Telomere length, health, and mortality in a cohort of older Black South African adults

METHODS SUPPLEMENT

Sample type, storage, extraction, and integrity

Leukocyte samples from venous blood draw were used for telomere length assays and stored at -20°C prior to extraction. The Qiagen DNeasy Blood and Tissue Kit was used to extract DNA. Five nanograms of genomic DNA were dried down in each 384-well plate and resuspended in 10µL of either the telomere or 36B4 polymerase chain reaction (PCR) reaction mixture and stored at 4°C up to 6 hours. 100% of samples used for Standard and QC tests were tested for DNA quality and integrity. DNA quality and integrity were documented using the GE NanoVue UV/visible spectrophotometer.

qPCR assay

Real Time qPCR was used to determine the ratio of telomere repeat copy number to single-copy gene copy number. This is referred to as the Telomere to Single Gene (T/S) ratio and its value should be proportional to the average telomere length. The T/S ratio value for all samples was then compared to that of a reference DNA sample to normalize for experimental variations. We used the Thermo Fisher PowerUp SYBR Green Master Mix, Thermo Fisher dried PCR primers, and Thermo Scientific HyClone Hypure Molecular Biograde water as the master mix and reagents. Final reaction volume was 10 ul per reaction. The telomere primer sequences were as follows:

Tel 1 GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT
Tel 2 TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA

Final concentrations of Telo-1 and Telo-2 were 270nM and 900nM, respectively.

The 36B4 single copy gene PCR primers were as follows:

36B4u CAGCAAGTGGGAAGGTGTAATCC
36B4d CCCATTCTATCATCAACGGGTACAA

Final concentrations of 36B4u and 36B4d were 300nM and 500nM, respectively. The full PCR programs for the telomere and single gene cycling are as follows:

Telomere Cycling:
HOLD: 50°C for 2 minutes 95°C for 2 minutes
PCR: 35 cycles as default. Run full 35 cycles for test plate and based on the plateau, you can change the # of cycle.
95°C for 15 seconds
54°C for 2 minutes (Data Collection)

36B4 Cycling:
HOLD: 50°C for 2 minutes 95°C for 2 minutes
PCR: 40 cycles as default. Run full 40 cycles for test plate and based on the plateau, you can change the # of cycle.
95°C for 15 seconds
58°C for 1 minute and 10 seconds (Data Collection)

The PCR efficiency of the single copy gene and telomere primers was between 93-100%. Each 384-well plate also contained a 6-point standard curve from 0.625ng to 20ng using pooled buffy-coat derived genomic DNA. The standard curve assessed and compensated for inter-plate variations in PCR efficiency. The slopes of the standard

curve for both the telomere and 36B4 reactions were -3.33±0.33 and the linear correlation coefficient (R^2) values for both reactions were over 0.99.

Data analysis

Mean T/S ratio was 0.77 (SD=0.1). All samples for both the telomere and single-copy gene (36B4) reactions were performed in triplicate on different plates. The T/S ratio (-dCt) for each sample was calculated by subtracting the average 36B4 Ct value from the average telomere Ct value. The relative T/S ratio (-ddCt) was determined by subtracting the T/S ratio value of the 5ng standard curve point from the T/S ratio of each unknown sample. The relative T/S ratio was exponentiated to adjust for skew.

Quality control samples were interspersed throughout the test samples to assess inter-plate and intra-plate variability of threshold cycle (Ct) values. A combined inter- and intra-assay coefficient of variation (CV) calculated from the relative T/S ratio (-ddCt) of quality control samples is 8.5%. If CV for triplicate values was ≥ 2.0, we deleted one value that was more than one standard deviation apart from the average of triplicate CT. If it was still ≥ 2.5, we failed the entire ID, as the data is considered not valid. Of the sample, 2% failed final QC and were thus excluded from data analysis.