**Mediation Results**

Intervention Condition Predicting Parent TL via Parenting, Interparental Conflict, and Alcohol Use

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Parenting | Interparental Conflict | Alcohol Use | Parent TL |
|  | *B* (SE) | *p* | *B* (SE) | *p* | *B* (SE) | *p* | *B* (SE) | *p* |
| Intervention  | .15 (.14) | .29 | .19 (.16) | .24 | -.24 (.14) | .09 | .28 (.14) | .06 |
| Interparental Conflict | - | - | - | - | - | - | -.04 (.17) | .84 |
| Alcohol Use | - | - | - | - | - | - | .20 (.14) | .17 |
| Positive Parenting | - | - | - | - | - | - | -.06 (.14) | .64 |
| ACEs1 | - | - | - | - | - | - | -.16 (.14) | .26 |
| Education | - | - | - | - | - | - | -.34 (.13) | **.02** |

1Parent ACEs was modeled as a correlate of intervention condition (*r* = .02, *p* = .91). TL = Telomere length, ACEs = Adverse childhood experiences.

Intervention Condition Predicting Child TL via Parenting, Interparental Conflict, and Alcohol Use

|  |  |  |  |
| --- | --- | --- | --- |
|  | Parenting | Interparental Conflict | Child TL |
|  | *B* (SE) | *p* | *B* (SE) | *p* | *B* (SE) | *p* |
| Intervention  | .09 (.18) | .62 | .27 (.17) | .14 | -.18 (.15) | .25 |
| Interparental Conflict | - | - | - | - | .10 (.18) | .59 |
| Positive Parenting | - | - | - | - | .31 (.17) | .09 |
| ACEs1 | - | - | - | - | .09 (.16) | .58 |
| Race | - | - | - | - | .37 (.14) | **.02** |

1Child ACEs was modeled as a correlate of intervention condition (*r* = .11, *p* = .45). TL = Telomere length, ACEs = Adverse childhood experiences.

**Saliva collection and tsimane telomere length laboratory methods and basic preliminary analysis**

Methods

Oragene OGR-500 saliva collection kits were mailed to families for saliva collection. Participants were provided with written and video instructions for providing the saliva samples by spitting into the collection kits. Participants were also provided a return envelope and biosafe bags for returning the collection kits. After collection was complete the sealed kits were mailed to the PI at (*University name masked for review*) upon which they were stored at room temperature for approximately 6 months. Saliva samples were shipped from (*University name masked for review*) in four cardboard storage boxes. Upon arrival at the (*Lab and university name masked for review*), storage boxes were immediately transferred to a secured BSL-2 room for storage until extraction. DNA was extracted from whole saliva using DNA Genotek’s extraction protocol. For all samples, DNA concentration and purity was measured by spectrophotometry using BioTek's Take3 plate and the Epoch microplate spectrophotometer (Winooski, VT, USA). DNA was then diluted with PCR-grade water to the working concentration (8 ng/ml) required for the monochrome multiplex quantitative PCR (MMQPCR) telomere length (TL) assay. Diluted working stock DNAs were housed in 8-tube strips sealed with strip caps and were kept at 4°C until assayed. Stock extracts were then transferred to -20°C. Remaining whole saliva samples were kept at room temperature.

Relative TL was assayed using a modified version of Cawthon’s MMQPCR assay on a CFX 384 real-time PCR detection system (Bio Rad, Hercules, CA). Reagents included PowerUp SYBR Green master mix (Applied Biosystems, Waltham, MA) and the telomere/albumin2 primer pairs published in Tackney et al., 2014. Master mix, primer, and water volumes/concentrations and PCR conditions are listed in Table 1. Upon completion of the TL assay, working stock DNA were stored at -20°C.

