Reviewer’s report

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

Version: 1 Date: 19 Dec 2019

Reviewer: Marco Lucarelli

Reviewer's report:

The Authors have significantly improved the manuscript. Only the following minor issues remain.

1) In the abstract, the following sentence has some wrong punctuation.
"CD individuals who had been diagnosed at least 2 years previously; were on a 38 GFD; and who were currently …"

2) Line 298. I think that also "from" should be canceled.

3) Although improved in the revised version, the Authors poorly describe methylation arrays, bisulfite treatment, pyrosequencing and Sanger sequencing, because these techniques have been performed by the Australian Genome Research Facility (AGRF). In fact, they said: "...therefore the exact details of the kits and processes used were not available to the authors." I leave to the Editor the decision if this approach may be acceptable. If someone would replicate the experiments of the Authors, he/she should ask to AGFR. The alternative would be that AGFR provided the details of methods used to be embedded in Materials and Methods of the article.

4) I do not think the Authors have to necessary deal with the topic of CpG and non-CpG methylation in this manuscript. Lot of papers do not. Therefore, the Editor can decide independently from this point. I wrote it just to highlight the problem.

The following is a previous Reviewer's observation: "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). "

This is the Authors' answer: "The Illuminia Infinium HumanMethylation450 Beadchip (HM450) array provides single base resolution genotyping of targeted CpG sites using probes on a microarray. The array uses two probe types with a different chemistry which each have a different dynamic range, which has the potential to affect DNA methylation measurements. Despite this, the epigenetic research community has widely used this array, and specific bioinformatics methods (embedded in the packages
used to analyse this dataset) have enable us to address the technical impact of the two probe designs. The Qiagen Pyromark platform is utilised by the Australian Genome Research Facility in their Targeted Sites and Methylation Validation service. We used this service to validate methylation findings identified on the HM450 array. This technology is a type of NGS technique. All pyrosequencing assays were designed, fully optimised, and performed by the Australian Genome Research Facility, a commercial fee for service provider. The references supplied by the reviewer refer to issues observed in older methodologies that were either not used in this study or have been addressed via specific primer design software utilised by AGRF.

This is my reply: Obviously, I know both approaches for methylation study. I did not ask to explain me the functioning of these approaches. I asked to highlight in discussion their limitations. In fact, what the Authors say is just the demonstration of the bias of both method: the first approach experimentally points only to CpG methylation; the second approach, although able to detect also non-CpG methylation, excludes it in the step of data analysis. The topic of CpG and non-CpG methylation is a modern subject and not, as referred by the Authors, linked to older methodologies. See for example the last paper on this topic: "Active Demethylation of Non-CpG Moieties in Animals: A Neglected Research Area. Int J Mol Sci. 2019 Dec 12;20(24). pii: E6272. doi: 10.3390/ijms20246272". Most of NGS-based methods failed to detect or to analyze non-CpG methylation which, however, may have great functional importance. For example, when only CpG methylation cannot support a functional correlation with expression and/or in case of negative results from CpG methylation.

**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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Please indicate the quality of language in the manuscript:

Acceptable

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