Author's response to reviews

Title: Telomere shortening in leukocyte subpopulations in depression

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Author's response to reviews: see over
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Detailed response to reviewers' comments on the manuscript:
Title: Telomere shortening in leukocyte subpopulations in depression
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Reviewer 1

Query 1: Although the authors acknowledge this in the Discussion, I believe they should temper their diagnoses of unipolar major depressive disorder in the Methods section by referring to it as “as determined by standard clinical evaluation.”

Response 1: We edited the Methods section and now provide the requested information highlighted in the revised manuscript.

Query 2: How were these 44 depressed individuals identified for inclusion? Had they all been inpatients when they received their diagnose?

Response 2: At the timepoint of study participation, all subjects with a lifetime diagnosis of depression were outpatients. They were not hospitalized for at least 6 months.

Query 3: What time of day were the blood samples obtained? Were the subjects fasted? Had any subjects had recent colds or infections or immunizations? Did any have significant elevations in the WBC?

Response 3: All blood samples were collected between 10 am and 1 pm. As participants were allowed to eat or drink in the morning, they did not fast. This information was included into the Methods section. Subjects who reported an inflammatory or immunological condition within the past two weeks before participation were excluded from participation.

Unfortunately, we did not assess the WBC composition in this study. However, we measured telomeres in the isolated subpopulations of white blood cells. Thereby, the possible influence of alterations in WBCs can not influence the results in this study. We have added this information to the methods section and included it in the discussion.

Query 4: How was “age at first depressive episode” and “total number of episodes” determined? How reliable are those? Do they have any information as to the combined duration of each subject’s episodes?

Response 4: Age at first depressive episode and the total number of episodes were taken from the clinical documentation. Indeed, it is unclear how reliable this information is. We included this information in the methods section and discuss the
problem of reliability of information on “age at first depressive episode” and “total number of episodes” in the limitations section. Further, we now report that the number of depressive episodes did not change the pattern of results or became significant when included in the models.

**Query 5:** The authors report on antidepressant use. Is this just current use or also lifetime use? Was this quantified over the subject’s lifetime? Are there records on other medications that are relevant: lithium, antipsychotics, statins, insulin sensitizers, omega-3 fatty acids, vitamins, etc? Are data available on BMI? I recognize that the authors do comment on several of these limitations.

Response 5: Unfortunately, we only asked participants about current medication. However, subjects did not report the intake of lithium, antipsychotics, statins etc. Other medication was for the treatment of hypertension and the thyroid. We therefore cannot say anything about lifetime medication.

As detailed in the discussion section, the BMI was unfortunately not assessed. Indeed, it is possible that a higher BMI in depressed individuals could be one pathway leading to telomere shortening, as a higher BMI is associated with higher levels of oxidative stress. We included this point in the limitations section and recommend investigating BMI and depression jointly in future studies.

**Query 6:** 6. Were all samples run in the same batch?

Response 6: No, samples were analyzed in sets of 10 samples + 1 reference (control sample of a 30 year old male). The three cell populations (CD4+, CD8+ and CD20+) were analyzed in series.

**Query 7:** If post hoc comparisons of individual groups were performed after the overall ANOVA significance, this should be presented on p. 7.

Response 7: Thank you very much for this point, which improved the results part of our manuscript. We included the posthoc analyses in the manuscript (see results section).

**Query 8:** What metric do they use for calculating an average acceleration of cell aging of 25-28 years?

Response 7: Our statistician (SK) who calculated all analyses included the following paragraph to clarify the analyses (compare page 7 and 8):

“To compare the TL reduction observed in the IS and RS to the one associated with aging, the coefficient estimate of the linear model for TL corresponding to each group (-.167 for IS, -.184 for RS), giving the group effect, was divided by the coefficient estimate for age (-.00657), giving the TL reduction per year of age. To assess the variability of this statistic, it was bootstrapped 10,000 times. The TL reduction in IS
(RS) participants corresponded to the reduction in control participants \(-167/-.00657 = 25.3 \text{ (}-184/-00657 = 27.9\text{)}\) years older (slight differences due to floating point arithmetic and rounding), bootstrapped 95% confidence interval 13.9-58.6 (12.3-66.9").

**Query 8b**: How long were the IS subjects in remission?

**Response 8b**: This is a very relevant point. Indeed, we assessed only that subjects had a lifetime diagnosis of depression and were at least six months in remission. Thus, we do not know how long they were in remission and cannot calculate corresponding analyses. This point was included in the limitations section. We will assess this question in future studies.

**Query 8c**: On p. 8, it is difficult to infer that the IS groups did not recover, since we do not know their pre-depression baseline. Perhaps they started with a lower telomere length. However, another study, which could be cited, also found no (Verhoeven et al., 2013).

**Response 8c**: Thank you very much for drawing our attention to this interesting study! Indeed, the reviewer is correct that we cannot draw conclusions in this crosssectional study design on whether the IR group recovered or not. We can only report that both groups (IS and RS) did not differ significantly and this is in line with the findings by Verhoeven and collegues (2013). This study is now cited in the manuscript and the results are discussed in the light of their findings.

**Reviewer 2**

Depression may arise from different causes and not all of them are known. Differences in etiology, however, may result in marked differences in pathophysiology, even if clinical symptoms look similar. One important factor, especially for depression, concerns the history of childhood maltreatment. Individuals with childhood adversities have an earlier onset, more severe course, more comorbidities, more remissions and they respond more poorly to treatments than their depressive counterparts without substantial childhood maltreatment. Investigations of the biological differences between subjects with and without a history of depression therefore must consider this factor. The present study confirmed that individuals with a history of unipolar depression (with and without current depressive symptoms) present substantially shorter telomeres in each of the three investigated lymphocyte subpopulations. This finding is of substantial interest and worth reporting. However, it seems a bit disappointing that a study from the Kolassa-lab with its high reputation in studies of molecular psychiatry and clinical psychology has not taken into account the findings on depression and childhood adversities.

**Response**: We completely agree with the reviewer that this factor should be considered in a study like this. Unfortunately, it was not possible to assess this information in this piloting sample assessed between 2008-2010. However, we currently assess this question in a large study on the consequences of childhood adversities.
maltreatment on mothers and their infants.