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Commentary: Raising the bar on telomere epidemiology

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Ehrlenbach and co-workers[1] report, in this issue of the *International Journal of Epidemiology*, findings of a longitudinal, population-based study in 510 individuals. They observed that individuals who died during a 10-year follow-up had shorter relative telomere length (RTL), measured by quantitative polymerase chain reaction (qPCR), than those who survived. They also confirmed from the previous work that the rate of leucocyte telomere length (LTL) shortening was proportional to baseline LTL.[2]

Though a controversy had existed about whether LTL predicts survival in the elderly,[3,4] more recent studies in same-sex elderly twins[5,6] showed that the co-twin with the shorter LTL was likely to die first. The same-sex twin model is a powerful tool to test the LTL–survival connection, since it avoids statistical adjustments for age and sex. Therefore, at first glance, the findings of Ehrlenbach lend further support to the thesis that, in the elderly, LTL predicts survival.

Still, it is worthwhile to probe the findings presented in this article because the authors assert that their qPCR method is superior to another method of telomere length measurement—the Southern blot analysis of the terminal restriction fragment (TRF) length. They indicate that 'apart from other numerous drawbacks, the TRF technique is time consuming, difficult to quantify, and requires large amounts of DNA'. In stating this opinion, the authors joined a growing cadre of qPCR users who reflexively criticize the Southern blot analysis. For instance, Shen *et al*.[7] articulated the familiar script about the disadvantages of the Southern blot method to justify the use of the qPCR, of which the coefficient of variation (CV) in their hands, expressed in replicates performed on different occasions, was 27%. This fact alone nullified any conclusion derived from the findings reported by the authors.

The first telling finding of the present work is that in spite of the age range of more than four decades at baseline (Figure 3 in Ehrlenbach *et al*.[1]), age explains <1% of the RTL of participants. This is a remarkably low value, considering the wide age range of the sample. In this regard, there is a discrepancy in data presented within Table 1, and between Table 1 and Figure 3.[1] 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[1] suggest that the age range of the sample was in fact ~45–85 years, which is in conflict with Table 1.[1]

The second perplexing finding is the vast inter-individual variation in RTL, expressed as telomere (T)/single-gene (S) ratio for individuals of about same age. For instance, the T/S ratio for participants in their 40s ranged from ~0.5 to 4 (Figure 3[1]). Such a large inter-individual variation in LTL is highly unusual when LTL is measured by Southern blots and it might explain why age accounts for <1% of the inter-individual variation in RTL among all participants. Part of the discrepancy between Southern blot results and the qPCR relates to the fact that whereas the qPCR strictly measures the canonic part of the telomeres (i.e. only TTAGGG repeats), the Southern blot results include the sub-telomeric (non-canonic region) up to the restriction site of the enzyme digest. However, this difference hardly explains the vast inter-individual variation in RTL for a given age observed in this study. A similar problem is often observed in other studies using the qPCR.[8]

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

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to assess reproducibility of assays in large-scale epidemiological studies. However, such a value is still unusually low even for intra-assay qPCR runs.

Another ambiguous finding of the present study is that participants who died during the follow-up period had a shorter RTL than those who survived. But participants who died were clearly older on average than the survivors (Table 1 and Figure 3)[1] and no information is provided about the sex distribution in surviving or deceased participants. Based on data presented in Table 1,[1] the age range in 1995 for deceased participants was 67–81 years. It is not enough to compare RTL in survivors aged >67 years with the deceased, since, on average, the age of survivors >67 years can still be younger than that of the deceased. Older persons are more likely to die than younger ones and the life expectancy of women is greater than that of men. The question, then, is whether the relationship between RTL and survival would hold after adjustment for age and sex, even though the effects of both on RTL were marginal, perhaps because of the use of the qPCR.

Clearly, the results of this paper display a puzzling dichotomy. While the authors claim that their qPCR method is highly accurate and reproducible, their concrete findings hardly reflect this assertion. The Southern blot analysis of the TRF is indeed labour intensive, costly, requires lots of DNA and demands experience and expertise, which explain why this method, considered the gold standard of telomere length measurements, is not now widely used in epidemiology. The qPCR method has become the favourite among epidemiologists and clinical researchers because of features such as high throughput and low cost, which have little to do with reproducibility and accuracy. But when shortcuts are taken for the sake of convenience and cost, over time they erode the fundamentals of scientific inquiry. 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.[9] Only then we will bolster the trust of the scientific community in telomere epidemiology and establish its prominence in ageing research.

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References

[1] Ehrlenbach S, Willeit P, Kiechl S *et al*. Influences on the reduction of relative telomere length over 10 years in the population-based Bruneck Study: introduction of a well-controlled high-throughput assay. *Int J Epidemiol* 2009;**38:**1725–34.

[2] Aviv A, Chen W, Gardner JP *et al*. Leukocyte telomere DYNAMICS: longitudinal findings in young adults of the Bogalusa Heart Study. *Am J Epidemiol* 2009;**169:** 323–29.

[3] Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 2003;**361:**393–95.

[4] Martin-Ruiz CM, Gussekloo J, van Heemst D, von Zglinicki T, Westendorp RG. Telomere length in white blood cells is not associated with morbidity or mortality in the oldest old: a population-based study. *Aging Cell* 2005;**4:**287–90.

[5] Kimura M, Hjelmborg JV, Gardner JP *et al*. Short leukocyte telomeres forecast mortality: a study in elderly Danish twins. *Am J Epidemiol* 2008;**167:**799–806.

[6] Bakaysa SL, Mucci LA, Slagboom PE *et al*. Telomere length predicts survival independent of genetic influences. *Aging Cell* 2007;**6:**769–74.

[7] Shen J, Terry MB, Gurvich I, Liao Y, Senie RT, Santella RM. Short telomere length and breast cancer risk: a study in sister sets. *Cancer Res* 2007;**67:**5538–44.

[8] Njajou OT, Cawthon RM, Damcott CM *et al*. Telomere length is paternally inherited and is associated with parental lifespan. *Proc Natl Acad Sci USA* 2007;**104:**12135–39.

[9] Aviv A. The epidemiology of human telomeres: faults and promises. *J Gerontol A Biol Sci Med Sci* 2008;**63:**979–83.