Reviewer's report

Title: Whole genome methylation array analysis reveals new aspects in Balkan Endemic Nephropathy etiology

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Reviewer: Eoin Brennan

Reviewer's report:

This study attempts to investigate the role of DNA methylation as an epigenetic regulatory mechanism in Balkan Endemic Nephropathy (BEN). This is a valid and important question to aid our understanding of the pathophysiology underlying BEN. The authors have performed whole genome DNA methylation analysis on PBL DNA pools from 159 BEN patients and 170 healthy controls, and have measured the methylation status of 27,000 CpG islands. Using this strategy, hypomethylation in SEC61G, IL17RA and HDAC11 was observed in patients versus controls.

Major Compulsory Revisions

1. Considering the large number of CpG islands assessed and the pooling strategy employed, it is important to confirm that the SEC61G, IL17RA and HDAC1 DMRs are indeed true signals. The authors should demonstrate the degree of correlation between DNA methylation and gene expression levels of the three identified genes (SEC61G, IL17RA and HDAC11) in BEN patients versus controls?

2. Presently Tables 2-3 are quite descriptive and lack relevant biological information. For the cohorts of DMRs identified in Tables 2 and 3, the authors should perform pathway analyses of the genes associated with these CpG islands to determine whether specific pathways or gene networks are strongly enriched in these DMRs. This would be an important strategy to infer some biological meaning to the emerging data.

3. For Table 1, the authors should include additional information on the patients selected for pools and also the controls, to make this Table much more comprehensive. For example: mean age at diagnosis of BEN ± SD; Mean duration of BEN ± SD; any relevant clinical parameters available, e.g. HbA1c, BMI. I believe this would greatly enhance the information available to the reader.

4. For Tables 2 and 3, please provide complete information on the DMR groups and their respective genes (such as listing all genes within the DMR groups, CpG island genomic location and methylation status). These could be included as Supplemental information in an excel table.

5. In the discussion section the major focus is on the top three candidate genes - SEC61G, IL17RA and HDAC11. Some of this information could be greatly
condensed and I would suggest discussing the pathway analysis results (see comment 2) to review the overall biological signal emerging from this study.

Minor Essential Revisions

6. Finally, there are a number of typographical and grammatical mistakes that need to be corrected throughout the manuscript.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests