



TELOMERE LENGTH, TELOMERASE ACTIVITY, AND AGE-RELATED DISEASE: ACTG NWCS 422

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Background

- Telomeres are nucleoproteins with TTAGGG repeats on chromosomes
- Shorten with cell division until critical length of senescence and dysfunction
- Telomere length (TL) maintained by telomerase -- ribonucleoprotein enzyme complex with reverse transcriptase (TERT)
- *In vitro* -- all NRTIs inhibit TERT: TFV>FTC>AZT>ABC
- *Ex vivo* -- TL shorter and TERT less active with NRTIs
- Short TL associated with death, CVD, Alzheimer's, ESRD and cancer in HIV-negative
- Which factors are associated with shorter TL or reduced telomerase activity?
- Are telomere length or telomerase activity associated with age-related disease among HIV-infected individuals on ART?

Methods

- Design: nested case-control study
- Data and banked PBMCs from ACTG Longitudinal Linked Randomized Trials (ALLRT) participants with sustained HIV RNA suppression to <200 copies/mL within 24 weeks of ART initiation without rebound for at least 96 weeks

Methods (cont.)

- Cases: non-accidental death or confirmed diagnosis of cancer, cardiovascular, liver, renal, neurocognitive, or pulmonary disease, osteoporosis/bone fractures, or diabetes mellitus; and banked PBMCs samples pre-ART and prior to event (closest within 6 months)
- Controls: 2 to 1 matched for sex, age, NRTI duration, and timing of PBMC samples without events listed above
- TL: qPCR with relative TL measured by quantifying a telomere repeat copy (T) versus single copy reference gene (S) ratio
- Telomerase activity: real-time quantitative telomeric repeat amplification protocol (RQ-TRAP)
- Multivariable logistic regression for matched case-control

Results

- Analyzed 351 ALLRT participants (117 cases and 234 controls) 117 Cases on ART for median of 4.3 years (Table 1): 14 (35%) diabetes, 33 (28%) renal disease, 18 (15%) cancer, 14 (12%) CVD, 7 (6%) death, and 4 (4%) bone fractures
- Short pre-ART TL (<0.4 T/S ratio) associated with pre-ART VL >10⁵ copies/mL (OR=1.9; 95% CI 1.2-3.0) and pre-ART TA in the lowest quartile (OR 1.8; 95% CI 1.0-3.2)
- Short pre-event TL (or matching time point) (<0.4 T/S ratio) associated with female sex (OR=2.8 ; 95% CI 1.5-4.9), non-white race (OR=1.8; 95% CI 1.2-2.8), ART with TDF (OR 1.6; 95% CI 1.0-2.5), and entry year (OR 1.1; 95% CI 1.03-1.14)
- Factors associated with age-related disease were earlier calendar study entry year, pre-ART CD4<200 cells/μl, higher pre-ART VL, initial ART regimen without TDF, lower CD4/CD8 ratio after 96 weeks on ART and smoking (Tables 1 and 2)
- TL and TA (pre-ART, pre-event and delta) were not associated with age-related disease in univariable or multivariable analyses (Table 1 and 2 and Figures)

