Author's response to reviews

Title: Cognitive performance and leukocyte telomere length in two narrow age-range cohorts: a population study

Authors:

Karen A. Mather (karen.mather@unsw.edu.au)
Anthony F Jorm (ajorm@unimelb.edu.au)
Kaarin KJ Anstey (k.anstey@anu.edu.au)
Peter J Milburn (Peter.Milburn@anu.edu.au)
Simon Easteal (s.easteal@anu.edu.au)
Helen Christensen (h.christensen@anu.edu.au)

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Author's response to reviews: see over
The authors thank the reviewers for their comments and appraisal of the manuscript. The reviewers’ queries are answered below and any changes to the text in the revised article are highlighted by track changes.

**Reviewer's report**

**Title:** Cognitive performance and leukocyte telomere length in two narrow age-range cohorts: a population study  
**Version:** 1  
**Date:** 12 May 2010  
**Reviewer:** Sarah Harris

**Reviewer's report:**

The paper investigates associations between telomere length and both cognitive function and cognitive change in two narrow age-range cohorts. The results provide little evidence for such associations. The study was generally well designed. I like the fact that two methods of telomere measurement were used and that they correlated highly. The paper is well written and easy to follow. The conclusions are appropriate. I hope that at the next wave of testing both telomere length and cognition are measured to allow an association of cognitive change with telomere length change to be investigated. I have only a few minor points.

**Minor essential revisions:**

1) In the background section of the abstract the authors state: “In this report, the relationships between baseline telomere length and cognitive performance and change are further assessed in two narrow age-range population cohorts.” Whereas in fact telomere length was measured at wave 2, not at baseline. This needs to be corrected.

This sentence on p.2 has been amended to:

"In this report, the relationships between telomere length and cognitive performance at wave 2 and cognitive change from wave 1 to wave 2 are assessed in two narrow age-range population cohorts."

2) In the statistical analyses section the authors state: “Due to sex differences on most measures, the analyses were also undertaken separately for men and women.” However, when carrying out analyses on the entire cohort they do not control for sex. I would like to see the analyses repeated controlling for sex.

Analyses controlling for sex in addition to the other covariates has been undertaken and the tables and text updated (see Table 2 & 3 & Methods p. 14).

**Level of interest:** An article whose findings are important to those with closely related research interests  
**Quality of written English:** Acceptable  
**Statistical review:** Yes, and I have assessed the statistics in my report.  
**Declaration of competing interests:**  
I declare that I have no competing interests
Reviewer's report
Title: Cognitive performance and leukocyte telomere length in two narrow age-range cohorts: a population study
Version: 1 Date: 24 May 2010
Reviewer: Joanna Collerton
Reviewer's report:
The association between telomere length and various health measures is an active area in gerontological research. Existing studies exploring the link between telomere length and cognitive performance have shown conflicting results. This manuscript explores the relationship between telomere length and cognitive performance in two narrow age band cohorts. It has the strengths of narrow age band cohort study design, the wide range of cognitive tests employed, and the availability of both cross-sectional and longitudinal cognitive data (although longitudinal telomere data is not available). However I have the following areas of concern: the coefficients of variation of the telomere length estimates; the data on the relationship between telomere length and change in cognitive function should be presented; and, as the authors state, the study was somewhat under-powered.

Author's General comment:
The authors thank Joanna Collerton for her detailed analysis of the manuscript and her comments. Her concerns have been addressed below and where relevant the manuscript has been altered.

Major compulsory revisions
1. Methods, telomere length measurement: The authors report mean inter-assay coefficients of variation (CV) of 3.4 and 8.8% for telomere length estimates for their 60+ and 40+ cohorts respectively. While the lower number might be acceptable, the higher one might seriously interfere with results. Typically, differences between groups in telomere length biomarker studies are in the order of 150 – 400 bp, equivalent to 4 – 8%. Also the authors did not give the variation of their mean CV, one might assume that at least one third of their estimates (at least in the younger group) actually showed a CV>10%. The authors do not state on how many samples these estimates are based; the difference in CVs between the younger and older groups is significant and might suggest the estimates are based on only a small number of samples. These are major weaknesses of the study and needs to be clearly indicated and discussed as such.

