**Reviewer's report**

**Title:** An association study on contrasting cystic fibrosis endophenotypes recognizes KRT8 but not KRT18 as a modifier of cystic fibrosis disease severity and CFTR mediated residual chloride secretion

**Version:** 1  **Date:** 26 January 2011

**Reviewer:** M Daniele Fallin

**Reviewer's report:**

In this article, the authors investigate two cytokeratin genes, KRT8 and KRT18, for a role in CF phenotype diversity by modifying trafficking of the CFTR-del508 mutant protein. CFTR-del508 is usually degraded before it reaches the epithelial membrane and is therefore unable to transport ions through the membrane. In some cases, CFTR-del508 can reach the epithelium and does function, albeit in a suboptimal manner. Residual CFTR function has been associated with a less severe CF disease phenotype. The authors use two phenotypic measures in this study: residual ion conductance of CFTR-del508 in the rectal epithelium and severe disease versus mild disease. The authors report a specific haplotype in KRT8 to be associated with mild CF disease and with residual ion conductance. They conclude that this “benign allele” could be important in targeting mutant CFTR to the epithelial membrane. While this work presents a previously under-investigated but potentially very interesting modifier candidate, the presentation of analytic design and results is unclear and replication/further biological examination of the KRT8-CFTR relationship is needed.

1) The authors do not fully introduce/consider other factors that have been reported to promote trafficking and partially restore del508 function at the plasma membrane such as over-expression, reduced temperature, compounds that affect the folding environment, and other mutations in cis with del508 (I539T, R553Q, and others). I expected some discussion of the other mutations and why their physiological effect may be (or may not be, as reported by members of your group previously) important in this analysis. It appears that other genotype data are available for these samples. It would be important to know if any of the potential cis acting CFTR modifying mutations are present among this group.

2) Along the lines of the point above, have you looked at KRT8 and ion conductance in severe CFTR mutations other than del508? What about other Class II mutations? Do you have access to another population of patients you could attempt to replicate this result in?

3) The introduction could benefit from a clearer explanation of functional consequences of delF508 and the variation of CFTR activity found in delF508 homozygotes. For example, do the rectal suction biopsies range from 0% function to 30% function? 50%? How much of a clinical impact does a CFTR-del508 protein functioning in the epithelial membrane have? I believe
having a more detailed sense of the variation in CFTR del-508 ion conductance will add to the understanding and clinical relevance of the results.

4) The design of this project is not clear. While the methods describe a twin/sibling sample, it is unclear how these were used for the association analyses presented. My interpretation of the description is that all concordant severe sib pairs were compared to all concordant mild sib pairs for the severity phenotype. However, there is no mention of what specific test was done or how the sibling correlations within each group were accommodated in the testing.

5) It is unclear why the results for the microsatellite analysis (first paragraph of results) are presented in allele comparisons for one phenotype but genotype comparison for the other. Were the results not significant for the genotype comparison?

Minor:

1) The term “CF basic defect” is used often in the text. It appears that the use is intended to refer to the mutation, but at times it appears that you specifically mean the physiologic consequence. This can be confusing at times in the presentation. I would suggest stating “impaired ion conductance” in some instances when explaining data or a process to make the idea you are trying to express more clear to the reader.

2) In your methods, please also cite the Mekus paper (Twin Res. 2000 Dec;3(4):277-93) when referencing the ranking algorithm.

3) Page 12, line 4 worded like KRT8 is the cause of the CF basic defect, which it is not. I would recommend “the modification of the CF basic defect in CFTR-del508 homozygotes.”

4) The phylogenetic age of the allele section seems irrelevant to the results reported. If it is important to the interpretation of this work, this should be more clearly discussed.

5) Pg 15, last line: I would suggest the use of “may be” instead of “apparently” since there is no biological data to back up this statement. Have you thought about trying to show the role of the KRT8 haplotypes and their effect on CFTR-del508 molecules in cell culture?

6) Figure 2: A. Is there a reason why you did not combine axis labels with the overall microsatellite graph? 
B. Show microsatellite distribution by haplotype (1122, 2211, and other)

7) Table 1: Change 0.000 to <0.001