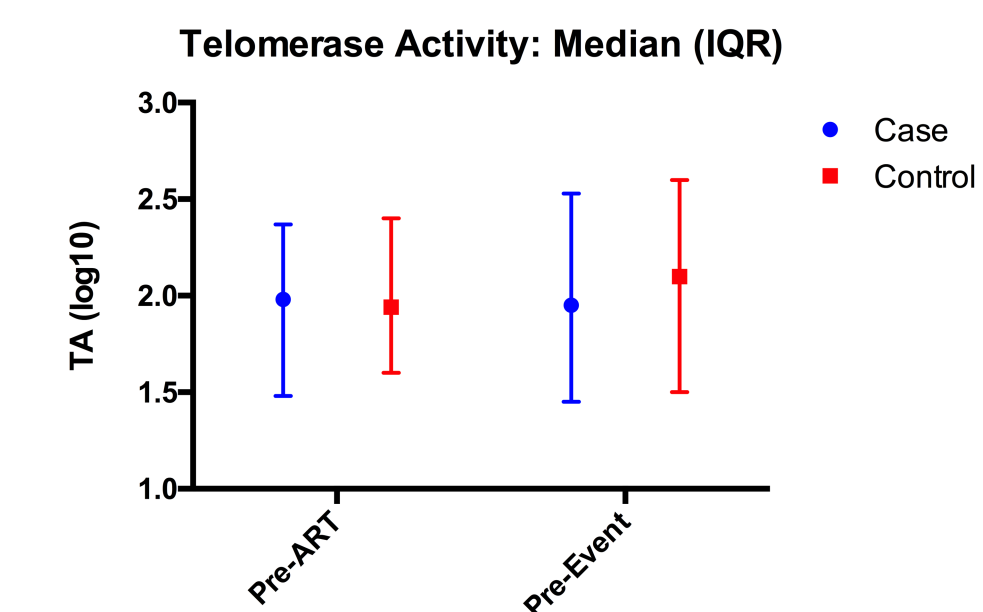
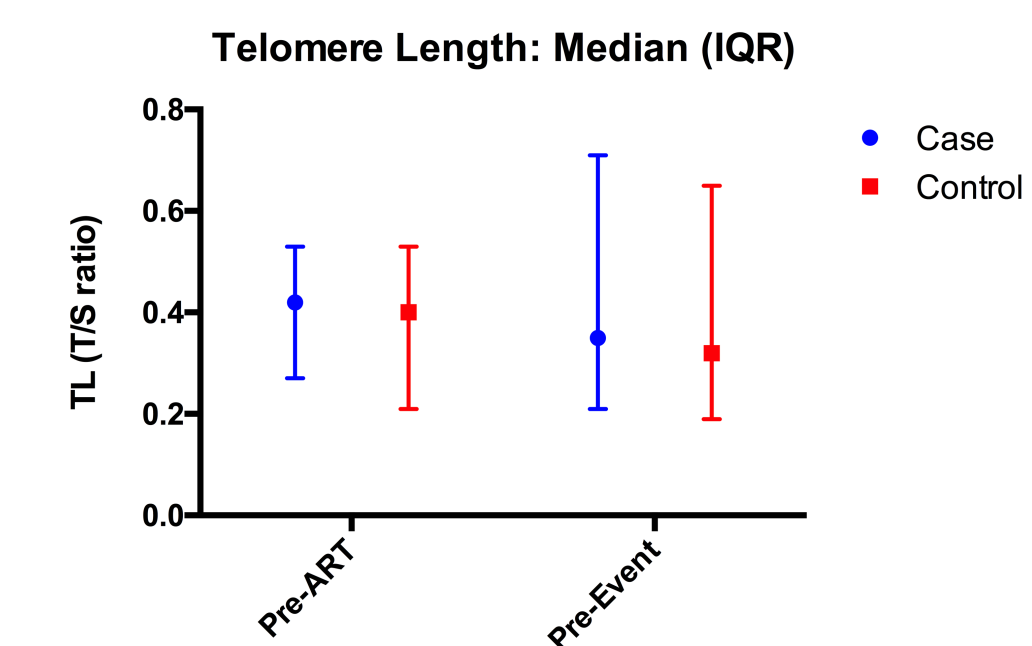
Table 1. Participant characteristics and comparison between cases and controls

| Characteristic | Case (n=117) | Control (n=234) | p-value* |
|-----------------------------------|--------------------------|--------------------------|------------------|
| Age | 43 (38, 50) | 42 (36, 49) | 0.15 |
| Female sex | 27 (23%) | 54 (23%) | 1.0 |
| White race | 54 (46%) | 110 (47%) | 0.88 |
| Smoking (ever) | 76 (65%) | 119 (51%) | 0.01 |
| Smoking (pack-yrs) | 7.5 (0, 22.5) | 0.13 (0, 10.6) | <0.001 |
| IDU | 14 (12%) | 15 (6%) | 0.07 |
| ART with TDF | 28 (24%) | 119 (51%) | <0.001 |
| Entry Year | 2002 (1999, 2003) | 2004 (2002, 2010) | <0.001 |
| Time to event (years) | 4.3 (2.7, 7.6) | -- | -- |
| <i>Pre-ART</i> | | | |
| CD4 (cells/μl) | 204 (77, 393) | 254 (89, 397) | 0.70 |
| <200 | 58 (50%) | 90 (38%) | 0.05 |
| CD8 (cells/μl) | 857 (545, 1156) | 752 (551, 1128) | 0.38 |
| CD4/CD8 | 0.23 (0.11, 0.41) | 0.33 (0.14, 0.45) | 0.29 |
| HIV RNA (log₁₀) | 4.9 (4.5, 5.5) | 4.7 (4.3, 5.3) | 0.01 |
| TL (T/S ratio) | 0.42 (0.27, 0.53) | 0.40 (0.21, 0.53) | 0.19 |
| TA (log ₁₀) | 1.98 (1.48, 2.37) | 1.94 (1.60, 2.40) | 0.67 |
| <i>Pre-Event**</i> | | | |
| TL (T/S ratio) | 0.35 (0.21, 0.71) | 0.32 (0.19, 0.65) | 0.30 |
| TA (log ₁₀) | 1.95 (1.45, 2.53) | 2.1 (1.5, 2.6) | 0.34 |
| <i>Delta***</i> | | | |
| TL (T/S ratio) | +0.0006 (-0.22, +0.37) | -0.01 (-0.26, +0.28) | 0.44 |
| TA (log ₁₀) | +0.07 (-0.53, +0.69) | +0.02 (-0.55, +0.76) | 0.86 |
| CD4/CD8 96 weeks | 0.59 (0.37, 0.89) | 0.72 (0.46, 1.0) | 0.02 |

