Reviewer’s report

Title: Comparison of DNA methylation profiles from saliva in Coeliac disease and non-Coeliac disease individuals

Version: 0 Date: 30 Oct 2019

Reviewer: Marco Lucarelli

Reviewer's report:

The manuscript compares DNA methylation from saliva in subjects affected by coeliac disease and non-coeliac controls by using the Illumina Infinium Methylation 450 Beadchip array and pyrosequencing. The aim of the work is to identify useful epigenetic biomarkers from easily accessible saliva samples, in respect to more difficult to obtain intestinal biopsies.

The work appears promising and original, since no previous methylation data obtained from saliva samples of coeliac subjects have been reported. This makes the manuscripts worth of publication although the data are still preliminary. However, some minor questions need to be addressed.

Abstract
1) The Abstract suffers from some unclear wording and punctuation in the Results section.

Results
2) The first two paragraphs of the Results (lines 85-99) and the paragraph from line 143 to line 150 could be moved to the Methods section, as description of case series.
3) The Authors should better describe what criteria they used to choose genes for additional experiments and, among these genes, how they choose CpG sites to be deeper analyzed. In synthesis, I think a better description of experimental planning is needed (an exemplificative scheme may be useful).
4) More attention is needed to explain the differences in methylation observed in the SLC17A3 gene between the array and the sequence in the two cohorts. Have the Authors tried to sequence the samples from the first cohort? Do discrepancies depend on differences between the two cohorts or between the two methods?
5) Cohorts. It is not clear what is the difference between the "illumina" cohort and the "pyrosequencing" cohort. Which is the cohort described in Methods? Is the illumina cohort a subgroup selected from the larger cohort described in table 4? If so, table 4 should be described first. Moreover, if so, Authors should explain whether the "pyrosequencing" cohort also include the individuals analyzed by the Illumina or not and discuss their rationale in this experimental design. In synthesis, I think a more ordinated presentation of case series in Methods is needed, with a link to the two tables, as well as a more detailed description of what has been done on one cohort in respect to the other and its rationale.
6) The Authors should discuss the hypothetical correlation between the methylation data and the expression of the differentially methylated genes: does the theoretical up-regulation (hypomethylation) or down-regulation (hypermethylation) have a possible role in the coeliac disease?
7) The Authors only report difference in CpG methylation, although evidence indicate that significant and functional CpN methylation may be masked by the use of PCR primers that do not allow to evidence it, with the indirect consequence of underestimating also differences in CpG methylation:

What kind of primers for illumina assay and pyrosequencing have the Authors used? Are these primers biased versus CpN methylation? These aspects should be discussed in relation to the obtained data (the indicated papers could be quoted as support).

Discussion
8) Discussion, line 191: Cpg -> CpG.

Methods
9) Line 266. The term "general population" is ambiguous in this context. Does the selection concern the cohort of affected patients or control subjects? If both, the term cannot be used.
10) Bisulfite modification. The bisulfite modification is poorly described. Authors should describe the kit used and the positive and negative controls they adopted for the bisulfite modification and subsequent sequencing protocol.
11) Line 365. The Authors say that Sanger sequencing was performed, with no other details, within a paragraph called "Bisulfite sequencing" including pyrosequencing. In this paragraph each sequencing method used, also if it was performed by a facility, should be described in greater detail. In addition, when in the text the Authors refer to results obtained by sequencing, which method was used should made more clear. For example, in the "Results" section of the present manuscript is some Sanger sequencing results reported?

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.

I am able to assess the statistics

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