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# Improving comparability between qPCR-based telomere studies

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**Abstract**

Comparability of findings from qPCR-based telomere studies is hampered by such measurement results being assay-specific, precluding a direct quantitative comparisons of observed differences and/or slopes of associations between studies. It is proposed that this can be partially alleviated by expressing qPCR-based telomere data as Z-scores.

**KEYWORDS**

comparability, qPCR, standardization, telomeres

Telomeres, the complex of the repeated DNA sequence TTAGGGn and associated proteins at the end of linear chromosomes, are attracting increasing attention because of associations of telomere length with morbidity and mortality (e.g., Boonekamp, Simons, Hemerik, & Verhulst, 2013; Verhulst et al., 2016). There are multiple techniques to measure telomeres, each with their own advantages and disadvantages (Aubert & Lansdorp, 2008; Nussey et al., 2014), but the majority of studies have measured telomere length using either real-time qPCR (Cawthon, 2002) or Southern blot (Kimura et al., 2010). Practical differences in these methods have been extensively discussed elsewhere (Aubert & Lansdorp, 2008; Nussey et al., 2014), but a relatively neglected consequence of the choice of technique is that qPCR yields information on telomere length in the form of a scale-free ratio between the number of telomeric repeats and a 'control gene' (rTL), while Southern blot yields an estimate of telomere length (TL) expressed in base pairs. Due to technical limitations, rTL values are not comparable between assays in different laboratories, or sometimes between different runs within laboratories (Martin-Ruiz et al., 2015). This stochastic element precludes a direct comparison of results between studies. For example, two qPCR-based studies may each report a sex difference in rTL, with the same sample size and the same statistical result, while the numerical sex-effect on rTL is very different (see below for an example). This contrast may

be due to a substantial difference in sex-effect between the studies, or, alternatively, it may be due to rTL values differing between assays/laboratories, and identifying the more likely explanation is not usually straightforward. Some studies report differences between groups as a percentage, but a percentage difference is meaningless with the base (i.e., a 100%) being an assay-linked number that contains no real information. Another solution opted for in some studies is to measure a selection of samples with another technique such as Southern blot and use the results to estimate telomere length in base pairs from the regression of Southern blot values on the qPCR values. While this may work in theory, in practice success is limited, with predicted TL values in some cases well outside the range normally encountered. A further disadvantage of this approach is that it is likely that the Southern blot measurement will have to be repeated for every data set because the general level of the rTL values varies from assay to assay (Martin-Ruiz, Gussekloo, van Heemst, von Zglinicki, & Westendorp, 2005).

The incomparability problem of qPCR-based telomere measurements can only be resolved through standardization of the qPCR measurements to the extent that assay-dependent variation becomes negligible. While such a standardization may at some point be achieved, in the meantime I here propose a simple method that at least mitigates the incomparability problem. Comparability between

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studies can be improved by transforming telomere measurements to a Z-score (also known as the standard normal distribution) prior to the statistical analysis. Transforming data to a Z-score is achieved by first subtracting the overall mean rTL from the raw data, and, second, dividing the resulting mean centred data by the standard deviation of rTL. The first step transforms mean rTL to equal zero, while the second step transforms the standard deviation of rTL to equal 1. The result of this transformation is that estimated differences and regression coefficients are expressed in standard deviations (SD), which are directly comparable between studies. To illustrate this, consider two studies that compared rTL between the sexes, with one study reporting females to have 0.18 longer rTL (Bosquet Enlow et al., 2019) and another study reporting a difference of 0.052 (Diez Roux et al., 2009). Thus, there was a 3.5-fold difference in observed sex-effect on rTL between these studies. However, when the differences are expressed in standard deviations (0.76 and 0.14 respectively), the sex differences are 0.24 and 0.37 respectively. These values are now directly comparable, and indeed the difference in estimated sex effect has become much smaller (and has reversed!). Moreover, they can be compared statistically using the standard errors of the estimates, which was not possible with the untransformed estimates.

Meta-analyses are an important tool to synthesize research results, and this analysis typically involves the transformation of observed differences to standardized effect sizes (Nakagawa & Cuthill, 2007). This in itself can be a complex procedure (e.g., Gardner et al., 2014), depending on which statistical information is reported, and hence is prone to error. Moreover, published studies often lack the information required to calculate standardized effect sizes, which then needs to be resolved through contacting the authors, which costs time, and will rarely be 100% successful (e.g., Gardner et al., 2014). Indeed, a non-negligible proportion of publications provided insufficient information to be included in a meta-analysis of associations between telomere length and exposure to stress and adversity (Pepper, Bateson, & Nettle, 2018), considerably reducing the impact of the omitted studies. Transforming qPCR-based telomere measurements to a Z-score alleviates these problems, increasing publication impact, because results are expressed as standardized effect sizes, and hence can in many cases be directly entered in a meta-analysis.

Generating rTL data from the raw qPCR data involves a number of calculations, which can involve corrections for methodological issues such as the type of buffer in which blood was stored when this varies between samples (Eastwood, Mulder, Verhulst, & Peters, 2018) or effects of position on the plates (Eisenberg, Kuzawa, & Hayes, 2015). The SD of the raw data will be larger than that of the data after correction for methodological effects, because the purpose of the corrections is to remove methodologically caused variance from the total variance. Because the corrections serve to remove noise from the data rather than biological variation it is preferable to transform the data after the corrections have been applied. Likewise, when the rTL distribution gives reason to log-transform the qPCR data, the transformation to Z-scores should be done after the

log-transformation. Lastly, it is worth noting that a Z-transformation does not by itself change the shape of the distribution and therefore does not affect what is the best statistical approach.