|  |
| --- |
| Table 1: Reagent concentrations, volumes utilized, and reaction conditions, for the MMQPCR relative TL assay |
| Storage | **Item** | **l/well** | **final conc** | **units** | **stock conc** |
| 4°C | **PowerUp SYBR Green mastermix** | 2.50 |  |  |  |
| -20°C | **telc** | 0.05 | 100 | nM | 10000 |
| -20°C | **telg** | 0.45 | 900 | nM | 10000 |
| -20°C | **albu** | 0.35 | 700 | nM | 10000 |
| -20°C | **albd** | 0.25 | 500 | nM | 10000 |
|  RT |  H2O | 0.40 |  |   |   |
|  |  |  |  |  |  |
|   |  **DNA** |  1.00 1.60 |  ng/μl | 8 |
|  |  |  |  |
| TOTAL REACTION VOLUME (μl) | **5** | **μl** |  |
|  |  |  |  |
| Cycling Profile, *Reference masked for review* |  |  |
| Hold (1x) | **Cycle 2 (x2)** | **Cycle 3 (x40)** | **Melt** |
| 15 min | 2 sec | 30 sec | 2 sec | 30 sec | 15 sec | 30 sec | 15 sec | 65-98, by 1 degree/step |
| 95°C | 98°C | 49°C | 98°C | 59°C | 74°C | 84°C | 85°C | wait 90 sec on first step |
|  |  |  |  | **Acquisition** |  |  | **Acquisition** | wait 5 seconds all other steps |

High quality DNA extracted from whole blood was used to create a six-point standard reference curve. A three-fold serial dilution was used. Standard curve concentrations ranged from 5 ng/ml for standard one to 0.02 ng/l for standard six. Standard DNA was diluted to working concentrations immediately prior to assay from a frozen stock. The same high quality DNA was diluted to 8 ng/l and used as one of three positive controls. The other two positive controls were derived from DNA extracted from whole saliva that we collected and processed in house prior to data generation.

Samples were assayed twice on two different plates on the same day. All DNA standards, samples, and negative controls were included in triplicate on each 384-we;; plate assayed. Average standard curve R2 across the two plates was 0.99 (SD = 0.01) for T and 0.98 (SD = 0.01) for S. Plate assay efficiency was 102.5% (SD = 1.8%) on average for T, 94.5% (SD < 0.1%) for S. Seven samples (n = 3 parents and 4 children) failed to amplify and were excluded.

T/S ratio is the unit of analysis for the relative TL assay. T/S ratio for each sample was calculated by dividing the estimated starting quantity (SQ) of its telomere amplicon (T) by that of a single copy gene – albumin (S) (SQT/SQS). SQ is determined by where the amplified sample falls on the standard curve. Well position was accounted for during these calculations using the method reported previously by (*reference masked for review*). T/S ratio was averaged across the sample triplicates from both plates and were included in the subsequent analyses. Average intra-sample CV was 0.075. Dixon’s Q test was used to identify significant outliers (n = 14), which were removed.

**Quality control (measures of external and internal validity)**

Correlations between both age-TL and parent TL-offspring TL are well documented in the literature and can be used as measures of external validity. After averaging T/S values across both plates, the observed relative TL-age correlation (r = -0.279, 95%CI = -0.461, -0.074) had overlapping 95% CIs with those from our cohorts from the Philippines (r = -0.18, 95%CI = -0.23, -0.13 and r = -0.19, 95%CI = -0.23, -0.15), albeit with much fewer samples. The parent TL-offspring TL correlation for these saliva samples was (r = 0.28, 95%CI = -0.06, 0.55), which also had overlapping 95% CIs with our Filipino cohort (r = 0.12, 95%CI = 0.05, 0.20), again, with a vastly smaller sample size.

T/S values from each individual plate were used to test for inter-assay reproducibility. We found good levels of reproducibility that actually exceeded those from our Filipino samples. Intra-class coefficient (ICC) across plates was calculated rather than CV. CV has been shown to be invalid for TL analysis. ICC1 (correlation of values measured for the same individual) = 0.93 (95%CI = 0.90, 0.95), ICC1k (correlation of average values measured for the same individual) = 0.97 (95%CI = 0.95, 0.98). ICCs in the Philippines for the 2016 and 2005 cohorts respectively were ICC1 = 0.77 (95%CI = 0.69, 0.83); 0.81 (95%CI = 0.79, 0.84) and ICC1k = 0.91 (95%CI = 0.87, 0.94); 0.89 (95%CI = CI 0.88–0.91)).

Tackney, J., Cawthon, R. M., Coxworth, J. E., & Hawkes, K. (2014). Blood cell telomere lengths and shortening rates of chimpanzee and human females. American Journal of Human Biology, 26(4), 452–460. http://doi.org/10.1002/ajhb.22538