Reviewer's report

Title: Molecular Analysis Using DHPLC of Cystic Fibrosis in Central Italy: Increase of the Mutation Detection Rate Among the Affected Population

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Reviewer: paolo arosio

Reviewer's report:

General
The authors use DHPLC to identify causative mutations of CFTR gene in 290 DNA samples from Italian patients diagnosed for cystic fibrosis. The system, based on a published procedure, identified rare mutations two of which have not been reported before. It seemed an improvement over the previous assays for 29 common mutations. However, 11% of the alleles analysed did not show any causative DNA variation. They concluded that DHPLC increases the standard "level of mutation detection". The manuscript can be largely improved.

Discretionary Revisions (which the author can choose to ignore)
The title could be improved: It's not clear what the increase of detection rate refers to.
Background, p 3 l 7. Please, describe briefly the 6 different classes.
Background: page 3 line 1. I would change into: "one of the most common genetic diseases" (since other diseases such as hereditary hemochromatosis claim to be even more common)

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
1- Throughout the text the authors should indicate the nucleotide substitutions as well as the amino acid mutations.
2- On p 4 line 3 from bottom the sentence should be modified to define whether the samples selected for further investigation are the 290 samples analysed by INNO-LiPa assay
3- Page 3 line 12: modify "her frequency" into "its frequency"
4- Page 4 line 5. the group of Ferec (ref 16) has applied DHPLC to detect mutation in all 27 exons of CFTR, not on a limited number of exons.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1- Table 2 should be completed to include most of the experimental results, including the nucleotide substitutions, the mutations detected by the INNO-LiPa assays and by DHPLC. This would provide direct evidence of the improvement in mutant detection by DHPLC. Perhaps the data of Table 3 could be included in table 2, specifying that are polymorphisms. It should be explained why R75Q and M470V are considered DNA variations rather than mutations
2- The discussion should be improved to stress the relevance of the findings, which is not obvious in the present text. For examples the expected structural/functional effects of the two new mutations, comments if there was a complete agreement between DHPLC and the alternative assays for mutation discovery; and why 69 of the 580 alleles analysed did not carry detectable mutations. Did DHPLC detect all mutations in the 27 exons?
3- It should be explained why novel primers were required for exons 6b and 9, and how this improved the method.
**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**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:** No

**Declaration of competing interests:**

none