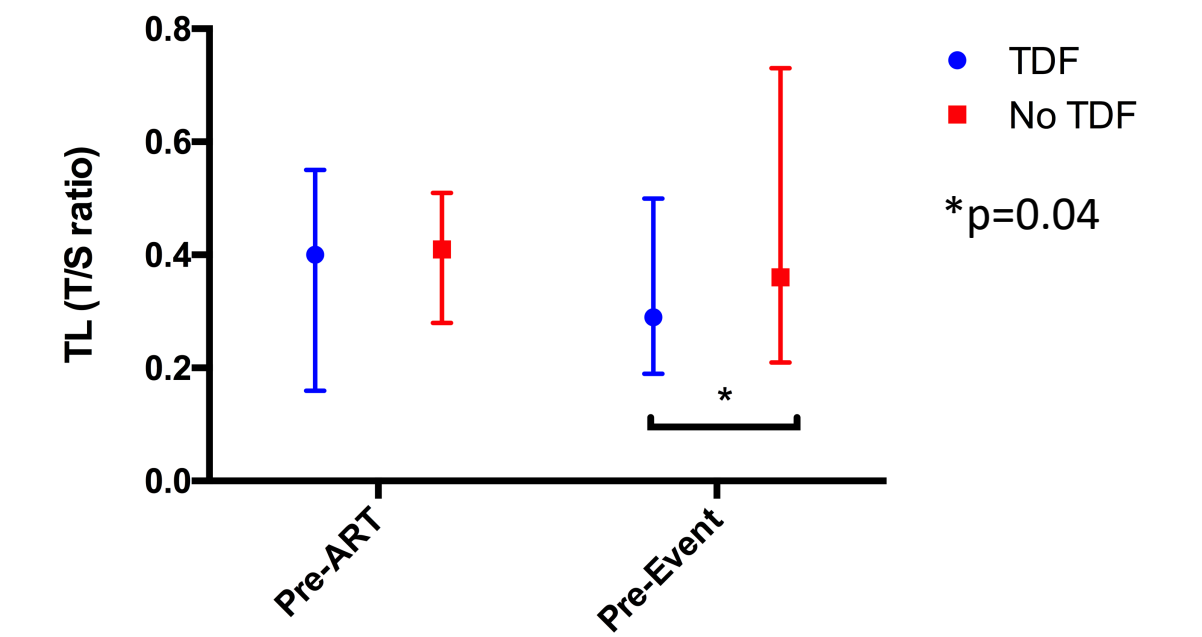
N (%) or median (IQR); *Comparison between cases and controls by Fischer exact test or Wilcoxon rank test; **For controls at matching time point; ***Change from pre-ART to pre-event or matching time point

Table 2. Multivariable case-control logistic regression

| | OR | 95% CI |
|-----------------------------------|------------|-----------------|
| Pre-Event TL (T/S ratio) | | |
| <0.19 | 1.0 | 0.50, 2.0 |
| 0.19 – 0.33 | 0.97 | 0.49, 1.9 |
| 0.34 – 0.65 | 0.93 | 0.47, 1.83 |
| >0.65 | 1.0 | -- |
| Smoking (ever) | 2.2 | 1.4, 3.7 |
| ART with TDF | 0.54 | 0.19, 1.56 |
| Entry Year | 0.95 | 0.84, 1.1 |
| Pre-ART CD4<200 | 1.1 | 0.61, 2.0 |
| Pre-ART HIV RNA per log copies/mL | 1.34 | 0.94, 1.9 |
| CD4/CD8 ratio 96 weeks | 0.93 | 0.47, 1.83 |



Telomere Length: Median (IQR) by initial ART with TDF



Conclusions

- Prior to ART, telomeres were significantly shorter among participants with higher HIV RNA and lower telomerase activity
- After median of 4 years on ART, telomeres declined (non-significant) and were significantly shorter among women, non-white race and those on ART with TDF
- TA did not change over time on ART
- We found no associations between TL, TA or changes in these biomarkers on ART, and age-related diseases
- The only independent factor associated with age-related disease in this study was smoking

Future Directions:

- Longer term follow-up (>10 years on ART) in older populations (>60 years) with more events and time points (ACTG HALLO)
- TL of CD8+CD28- T cells expressing CD57
- TL/TA after TAF switch -- intracellular TFV 7-10x higher