For the 40+ cohort, the inter-assay mean CV for the positive controls (n = 4), which were run in quadruplicate on each plate (n = 5) was 8.8% (range 6.5-12.1). For the 60+ cohort, the inter-assay mean CV for the positive controls that were run on every plate (n = 3) across all of the plates (n=5) was 3.4% (range 1.6-5.8). The authors acknowledge that the number of samples that the CV was derived from is small. However, Woo et al. (2008) also report CVs based on four samples and found the mean CV to be higher than our estimates (11.1%). Most published studies report mean inter-assay CVs that are less than 10%. The current study, especially the result for the 60+ cohort, compares well with that reported by Nordfjall et al. [1]of 5.27%, Cawthon [2]of 5.8%, O’Sullivan et al. [3] of 7%, Jang et al. [4] of 7.5%, Xu et al. [5] of 8.5%, Farzaneh-Far et al., [6] of 9.5% and Woo et al. [7] of 11.1%. Indeed, one published study has reported an inter-assay CV of 28% [8]. Some studies do not report the
inter-assay CV [e.g. 9]. In addition, the calculation of the CV can vary between studies, making comparisons difficult. For example, some studies report CVs for duplicate samples [e.g. 10], which is inadequate since the calculation of the CV is derived from the mean and standard deviation. Other studies give the CV for the telomere and single-copy gene Q-PCR and not for the final outcome measure of relative telomere length. The authors agree with the reviewer that our results suggest that the measurement error for telomere estimation is less for the 60+ cohort.

Minimisation of telomere length measurement error is important and the authors acknowledge that large measurement error may obscure any associations, particularly those of small effect size. For example, Kimura et al. [11] have suggested that the significant findings observed in a telomere study using the TRF method and not an earlier study were due to a revised telomere length estimation method that improved the inter-assay CV from 12 to 3.4%. Since most of our analyses were undertaken within each age cohort, the results for our 60+ cohort that had a lower inter-assay CV compared to most other Q-PCR studies, suggests that our observed results for the 60+ cohort are more valid than many prior studies. In addition, the current study has taken a cautious approach to the conclusions drawn from this work by suggesting that more studies need to be undertaken in this area.

The following has been added to the methods section (p... 10)
“....assessed for a set of positive controls (human cell line and saliva DNA, $n = 4$ for the 40+ cohort and $n = 3$ for the 60+ cohort)....”

The following has been added to the discussion (limitations paragraph):

“.....The variability of telomere length estimation as measured by the mean inter-assay coefficient of variation (CV) differed for the two cohorts. The larger measurement error for the 40+ cohort (8.8%) may have attenuated any significant relationships of small to medium effect size. Nevertheless, the inter-assay CV results of the present study fall within the range reported in prior studies, which is generally less than 10% but can range from 5.3 to 28% [1, 3, 6, 8]. The comparatively low inter-assay CV for the 60+ cohort (3.4%) and the lack of significant relationships in general for this cohort, suggests that there are either no associations between the cognitive measures and telomere length or they are of small effect size. The minimisation of telomere length measurement error should be an important priority in future telomere studies.”

2. Results, Table 2: The values given for the correlation coefficients in the table differ from those quoted in the text which raises some concerns about the accuracy of the figures in general.

The values in all of the tables have been double-checked and any inaccuracies corrected.

3. Results: the data on change in cognitive performance between waves 1 and 2 should be shown.
A new table (Table 3) has been added that shows the data on change in cognitive performance between waves 1 and 2.

4. Methods, Participants: A flow chart showing how the telomere 40 and 60 cohorts relate to the overall PATH cohort should be included.

Figure 1 has been added, which shows a flow chart indicating the relationships between the 40+ and 60+ cohorts with the PATH sample.

**Minor essential revisions**

1. Abstract, methods section: This should include the setting for the study (Australia- specifying region) and a list of cognitive measures used.

The abstract has been amended as below and on p. 2.

