SWAN Repository Dataset Documentation

Study: App019, "Weathering and telomeres: A novel approach to the study of racial disparities in health", Geronimus
Dataset: SWANRep019_Telomeres
Cohort: SWAN; Michigan, MGH, Chicago & Pittsburgh Sites

DNA samples from non-Hispanic black and white women from the SWAN Repository were received for the calculation of telomere length using qPCR methods. Published results are based on data from the triplicate qPCR runs conducted on 239 women.

The methods used from qPCR telomere length determination are based on Cawthon (2002) and published in Geronimus et al (2010). Excerpts from the methods section and appendices of Geronimus et al (2010) follow.

We measured telomere length by a ratio of telomere repeat copy number to a known single copy gene (36B4). Laboratory personnel conducting the telomere length measurements were completely blinded to all known characteristics of the SWAN participants. DNA was quantified using the ABI Quantifiler Human DNA Quantification Kit followed by analysis on the ABI 7500 Real-Time PCR instrument according to the manufacturer's protocol (Green, Roinestad, Boland, & Hennessy, 2005). We measured telomere length using real-time qPCR (Cawthon, 2002) with the several modifications. The ratio of telomere repeat copy number to 36B4 was compared to samples whose telomere lengths were known, as measured by the traditional Southern Blot method (Cawthon, 2002; Slagboom, Droog, & Boomsma, 1994). Separate amplification reactions were prepared for analysis of the telomeric repeat units and the 36B4 gene; all samples were analyzed in triplicate.

The telomere amplification reaction for each sample included 35ng of sample DNA, 25µl of Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA), 40ng of E. Coli DNA, and the telomere primers, which have been specifically designed to prevent the formation of primer dimmers (Cawthon, 2002). Final reaction volume was 50µl. Telomere primer sequences were: tel 1b 5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3' (final concentration of 100nM) and tel 2b 5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3' (final concentration of 900 nM) (Gil & Coetzer, 2004). The single copy gene amplification reaction for each sample included 35ng of sample DNA, 25µl of Power SYBR® Green PCR Master Mix, 36B4 primers, and deionized, distilled water to a final volume of 50µl.   The 36B4 primer sequences are: 36B4u 5'-CAGCAAGTGGGAAGGTGTAATCC-3' (final concentration of 300 nM) and 36B4d 5'- CCCATTCTATCATCAACGGGTACAA-3' (final concentration of 500 nM). All amplification runs were prepared in MicroAmp Optical 96-well reaction plates (Applied Biosystems, Foster City, CA). For each amplification run, known standards from

a single DNA reference sample were also amplified (as described above) to ensure that the amplification was functioning as expected. Following the addition of all sample or standard DNA and reagents, the plates were sealed with a MicroAmp Optical Adhesive film (Applied Biosystems, Foster City, CA) and centrifuged at 3,000 rpm for approximately 20 seconds.

All reactions were performed on the ABI 7500 Real-Time qPCR system. Both amplifications (telomere and 36B4) included a heat-activation step at 95°C for 10 minutes. For the telomere amplification, this was followed by 25 cycles of 95°C for 15 seconds and 54°C for one minute. For the 36B4 amplification, this was followed by 30 cycles of 95°C for 15 seconds, 58°C for one minute. Fluorescence data was collected during the annealing/extension steps of both reactions. The instrument was set to run in 9600 emulation mode with auto ramping. Resulting data was analyzed with ABI's SDS v1.2 software package using a manual C_t of 0.06 and the auto baseline setting. Telomere: 36B4 C_t ratios and telomere length were calculated using Cawthon's (2002) formula.

Note that because the relative T/S is based on the average DCt of the sample included in the analyses, relative T/S changes slightly when the sample is changed. For example, if one regression model includes 300 women, each of these women will have a particular relative T/S ratio. However, for another regression model that includes only 250 women, each of these women will a slightly different relative T/S ratio.

Dataset contents

#	Variable	Type	Description
1	ARCHID	Char	Encrypted SWAN Subject ID
2	T	Num	Telomere amplification Ct
3	S	Num	36B4 (single copy gene) amplification Ct
4	_Ct	Num	ΔCt
5	T_S	Num	T/S
6	RelT_S	Num	Relative T/S
7	TRFLength	Num	TRF Length
8	AvgTrip_T_S	Num	Average of triplicate run, T/S
9	AvgTrip_RelT_S	Num	Average of triplicate run, Relative T/S
10	AvgTrip_RelT_S_sd	Num	Average of triplicate run, Relative T/S sd
11	AvgTrip_TRFLength	Num	Average of triplicate run, TRF length

T and S data are Ct values, using fixed thresholds of .06