



TRN Recommendations for the measurement of telomere length in population studies

Background: The Telomere Research Network is a collaborative effort between telomere researchers and the NIH to establish best practices and methodologic guidelines for population based studies of telomere length in relation to psychosocial and environmental exposures and a predictor of later health outcomes. As this multi-year effort conducts methodologically rigorous cross laboratory and cross method comparison studies, we regularly seek to provide data-based recommendations to enhance the reproducibility and rigor of telomere length studies.

RECOMMENDATION 2: All DNA samples for a specific cohort or study should be extracted with the same extraction method utilizing a single protocol. In cohorts where samples have already been extracted investigators should analytically account for this variability and proceed with caution, acknowledging the potential that variability in sample processes may impact the affect the feasibility of telomere length studies in some cohorts.

Key considerations: Several previous studies have reported variability in telomere length measurement in different samples types and species as a function of different DNA extraction methods [1-7]. Universally these studies have focused on the impact of DNA extraction methodology on qPCR-based methods performed in a single laboratory. The TRN is conducting a systematic investigation of the effect of DNA extraction methods in whole blood samples to evaluate if there is a preferred methodological approach (e.g. salting out, silica membrane and magnetic beads) that maximizes assay repeatability and replicability and expects to provide specific recommendations as data becomes available. Given existing published data and the TRN preliminary results, there is sufficient evidence to conclude that, particularly for qPCR-based studies but likely for other methodologies as well, inclusion of different DNA extraction methods introduces additional sources of variability in the repeatability and replicability of TL measurement. We note that the quality of DNA extracted for other genomic assays, such as genotyping, DNA sequencing, or DNA methylation may not be of sufficient quality for telomere length assays.

For data sets where variation in protocol cannot be avoided, two options to nevertheless combine the data sets are the following:

(1) By subjecting a few samples (e.g. 20-30) to multiple protocols, data can be generated that allow direct estimation of the 'protocol effect'. This estimate can be calculated directly, comparing samples subjected to more than one protocol, to adjust the data accordingly. Alternatively, protocol can be included as a fixed effect in the statistical models, together with sample identity as random effect (i.e. including data multiple data points per sample, one for each protocol). This solution has the advantage that it allows estimation of differences, e.g. time effects, between the data sets, which may be of relevance for longitudinal data. Care should be taken to verify whether the adjustment should be applied to the mean, the standard deviation, or both.



(2) When option 1 is not possible, the remaining option is to include protocol as fixed effect in the statistical models. It is recommended to Z-transform the data prior to analysis for each protocol separately, in effect assuming the variance is the same for each protocol. Using this approach, changes from one time-point (protocol) to the next cannot be estimated, but it remains possible to test for associations with traits and to test interactions between traits and 'time', i.e. investigate causes of variation in telomere dynamics.

Note that these options can be applied to both cross-sectional and longitudinal data.

References:

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