“Methods: We tested the hypothesis that leukocyte telomere length correlates positively with cognitive performance and cognitive decline in two community cohorts of middle-aged (n = 311, 44-49 years) and older (n = 295, 64-70 years) adults, who participated in two waves of a longitudinal study undertaken in the Canberra- Queanbeyan region of Australia. Telomere length was estimated at Wave 2. Cognitive performance was measured using the Symbol Digit Modalities Test, the immediate recall test of the California Verbal Learning Test, reaction time (simple & choice) and the Trails Test Part B.”

2. Background, paragraph 2, line 8: ‘M’ should be explained.

The ‘M’ denotes mean and has now been spelt out:
“... using a larger and younger female sample with a wide age range (mean age 50 yrs, range 19-78 yrs).....”

3. Methods, Participants: The timeframe for waves 1 and 2 should be stated.

The text on p.7 has been amended to:
“Participants were drawn from a large longitudinal Australian population-based study, the PATH Through Life Project (PATH), which began in 1999 and collects data every four years.”

4. Methods, Participants: .......self-report data was collected using hand held computers...was this collected by the same trained interviewers who administered the physical and cognitive tests?

The self-report data was entered by the participants under the supervision of the trained interviewers who also administered the physical & cognitive tests.

The text has been changed to clarify this point on p.7:
"...The majority of the interviews occurred in the participant’s home and participants entered self-report data using hand-held computers under the supervision of trained interviewers who also administered the physical and cognitive tests...."

5. Methods, Participants: a comment on whether the PATH cohort was representative of the local source population could be included.

Information regarding the representativeness of the sample compared to the recruitment population has been added to p.7:
“..... When the entire PATH sample was compared with census information from the recruitment areas, participants were similar in marital status but tended to have greater rates of employment and higher education levels [12]. “

6. Methods, Measures: This section should be separated into cognitive measures and then other measures.

This change has been made to the Methods text (see p.11 & 12)

7. Methods, Measures- it is usual to have a time period assigned to the FEV e.g. FEV1- forced expiratory volume in 1 second.

A time period has been assigned to the FEV measurement used in this study. See p. 13: '.... A measure of lung function, the forced expiratory volume in the first second (FEV1), ..'

8. Methods, Statistical analysis, line 3: Should ‘complex’ read ‘choice’?

'Complex' has been changed to 'choice' to be consistent with the tables.

9. Results, Table 1: Having a mix of n (%), median (IQR) and mean (SD) within the table can confuse; suggest either separate columns for each or footnotes to identify which is which. Also some means have SD written next to them whilst others don’t; approach needs to be consistent.

The table has been amended according to the reviewer’s recommendations. See Table 1.

10. Results, Table 1: The IQR should give the lower quartile value and the upper quartile value rather than the actual range.

The IQR values have been changed to the actual values rather than the range.

11. Results, Table 1: Is hypertension defined as EITHER current use of antihypertensive drugs OR measured BP levels above a cut point? This is not clear.

The wording has been changed in Table 1 (p.35) to clarify the definition:
'Hypertension is defined as either the current use of anti-hypertensive medication or having a mean systolic blood pressure measurement ≥ 160mm Hg and/or a mean diastolic blood pressure ≥95mm Hg.'
12. Discussion, Paragraph 1: The gender differences in the findings in the older cohort should also be mentioned.

These differences are now mentioned in paragraph 1 of the discussion:

'...... Sex differences were also observed, with a significant correlation between telomere length and SDMT scores found for men only whereas a significant association between telomere length and simple reaction time was observed for women only. Telomere length at Wave 2 was not correlated with cognitive change over a four-year period from Wave 1 to Wave 2, except for a borderline significant association (immediate recall) and an association of small effect size in the contrary direction to that hypothesised for women of the older cohort for choice reaction time. '

13. Discussion, Paragraph 2: DSST should be spelt out.

This acronym has now been defined on p. 5

'...... was associated with better baseline performance on the Digit Symbol Substitution Test (DSST).'

Discretionary revisions
General point: the manuscript is rather wordy and could be tightened up e.g.
Methods, Participants: The last sentence repeats the end of the background section; Discussion, Paragraphs 2/3: this section is somewhat repetitive of the background section.

The manuscript has been ‘tightened up’ particularly in the sections mentioned above to avoid repetition.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
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

References