The benefits of transforming data to a Z-score are not restricted to qPCR-based TL measurements, and this procedure has previously been advocated in many other fields (e.g., Cheadle, Vawter, Freed, & Becker, 2003; Curtis, Smith, Ziganshin, & Elefteriades, 2018). In general, transforming data to a Z-score will improve the comparability of results whenever a direct comparison between raw data is not informative. This can arise whenever there are non-negligible effects of batch, assay type or laboratory, making comparisons between raw data uninformative.

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## REFERENCES

- Aubert, G., & Lansdorp, P. (2008). Telomeres and aging. *Physiological Reviews*, 88, 557–579. <https://doi.org/10.1152/physrev.00026.2007>
- Boonekamp, J. J., Simons, M. J. P., Hemerik, L., & Verhulst, S. (2013). Telomeres behave as measure of somatic redundancy rather than biological age. *Aging Cell*, 12, 330–332.
- Bosquet Enlow, M., Sideridis, G., Bollati, V., Hoxha, M., Hacker, M. R., & Wright, R. J. (2019). Maternal cortisol output in pregnancy and newborn telomere length: Evidence for sex-specific effects. *Psychoneuroendocrinology*, 102, 225–235. <https://doi.org/10.1016/j.psyneuen.2018.12.222>
- Cawthon, R. (2002). Telomere measurement by quantitative PCR. *Nucleic Acids Research*, 30, e47. <https://doi.org/10.1093/nar/30.10.e47>
- Cheadle, C., Vawter, M. P., Freed, W. J., & Becker, K. G. (2003). Analysis of microarray data using Z score transformation. *The Journal of Molecular Diagnostics*, 5, 73–81. [https://doi.org/10.1016/S1525-1578\(10\)60455-2](https://doi.org/10.1016/S1525-1578(10)60455-2)
- Curtis, A., Smith, T., Ziganshin, B., & Elefteriades, J. (2018). The mystery of the Z-score. *Aorta*, 4, 124–130.
- Diez Roux, A. V., Ranjit, N., Jenny, N. S., Shea, S., Cushman, M., Fitzpatrick, A., & Seeman, T. (2009). Race/ethnicity and telomere length in the Multi-Ethnic Study of Atherosclerosis. *Aging Cell*, 8, 251–257. <https://doi.org/10.1111/j.1474-9726.2009.00470.x>
- Eastwood, J. R., Mulder, E., Verhulst, S., & Peters, A. (2018). Increasing the accuracy and precision of relative telomere length estimates by RT qPCR. *Molecular Ecology Resources*, 18, 68–78. <https://doi.org/10.1111/1755-0998.12711>
- Eisenberg, D. T. A., Kuzawa, C. W., & Hayes, M. G. (2015). Improving qPCR telomere length assays: Controlling for well position effects increases statistical power. *American Journal of Human Biology*, 27, 570–575. <https://doi.org/10.1002/ajhb.22690>
- Gardner, M., Bann, D., Wiley, L., Cooper, R., Hardy, R., Nitsch, D., ... Ben-Shlomo, Y. (2014). Gender and telomere length: Systematic review and meta-analysis. *Experimental Gerontology*, 51, 15–27. <https://doi.org/10.1016/j.exger.2013.12.004>
- Kimura, M., Stone, R. C., Hunt, S. C., Skurnick, J., Lu, X., Cao, X., ... Aviv, A. (2010). Measurement of telomere length by the Southern blot analysis of terminal restriction fragment lengths. *Nature Protocols*, 5, 1586–1607. <https://doi.org/10.1038/nprot.2010.124>
- Martin-Ruiz, C. M., Baird, D., Roger, L., Boukamp, P., Krunic, D., Cawthon, R., ... von Zglinicki, T. (2015). Reproducibility of telomere length assessment: An international collaborative study. *International Journal of Epidemiology*, 44, 1673–1683. <https://doi.org/10.1093/ije/dyu191>
- Martin-Ruiz, C., Gussekloo, J., van Heemst, D., von Zglinicki, T., & Westendorp, R. (2005). Telomere length in white blood cells

- is not associated with morbidity or mortality in the oldest old: A population-based study. *Aging Cell*, 4, 287–290. <https://doi.org/10.1111/j.1474-9726.2005.00171.x>
- Nakagawa, S., & Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: A practical guide for biologists. *Biological Reviews*, 82, 591–605. <https://doi.org/10.1111/j.1469-185X.2007.00027.x>
- Nussey, D. H., Baird, D., Barrett, E., Boner, W., Fairlie, J., Gemmell, N., ... Monaghan, P. (2014). Measuring telomere length and telomere dynamics in evolutionary biology and ecology. *Methods in Ecology and Evolution*, 5, 299–310. <https://doi.org/10.1111/2041-210X.12161>
- Pepper, G. V., Bateson, M., & Nettle, D. (2018). Telomeres as integrative markers of exposure to stress and adversity: A systematic review and meta-analysis. *Royal Society Open Science*, 5, 180744. <https://doi.org/10.1098/rsos.180744>
- Verhulst, S., Dalgård, C., Labat, C., Kark, J. D., Kimura, M., Christensen, K., ... Benetos, A. (2016). A short leucocyte telomere length is associated with development of insulin resistance. *Diabetologia*, 59, 1258–1265. <https://doi.org/10.1007/s00125-016-3915-6>

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