Letters to the Editor

Raising the bar on telomere epidemiology

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In his commentary ‘Raising the bar on telomere epidemiology’,1 Dr Aviv reported an alleged inconsistency in Table 1 from our study on telomere shortening:2 ‘In this regard, there is a discrepancy in data presented within Table 1, and between Table 1 and Figure 3. The table shows that in 1995, the age of all participants, i.e. “all samples”, was 53–71 years and that of the deceased was 67–81 years. How could that be? Moreover, data displayed in Figure 3 suggest that the age range of the sample was in fact ~45–85 years, which is in conflict with Table 1.’ As a matter of fact, the numbers that Dr Aviv obviously interpreted as the full age range of the sample, were interquartile ranges as unequivocally described in the legend to Table 1.2 The true full age range of 45–84 years was depicted several times in the text and Figure 3, so there is little reason for misinterpretation and clearly no contradiction as claimed by Dr Aviv.1

The discussion on this putative contradiction continues on the second page of this commentary: ‘Another ambiguous finding of the present study is that participants who died during the follow-up period had a shorter relative telomere length (RTL) than those who survived. But participants who died were clearly older on average than the survivors (Table 1 and Figure 3) and no information is provided about the sex distribution in surviving or deceased participants’. We are well aware of the fact that age is a key determinant of telomere length and shortening, and that is why we compared the RTL of participants who survived and died in subgroups of comparable average age. Alternative approaches like a survival analysis formally adjusted for age and sex (Cox models) or comparison of RTL between individuals who died and age- and sex-matched controls confirmed the presence of a highly significant and independent association between RTL and mortality. Again, the interval of 67–81 years represents the interquartile range and not the full range.

Another aspect of the commentary was the discussion on the comparability of quantitative PCR (qPCR) to Southern blot analysis of the terminal restriction fragment (TRF) length. Dr Aviv suggested in his commentary: ‘To resolve the contentious debate about the optimal method to measure telomere length in epidemiological research, we must marshal the resources for impartial and rigorous comparisons in large-scale epidemiological studies between the qPCR and Southern blot methods’. Dr Aviv might have missed that we applied both approaches in a subgroup of the investigated subjects for asserting the comparability of the two approaches. In our study and in a large survey within the West of Scotland Coronary Prevention (WOSCOP) Study, a good correlation between TRF measurements (Southern blot) and RTL (qPCR) was demonstrated with very similar correlation coefficients obtained ($r = 0.63$ in Ehrlenbach et al.2 and $r = 0.65$ in Brouilette et al.5). Both methods actually have their strengths and limitations and epidemiological studies focusing on cardiovascular disease yielded very similar findings irrespective of the method applied.

The next aspect to emphasize is the coefficient of variation (CV) values. Dr Aviv stated the following: ‘How can these “problems” be reconciled with the authors’ statement that after exclusion of outliers, the CV of RTL replicates was only 0.9%? I presume that this value reflects the CV of four replicates of the same run, carried out in the same 384-well plate, rather than runs performed on different plates on different occasions, which is the only reliable way to assess reproducibility of assays in large-scale epidemiological studies. However, such a value is still unusually low even for intra-assay qPCR runs’. In our study, each sample was run in quadruple on the same 384-well plate to minimize intra-assay variability. With this elaborate procedure and by applying the efficiency correction method,4 the intra-assay CV values could indeed be kept as low as 0.9%, which considerably increases the confidence in the paired comparison of samples that were always analyzed on the same assay plate with close proximity of the paired samples to even exclude drift effects over a single plate. The exact protocol was described in the Supplementary data to ensure that other researchers
can technically reproduce the procedure. Furthermore, and most importantly, each sample was normalized to a control DNA sample to minimize the inter-assay variability. The inter-assay CV as determined from this control DNA sample was 2.4%. The major focus of our work was to introduce a well-controlled high-throughput genotyping method to obtain reliable estimates for relative telomere length with low amounts of DNA. This is very similar to the performance of a high-quality enzyme linked immuno-sorbent assay (ELISA) for measurement of standard biomarkers.

With respect to the reliability of the qPCR approach in general, we would like to point to another very recent article on telomere attrition rates over 10 years, which had a very similar study design: Nordfjäll et al.6 investigated the dependency of the telomere attrition rate on the baseline telomere length and found a correlation coefficient nearly identical to ours (r = 0.752 in Nordfjäll et al.6 versus r = 0.674 in Ehrlenbach et al.2). This is another strong indication that the qPCR approach is highly reliable, valid and replicable. In addition, recent papers in high-ranking journals (e.g. Lancet3, PLOS Genetics6) on telomere dynamics used the qPCR approach instead of the TRF methodology for association studies, thus indicating that qPCR is becoming a state-of-the-art technology for inferring relative telomere length and associated phenotypic consequences.

We certainly respect that researchers like Dr Aviv passionately advance their view. However, ‘raising the bar on telomere biology’ does not necessarily mean sticking to old, but still valuable, methods, but to take a step forward towards new technologies, thus enabling assessment of novel risk conditions in a large number of epidemiological studies.

References

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Chronic disease prevention: the importance of calls to action

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We welcome the stimulating editorial by Shah Ebrahim on our call to action to scale up the prevention and control of chronic diseases.1 It is an important contribution to efforts to ensure that chronic diseases receive the global and national attention commensurate with their enormous health and economic burdens, especially in low- and middle-income countries.2

Our call for action is 2-fold: an urgent call to implement policies where we already have the evidence on intervention cost-effectiveness (salt reduction, tobacco control and a multiple drug regime for managing high cardiovascular risk); and a similar call to collect rigorous evidence on other beneficial interventions with the potential for making a difference in populations. Regarding the latter, the editorial confirms...