appear to be skewed, implying a non-normal distribution. If that is the case, a rank test (Mann-Whitney or Kruskal-Wallis) would be appropriate while the t-test is not applicable to these kind of data. The software used by the authors (SPSS, R) should be able to handle the nonparametric test.

4. Results and discussion, second paragraph on comorbidities, page 6: the authors stress in their cover letter that some of these comorbidities do not match the observation from other CF populations and that the authors choose not to discuss this as it might distract the reader from the principle findings. However, if the associations described here (ACE vs onset and ACE vs B. cepacia) concern traits for which the studied population differs significantly, this must be emphasized as a reader from the human genetics field is unlikely to be aware of the distribution of these phenotypes in other CF population.

5. Results and discussion: as noted above, the authors have consistently identified the D allele as a risk factor. This finding needs to be emphasized somewhere towards the end of the results & discussion section as it demonstrates convincingly (and better that the P-values and ORs) that the finding is sound. Along the same line of thought: is this a successful replication of a finding from another CF population, in other words: have references [8, 30, 31] also identified the D allele as a risk factor? Unfortunately, the current paragraph within the results and discussion section that compares the Bartlett study seems to imply that the I allele should dispose to severe disease, i.e. liver disease – why (text reads on page 8: “The I/I genotype was not associated with the presence of liver disease”)?

6. Even though the revised manuscript was edited, some phrases remain awkward and/or imprecise. Maybe BMC can provide assistance in these matters? Examples are:

6.1. Abstract – page 2: text reads “There was an association of the D/D genotype with ….. in the different groups of mutations in the CFTR gene.” The association was observed in subgroups of patients which were defined by their CFTR mutation genotype (all patients / subgroup I: no mutation detected; subgroup II: one CFTR allele resolved; subgroup III: both CFTR alleles resolved).

6.2. Patient and methods – page 4: text reads “Other identified mutations in class IV (P205S e R334W) were not included in the statistical analysis.” Does this imply that 180 – 2 = 178 patients were analysed? Does this mean that two compound heterozygotes carrying P205S and R334W together with a class I, II or III mutation were not summarized in the subgroup “both CFTR alleles resolved”?

6.3. Results and discussion, page 6: text reads “The genotypes to mutations identified in the CFTR gene were ….“ Suggestion for rephrasing: „The patients’ CFTR genotypes were .....

6.4. Results and discussion, page 7: text reads „The ACE gene D/I polymorphism showed a higher frequency for ACE*D (228 patients) compared with ACE*I (132
patients).” Of course, the number of patients within a study does not increase if alleles are counted – text must read “228/360 alleles” and “132/360 alleles”.

6.5. Same paragraph as #6.4: text reads “The analyses of the ACE gene D/I polymorphism with the clinical variables are denoted in Table 2, where it can be observed every association possible between the clinical trial, CFTR mutation identified and ACE gene D/I polymorphism.” The phrase “where it can be observed every association possible” is grammatically wrong and needs to be edited appropriately.

6.6. Results and discussion, page 7: text reads “…..when only one mutation in the CFTR gene was identified.” Suggestion for rephrasing: “…. in the subgroup of patients with one identified CFTR mutation.”

6.7. Maybe the tables have escaped the editing process? Table 2 contains the following typographical errors: “pacients” (patients), “ileous” (ileus); table 4: “ausense” (absence)

6.8. Maybe the legend to figure 1 has also escaped the editing process as it is very cumbersome to read. “…patients without the genotype distribution….?” likely refers to “…. In the subgroup of patients for whom no CFTR mutation could be identified ….?” And similarly, “… patients with two mutations identified in the CFTR gene ….” likely refers to “…. In the subgroup of patients for whom two class I, II and III mutation have been identified ….”. Likewise, instead of “Analysis performed group by group by t-student test, considering p value of alpha to 0.05 as statistically significant.”, the text might be reworded to read: “Analysis was performed by student’s T test considering a p-value of 0.05 as statistically significant.”

---

**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.