Reviewer’s report

Title: Distinct DNA methylation patterns of cognitive impairment and Trisomy 21 in Down Syndrome

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Reviewer: Paolo Garagnani

Reviewer’s report:

Major compulsory revisions

The manuscript by Jones et al. is potentially of great interest in the study of Down Syndrome and, more in general, of diseases associated with cognitive impairment. However I believe that the study in its current form suffers of two major drawbacks:

1) The cohort under study is too small and not evenly balanced between cases (10 subjects) and controls (5 subjects). The authors are therefore requested to replicate the major findings on a larger and balanced cohort, possibly referring to a statistician to decide the minimum number of samples to analyze on the basis of the variability of the data.

2) Contamination of buccal swabs by blood cells. I do not enter into the merits of contamination correction by subtraction of PC1 values, for which I suggest to refer to a statistician. In any case, this procedure should be better explained in the Methods section. Although the authors insist on the success of data correction both in the Results and in the Discussion section, I am not fully convinced by their arguments and by the presented results. Above all, I am surprised that the authors find only a small overlapping between the probes differentially methylated between DS and controls, identified by a linear model fitting method, and the probes that correlate with the BPT (which moreover are more numerous than those identified by the linear fitting method, which looks quite strange). Indeed, authors themselves state in the Results section that in Figure 3c there is a clear division between DS and controls: this means that also the correlation analysis identifies probes that differ between the two groups. So, why there is no overlap with those identified using the linear model? I am afraid that this is due to a different “performance” of the two statistical approaches, which return a different set of differentially methylated probes. Therefore, the lack of overlapping cannot be used to state that PC1 correction was successful, as the authors do in the Discussion section. Considering the distribution of BPT values between DS and controls, you can note a gap between the DS scores, ranging from 64 to 80 (with one subject with score=35), and the controls scores, which are all 100. Therefore, I do not think that it is appropriate to perform the Spearman correlation test using both DS and controls, but it would be more appropriate to perform it only within the DS group: this result should provide the probes that actually correlate with cognitive status. Summarizing, I suggest the author:
to perform the Spearman correlation test only within the DS group
- to use a linear model to compare DS with high BPT, DS with low BPT and controls. In this way they can demonstrate that the probes differentially methylated between DS with low BPT and DS with high BPT/controls do not belong to blood related-GO. Moreover, it could be interesting to use available data in GEO datasets to demonstrate that the identified probes do not differ between epithelial and blood cells (for example, using the data by Lowe et al.)

Minor Essential Revisions

In the introduction it is stated that almost all individuals with DS have AD-like neuropathological changes. To my knowledge, current literature has downsized the prevalence of AD among DS (see the recent review by Zigman WB, “Atypical aging in down syndrome”). Therefore, please correct the paragraph on the basis of more recent literature.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests