Telomere Length Declines In Persons Living With HIV Before Antiretroviral Therapy Start But Not After Viral Suppression: A Longitudinal Study Over >17 Years

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Keywords: HIV infection, telomere length, multivariable analysis, antiretroviral therapy

Key Points: 107 Swiss HIV Cohort Study participants contributed longitudinal samples. During untreated chronic HIV infection (median observation time, 7.7 years) but not during suppressive antiretroviral therapy (median observation time, 9.8 years) we recorded significant telomere length decline.

Abstract

Background: In people living with HIV (PWH), long-term telomere length (TL) change without/with suppressive antiretroviral therapy (ART) and the contribution of genetic background to TL are incompletely understood.

Methods: We measured TL change in peripheral blood mononuclear cells by quantitative PCR in 107 Swiss HIV Cohort Study participants with longitudinal samples available both before and during suppressive ART. We applied mixed effects multi-level regression to obtain uni-/multivariable estimates for longitudinal TL dynamics including age, sex, and CD4:CD8 ratio. We assessed the effect of individual antiretrovirals and of an individual TL-polygenic risk score (TL-PRS; based on 239 single nucleotide polymorphisms) on TL in 798 additional participants from our previous longitudinal studies.

Results: During untreated HIV infection (median observation, 7.7 [interquartile range, IQR, 4.7-11] years), TL declined significantly (median -2.12%/year; IQR, -3.48% to -0.76%/year; p=0.002). During suppressive ART (median observation, 9.8 [IQR, 7.1-11.1] years), there was no evidence of TL decline or increase (median +0.54%/year; IQR, -0.55% to +1.63%/year; p=0.329). TL-PRS contributed to TL change (global p=0.019) but particular antiretrovirals did not (all p>0.15).

Discussion: In PWH, TL is associated with an individual polygenic risk score. TL declined significantly during untreated chronic HIV infection but no TL change occurred during suppressive ART.

Key words: HIV, longitudinal study, telomere length, polygenic risk score, aging, antiretroviral therapy

Introduction

Telomere length (TL) shortens with age and short TL is associated with coronary artery disease (CAD) and all-cause mortality in the general population [1,2]. In people living with HIV (PWH), we and others have reported associations of short TL with CAD events [3], metabolic syndrome [4], and neurocognitive impairment [5]. This is of particular relevance because PWH have shorter TL [6-8], and may have accelerated or accentuated aging and an increased risk of age-associated diseases compared to HIV-negative persons [9,10]. Mechanisms that contribute to TL shortening in PWH may include uncontrolled viral replication [11] which is associated with sustained immune activation [8] and immunosenescence [8,12], and the inhibition of telomerase (the main enzyme involved in TL maintenance) by HIV proteins [13,14] and by certain antiretrovirals [15,16]. A large proportion of TL shortening in PWH may occur early during HIV infection, particularly during HIV seroconversion [17,18]. We recently reported that delaying antiretroviral therapy (ART) start in primary HIV infection for a matter of weeks is associated with significant and sustained TL shortening, compared to early ART start [19].

Longitudinal studies suggest that initiation of ART in chronic HIV infection may be associated with TL gain over 96 weeks follow-up [20,21]. It is unknown whether TL gain continues during periods of ART >96 weeks, and no longitudinal studies have compared TL change during untreated chronic HIV infection and after ART start in the same individuals. The aim of this study was to measure the rate of TL change during ≥3 years untreated chronic HIV infection in participants of the Swiss HIV Cohort Study (SHCS; www.shcs.ch [22]) and to assess in these same PWH whether the rate of TL change continues to be affected during ≥3 years of suppressive ART. In addition, we aimed to estimate the impact of clinical and HIV-associated factors including particular antiretrovirals on longitudinal TL dynamics. Finally, because genome-wide association studies (GWAS) have shown that TL is in part genetically determined [23-26], we also quantified the contribution of genetic background to TL. This

is the first comprehensive study to apply GWAS genotyping and a longitudinal approach that includes clinical and antiretroviral risk factors to TL in PWH.

Methods

Ethics, Consent. The study was approved by the local ethics committees. Participants provided written informed consent including for genetic testing.

Telomere length. We measured TL by quantitative PCR in stored peripheral blood mononuclear cells (PBMCs), and used as control the single copy albumin gene, as previously reported [8,19] (**Supplementary Methods**). We report TL values as relative values expressed as the T/S ratio (amplification of the telomere product/amplification of the single copy albumin gene). Samples were analyzed in duplicate. Within the same run, variation (SD/mean*100) was <1% between duplicate measurements.

Study Design. We provide data from 2 separate study populations (**Supplementary Figure 1**). First, we analyze TL change pre-ART and on-suppressive-ART in a highly selected population of participants (final n=107). Second, because of limited study population size, we investigated the potential effects of genetic background and of ARVs on longitudinal TL change in 798 additional participants from our previous longitudinal studies [3,32] who had $\geq$2 TL measurements available. For the first study population, we selected 111 participants (all ethnicities) who had a longitudinal set of PBMC samples available for TL measurement at the 4 time points (T1-T4) indicated in **Figure 1**, i.e. we measured TL in the first available sample before ART start (T1); in the last available sample before ART start (T2); in the first available sample after viral suppression was obtained (T3, i.e. in the first sample with concomitantly measured HIV RNA <20 copies/mL); and in the last available sample on suppressive ART (T4). All HIV RNA values measured between T3 and T4 had to be <100 copies/mL. To minimize the impact of short term intra-individual variability and assay variability on measured

TL, T1 and T2, as well as T3 and T4, had to be ≥3 years apart [27,28]. We measured and compared TL change between T1 and T2 (pre-ART) and TL change between T3 and T4 (on-suppressive-ART). Baseline was defined as the time of ART start and characteristics were taken from the last routine clinic visit before or at ART start. We excluded elite controllers (defined as participants with all HIV RNA values <100 copies/mL) because of confused time points, TL outliers (defined as relative TL>4), and samples that did not meet quality checks. In a pre-specified sensitivity analysis, we included the 81 participants with samples available at all 4 time points.

Genotyping. DNA was extracted from PBMCs and genotyped with the GWAS Global Screening Array v2.0 + MD (Illumina), or in the setting of previous SHCS genetic studies. Each batch of samples underwent quality control, filtering and imputation steps independently prior to merging, as described in the **Supplementary Methods**. For the final merged dataset, rare variants (minor allele frequency <5%), high missingness (>10%) or excessive deviation from Hardy-Weinberg Equilibrium (P_{HWE}<1e-6) were removed prior to calculating the PRS. We excluded individuals of non-European ancestry from the genetic analyses, as determined by principal component analysis with EIGENSTRAT (v6.1.4), together with the HapMap3 reference panel.

Calculating the Genome-Wide Polygenic Risk Score (PRS) for TL. We calculated the PRS for TL (TL-PRS) using the pruning and thresholding method implemented in PRSice (version 2.3.3). We used summary statistics for variants from a genome-wide meta-analysis on TL (n=78,592 individuals) [29]. After matching between the genotype data and summary statistics, the variants were clumped using windows of 250 kilobases and an $r2$ value of 0.1. The best-fit model with n=239 independent genome-wide significant SNPs (P<0.01) was then found by P-value thresholding using PRSice.

Statistical analyses. To estimate TL change over time, we used mixed-effects multilevel regression with random TL slope and intercept accounting for multiple time-points per patient. Best fitting models were identified based on Akaike and Bayesian information criteria (AIC, BIC) and

interactions/effect modifications were tested with likelihood-ratio tests (**Supplementary Methods**). We considered as covariables age, sex, BMI category, HIV transmission category, smoking, CMV seropositivity, hepatitis C (HCV) seropositivity, CD4 nadir, as well as CD4, CD8, CD4:CD8 ratio, log10 HIV RNA at ART start, and quintiles of TL-PRS [30,31]. Because regression coefficients of variables in models with interaction terms are difficult to interpret, we tabulated AIC, BIC and likelihood-ratio test p-values of the different models and used marginal predictions of the final model for visualisation. We also present linear predictions from average marginal effects for being on ART by sex on the TL slope over time and contrasts between male and female participants. Baseline characteristics of men and women were compared using Wilcoxon rank-sum tests (continuous variables) and Fisher's exact test (categorical variables). Data management and all analyses were done with Stata/SE 16.1 (StataCorp, College Station, Texas, USA).

Association of TL change with TL-PRS and with exposure to particular antiretrovirals (ARVs). To increase power, we investigated the association of TL change with TL-PRS and with exposure to particular ARVs in our participants plus participants of our previous longitudinal studies [3,32]. The majority of these participants contributed two data points which were unrelated to the ART start date. Mixed effects multilevel regression was applied to estimate TL change over time, adjusted for age, sex, and CD4:CD8 ratio. Cumulative exposure to the various ARVs was then added to this basic TL change model to check for effect modifications including interactions with TL change. We did not adjust significance levels for multiple testing.

Results

Participants, Time Intervals between TL Measurements. We selected 111 participants with 444 PBMC samples potentially available for TL measurement at the 4 defined time points indicated in **Figure 1**. At 39 (9%) time points, samples had already been used up, resulting in 405 analyzed samples. Of these, we excluded 8 samples from 2 elite controllers, 1 TL outlier sample and 2 samples from 2 participants from 1 center that did not meet quality checks. All following analyses are therefore based on 107 participants and 394 samples. Time points T1, T2, T3 and T4 were evenly populated with 25%, 26%, 25% and 24% of samples. The baseline characteristics of participants are shown in **Table 1** (30% women, 96% white, median age 44 years, 37% men who have sex with men, 38% heterosexual, 76% and 28% CMV- and HCV-seropositive, respectively). The number of participants contributing TL measurements at 1, 2, 3, and all 4 time points was 2, 4, 20, and 81, respectively. The median (interquartile range, IQR) time interval between time points T1 and T2, between T2 and T3, and between T3 and T4 was 7.68 (4.64-10.97) years, 1.31 (1.01-2.32) years, and 9.82 (7.13-11.05) years, respectively.

Visualization of TL trajectories of individual participants, observed data. TL trajectories showed considerable intra- and interindividual variability (**Figure 2**). Separate visualization of the "transition" period from T2 to T3, from immediately before ART start to when HIV suppression was first attained, shows that TL variability (TL amplitude on the y-axis) around the time of ART start is in fact similar to TL variability pre-ART and on-suppressive ART. In **Figure 3**, we show TL trajectories before/after HIV suppression was attained (i.e. before/after time point T3). Individual TL trajectories according to sex (**Figure 3**, **bottom panels**) suggest a similar TL decline pre-ART in men and women. During suppressive ART, a visual trend is apparent towards a TL increase in men and towards continued TL decrease in women.

Longitudinal Telomere Length Dynamics, Model Selection. To optimize estimates of TL change over time, we compared different mixed models. Model 3 (including sex interacting with intercept and slope) was superior (in terms of AIC and BIC) to model 1 without sex and was chosen as *basic multivariable model*. (**Supplementary Table 1**). Baseline CD4:CD8 ratio was significantly associated with baseline TL (per 1 unit of CD4:CD8 ratio higher, 28.8% longer TL; 95% CI, 12.8% to 44.7%; p<0.001), and inclusion of CD4:CD8 ratio improved model fit (likelihood ratio test, p<0.001). We found no evidence for significant effect modification of longitudinal TL change when we added other clinical variables to the models, including hepatitis C co-infection (**Supplementary Table 1**). Due to correlation between CD4, CD4 nadir and CD4:CD8 ratio, we added only CD4:CD8 ratio in the final *best fitting model* (model 3 in **Supplementary Table 1**). There were only very weak correlations between TL and closest CD4 (rho=0.095, p=0.06), closest CD8 (rho=0.012, p=0.82) or closest CD4:CD8 ratio (rho=0.076. p=0.13). Adding closest CD4 to the model did not affect results (p-value of CD4 coefficient=0.472).

Longitudinal Telomere Length Dynamics Pre-ART, Best Fitting Model (Table 2, Figure 4). Median (95% confidence interval [CI]) baseline TL was 0.975 (0.888-1.062) in men. In women, baseline TL was 19.2% (95% CI, 6.9% to 31.5%; p=0.002) shorter than in men. Pre-ART, TL shortened significantly (annualized TL change, -2.12% (95%CI, -0.76% to -3.48%; p=0.002) in men, with no evidence for any difference in TL decline by sex (difference in annualized TL change in women vs. men, 0.02%; 95% CI, -2.45% to 2.09%; p=0.88). On-suppressive-ART, there was no evidence for any further TL shortening (annualized TL change, 0.54%; 95%CI, -0.55% to 1.63%; p=0.33 in men), and no evidence for any difference in TL change by sex (difference in annualized TL change in women vs. men, -1.56%; 95% CI, -3.55% to 0.43%; p=0.13).

Sensitivity Analysis including level of HIV RNA and injection drug use (IDU) in the model. Baseline TL was inversely associated with the level of HIV RNA (p=0.093), and IDU had a trend towards lower

baseline TL (p=0.059). However, there was no evidence for any effect modification of TL change pre-ART or on-suppressive-ART when adding HIV RNA or IDU to the model (**Supplementary Results**).

Sensitivity Analyses, 81 participants with samples available at all 4 time points. When we restricted the analyses to 81 participants (39% women) with samples available at all 4 time points (T1-T4), results were essentially unchanged (**Table 2**).

Contribution of cumulative ART exposure to TL change. In the second study population (**Table 1**; n=905), 67, 582, 88, 168 participants provided 1, 2, 3, ≥4 samples respectively. The median interval between first and last TL measurement was 9.0 (IQR, 4.1 to 14.2) years, and 729/905 participants (80.6%) had samples >3 years apart available. The relationship between TL and age is shown in **Supplementary Figure 3**. We found no evidence of any association of cumulative exposure to each of 31 individual ART agents with longitudinal TL change, including tenofovir disoproxil fumarate and other nucleoside reverse transcriptase inhibitors (all likelihood-ratio tests p>0.15).

Association of TL change with polygenic risk score for TL. 658/905 participants in the second study population (**Table 1**) had GWAS genotyping available and 591/658 (89.8%) participants had samples >3 years apart available. Annualized TL change while ART-naïve and on-suppressive-ART in the genotyped participants was consistent with the first study population (**Table 2**). TL-polygenic risk score (TL-PRS) significantly contributed to TL change (global p-value, 0.019; **Table 2**). A significant genetic dose response relation was apparent when we restricted the analyses to 581 TL measurements in 350 participants while they were ART-naïve (**Table 2**): Compared to the first TL-PRS quintile (most favorable genetic background), median (IQR) TL in participants in the second, third, fourth, and fifth (most unfavorable) quintiles was 6.7% shorter (7.9% longer to 21.2% shorter), 9.4% shorter (4.9% longer to 23.6% shorter), 14.3% shorter (0.8% longer to 29.4% shorter), and 20.5% (4.9 longer to 36.1%) shorter, respectively. When we restricted the analyses to 1079 TL measurements in 586 participants while they were virologically suppressed, results were less consistent and the

association of TL-PRS quintiles with TL was U-shaped (**Table 2**).

Discussion

To our knowledge, this is the first study to provide longitudinal, quantitative evidence on TL change in PLW who served as their own controls during a median duration of almost 8 years of untreated and almost 10 years of well-controlled chronic HIV infection. Our study has 5 major findings. First, untreated HIV infection was accompanied by significant TL decline (median TL attrition, 2.12% per year), with no evidence of any difference between men and women. Second, we found no evidence of any further TL change during long-term suppressive ART. This suggests that successful ART attenuates TL attrition significantly, but we found no evidence of any TL *increase* during almost 10 years of suppressive ART. Third, our findings appear robust, because we found no evidence of any relevant effect modification either pre-ART or on-suppressive-ART when we considered multiple HIV-related and immunological variables. Fourth, in the extended study population, an unfavorable polygenic risk score was associated with shorter TL, especially during untreated HIV infection. Fifth, suppressive ART had a beneficial effect on TL attrition, irrespective of the particular antiretroviral agents used.

Prior evidence has suggested that TL is shorter in PWH compared to the general population. Our study confirms and extends previous reports by others [17,18] and us [19] that have shown significant TL decline occurring during HIV seroconversion [17,18], with ART initiation delay during primary HIV infection [19], and during untreated or suboptimally treated chronic HIV infection [33]. Each of these clinical settings correspond to periods of strong immune activation. Following ART start [34], a benefit of ART on aging biomarkers has been suggested by studies documenting a TL increase after 96 weeks of ART [20,21], with T-lymphocyte cellular shifts contributing to this TL

increase. In the same studies, epigenetic age acceleration present before ART start was reduced after 96 weeks [35] and after 4 years of suppressive ART [36]. We extend these findings here, by recording a -2.12% median TL decline during untreated HIV infection over a median observation period of almost 8 years. In the same individuals, we found no further median TL decline during suppressive ART. Moreover, we find no evidence of any TL increase over a median suppressive ART duration of almost 10 years, after excluding the early time period after ART start (median, 1.31 years) before viral suppression is achieved. The previously noted TL increase in the 96 weeks following ART start [20,21] may therefore represent an early benefit of ART initiation due to immune cell shifts. However, we see no evidence in our study that early TL increases following ART start are necessarily sustained over 10 years of suppressive ART. The prevalent notion in the general population is that TL decreases over 10 years, but there are few large and few longitudinal studies [37], and TL decline may not be strictly linear during the entire adult lifetime [38,39]. In one large longitudinal study (4576 individuals), almost half of participants had TL gain recorded during 10 years [24].

There is considerable concern about accelerated or accentuated aging and the occurrence of aging-associated diseases in PWH compared with HIV-negative persons [9,10]. Our findings may contribute to a better understanding of the aging process in PWH, by showing that untreated HIV infection was associated with significant TL attrition over almost 8 years. In contrast, suppressive ART had a clear beneficial effect on TL over almost 10 years. Our finding that TL-PRS was associated with TL decline particularly during *untreated* HIV infection is interesting, and might suggest that suppressive ART is a strong environmental factor with a larger effect on TL dynamics than genetic background. Because we assessed the TL-PRS in a convenience sample of previously assembled study populations [3,32], our genetic findings should be interpreted cautiously.

Strengths of our study include the exploitation of the rich, longitudinal database of the well-established SHCS, allowing each participant to serve as their own control during untreated and

suppressed HIV infection during more than 17 years. We selected extended time periods between TL measurements in response to the well-recognized dilemmas in TL studies, i.e. in order to minimize false positive results attributable to TL assay variability or short-term intra-individual TL variability. Our finding of TL decline in PWH before ART start appears clinically relevant because of its large effect size. A 2.12% annual TL decline during untreated HIV might translate into 11% shorter TL over 5 years. This compares to 8.2% shorter TL (the effect of being 10 years older) and 17%–22% shorter TL (the effect of delayed ART start during primary HIV infection) in our previous study in Swiss PWH [19]. In addition, a 2.8-fold shorter TL was associated with an approximately two-fold increased coronary artery disease event risk in Swiss PWH [3]. Our result of a beneficial effect of ART on TL appears robust, with no evidence for effect modification by clinical factors that can influence TL in vivo, including age, smoking, and others, as previously reported [20,21].

Our results have limitations. Even though the SHCS is one of the largest and best characterized cohorts of PWH, including a systematic biobank, our sample size was limited. This is because we applied stringent participant selection criteria (longitudinal samples in the same participant available at 4 defined time points, each $\geq$3 years apart). As in all TL studies, there was considerable inter-individual variability in TL dynamics. Our study population consisted predominantly of relatively young white participants, and two thirds were men. Results should therefore be cautiously extrapolated to other populations. Although we identified no significant effect of cumulative exposure to particular antiretroviral *agents* on longitudinal TL dynamics in 905 participants over 9 years, this finding needs to be confirmed in other populations. Because 488 different ART combinations were each used for $\geq$6 months in these participants, we were unable to assess any potential TL association with any particular ART *regimens*.

Importantly, we found no evidence of any significant differences in longitudinal TL *slope* between men and women in our study. Our finding that women had shorter TL compared to men may appear unexpected but is consistent with previous large studies and meta-analyses [40]. Men may have

longer TL than women before the age of 45-50 years [38] (which applies to the majority of our participants) and men may have shorter TL than women only thereafter. In addition, the annual TL shortening rate in women may decrease with the onset of menopause [39], and potential TL differences between men and women also seem to depend on the method of TL measurement [40] (differences being apparent in studies using Southern blot but not quantitative PCR, as done here), and the sample material (women may have longer TL than men when TL is measured in whole blood but not in PBMC) [40].

In conclusion, we show here that TL declines significantly during almost 8 years of untreated HIV infection, with a significant association with an individual TL-PRS. TL is stable during suppressive ART when measured longitudinally in the same participants, with no evidence of further TL decline or increase during almost 10 years following viral suppression. The effects of untreated HIV and suppressive ART on TL change appear large and thus clinically relevant. By contributing to TL preservation, suppressive ART may have a favorable effect on biological aging and the risk of aging-associated co-morbidities in PWH.

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Figure 1. Study Hypothesis and Time Points T1-T4 for Telomere Length Measurement Pre-ART and On Suppressive ART.

We measured telomere length at the 4 time points (T1-T4), i.e. in the first available blood sample before initiation of antiretroviral therapy (T1), the last available blood sample pre-ART (T2), the first available blood sample on suppressive ART (T3) (HIV RNA < 20 copies/ml) and the last available sample on suppressive ART (T4). Note that we required time points T1 and T2 (pre-ART time period) and time points T3 and T4 (on suppressive ART time period) to be at least 3 years apart. We hypothesized that telomere length decline over time is significantly attenuated after HIV viral suppression is attained, as symbolized by the lesser "steepness" of the TL slope on suppressive ART compared to pre-ART. ART start is indicated by the asterisk. The transition period refers to the time after ART start during which viral suppression is not yet attained.

Abbreviations. ART, antiretroviral therapy. IQR, interquartile range.

Figure 2. Telomere Length over Time, Observed data pre-ART and on-suppressive-ART, with Transition Period

First study population. Separate Spaghetti-plots for pre-ART period (time point T1 to T2), transition period (time point T2 to T3), and on-suppressive-ART (time point T3 to T4). Solid linear regression lines across all time points with 95% CI (shaded area) do not take multiple measures per patient into account. To minimize the impact of assay variability on measured TL, time points T1 and T2, as well as time points T3 and T4, had to be ≥3 years apart.

Figure 3. Telomere Length over Time, Observed data pre-ART and on-suppressive-ART

First study population. Spaghetti-plots for 2 phases across all patients (top panel) and separately for males and females (bottom panels). Solid linear regression lines across all time points with 95% CI (shaded area) do not take multiple measures per patient into account.

Figure 4. Telomere Length over Time, Best fitting model

First study population (n=107 participants). Mixed model including 2 TL measurements before ART start and 2 TL measurements after viral suppression was attained. Variables include pre-ART period, on-ART period, sex, and CD4:CD8 ratio. All TL measurements were ≥3 years apart. Results are presented as predicted telomere length intercept and slope in males (blue lines) and females (red lines). The shaded areas denote the 95% confidence intervals. The visually apparent difference in annualized TL change in the on-ART period between men and women was not statistically significant (p=0.13).

Table 1. Characteristics of Study Participants at Baseline[a]

	First study population (n=107) (Multivariable Models without Polygenic Risk Score, without antiretroviral agents)			Second study population (previously assembled study populations[3,32])	
	Males (n=75)	**Females** (n= 32)	**p-value**	**Multivariable Models with Antiretrovirals** (n=905)	**Multivariable Models with Polygenic Risk Score** (n=658 participants with GWAS available)
Male sex,	-	-		748 (83%)	555 (84%)
Age, median (IQR), years	45 (41-49)	40.5 (39-45)	P=0.016[b]	44 (37-53)	43 (36-52)
Ethnicity white	75 (100%)	28 (87.5%)	P=0.007[c]	869 (96%)	656 (99.5%)
black		1 (3.1%)		20 (2.2%)	
latinx		1 (3.1%)		10 (1.1%)	2 (0.5%)
asian		2 (6.3%)		6 (0.7%)	
BMI, median (IQR), kg/m2	23.2 (21.4-26.1)	22.1 (19.5-26.3)	P=0.082[b]	23.4 (21.3-25.7) [d]	23.4 (21.3-25.7) [d]
BMI < 18.5 (Underweight)	3 (4%)	4 (12.5%)	P=0.371[c]	34 (3.8%)	20 (3%)
18.5 - 24.9 (Normal)	48 (64%)	19 (59.4%)		567 (63.1%)	412 (63%)
25 -29.9 (Overweight)	19 (25.3%)	6 (18.8%)		243 (27.1%)	176 (27%)
≥ 30.0 (Obese)	5 (6.7%)	3 (9.4%)		54 (6%)	44 (7%)
Mode of HIV Transmission			P<0.001[c]		
Heterosexual	18 (24%)	23 (71.9%)		297 (32.8%)	203 (31%)
Men who have sex with men	40 (53.3%)	n.a.		405 (44.8%)	311 (47%)
Injection Drug Use / other	17 (22.7%)	9 (25%)		203 (22,4%)	144 (22%)
CD4 count, cells/µL, median (IQR)	260 (195-359)	252.5 (160.5-342)	P=0.360[b]	382 (240-555)	398.5 (254-566)
CD8 count, cells/µL, median (IQR)	999 (660-1320)	829.5 (513-1118.5)	P=0.086[b]	827 (571-1143)	820.5 (580-1160)
CD4 nadir (cells/µL), median	233 (162-315)	203 (133.5-	P=0.223[b]	268.5 (110-	286 (135-463)

(IQR)		286.5)		430)	
CD4:CD8 Ratio, median (IQR)	0.27 (0.18-0.38)	0.31 (0.17-0.44)	P=0.605[b]	0.44 (0.26-0.69)	0.46 (0.28-0.72)
Estimated duration of HIV infection at first sample, median (IQR), years	6.97 (5.53-9.74)	7.36 (5.36-9.01)	p=0.99[b]	6.62 (4.06-10.5)	6.40 (3.92-10.4)
HIV RNA, log copies/mL, median (IQR)	4.80 (4.37-5.15)	4.72 (4.17-5.16)	P=0.921[b]	n.a.[e]	n.a.[e]
Smoking never	18 (24%)	11 (34.4%)	P=0.096[c]	450 (49.7%)	329 (50%)
current	36 (48%)	18 (56.3%)		341 (37.7%)	247 (38%)
past	21 (28%)	3 (9.4%)		114 (12.6%)	82 (12%)
CMV seropositivity	58 (77.3%)	23 (71.9%)	0.624[c]	748 (82.7%)	537 (82%)
HCV seropositivity	20 (26.7%)	10 (31.3%)	0.644[c]	164 (18.1%)	120 (18%)

Notes. [a] defined as the last SHCS routine visit before or at ART Start; [b] Wilcoxon rank-sum test; [c] Fisher's exact test. [d] BMI Measurements were recorded in 898 participants; [e] Not reported because some participants already on ART at first TL time point. **Abbreviations.** IQR, interquartile range

	First study population (n=107): Multivariable Models <u>without</u> Polygenic Risk Score, without antiretroviral agents		**Second study population** (n=658; previously assembled study populations[3,32]): Multivariable Models <u>with</u> Polygenic Risk Score		
	Best Fitting Model[b]	**Participants with all 4 samples available**	**All TL measurements**	**TL while ART-naïve**	**TL while On-suppressive-ART**[a]
Number of participants	107	81	658	350	586
Number of TL measurements	394	324	1922	581	1079
Age at baseline, per 10 years older	---		-4.56% (-7.01% to -2.10%); p<0.001	-1.31% (-6.02% to 3.40%); p=0.585	-7.26% (-10.65% to -3.87%); p<0.001
CD4:CD8 ratio, per unit higher	28.76% (12.83% to 44.68%); p<0.001	27.37% (11.08% to 43.66%); p=0.001	9.10% (2.86% to 15.27%); p=0.004	8.83% (-3.60% to 21.26%); p=0.164	9.38% (1.77% to 16.98%); p=0.016
Female Sex	-19.16% (-31.46% to -8.63%); p=0.002	-18.97% (-32.28% to -5.67%); p=0.005	-34.25% (-41.43% to -27.07%); p<0.001	-38.87% (-52.97% to -24.78%); p<0.001	-32.61% (-42.03% to -23.18%); p<0.001
Annualized TL change	---	---	-1.11% (-1.41% to -0.67%); p<0.001	-1.96% (-3.26% to -6.51%); p=0.003	-0.22% (-0.90% to 0.47%); p=0.534
Annualized TL change - pre-ART, men	-2.12% (-3.48% to -0.76%); p=0.002	-2.30% (-3.82% to -0.78%); p=0.003	---	---	---
- pre-ART, difference women vs. men	0.02% (-2.45% to 2.09%); p=0.875	-0.22% (-2.67% to 2.22%); p=0.859	---	---	---
- on-suppressive-ART, men	0.54% (-0.55% to 1.63%); p=0.329	0.22% (-1.03% to 1.47%); p=0.733	---	---	---
- on-suppressive-ART, difference women vs. men	-1.56% (-3.55% to 0.43%); p=0.125	-1.49% (-3.66% to 0.69%), p=0.180	---	---	---
Contribution of TL-PRS to model	---	---	Global p=0.019	Global p=0.107	Global p=0.209
TL-PRS, 1st quintile (most favorable)	---	---	reference	reference	reference
- 2nd vs. 1st quintile	---	---	-10.09% (-17.83% to -2.35%); p=0.011	-6.67% (-21.22% to 7.89%); p=0.370	-8.88% (-18.91% to 1.16%); p=0.083
- 3rd vs. 1st quintile	---	---	-10.31% (-17.88% to -2.74%); p=0.008	-9.39% (-23.63% to 4.85%); p=0.196	-10.43% (-20.19% to -0.67%) p=0.036
- 4th vs. 1st quintile	---	---	-11.73% (-19.47% to -3.99%); p=0.003	-14.31% (-29.40% to 0.78%); p=0.063	-7.60% (-17.30% to 0.21%); p=0.124
- 5th (most unfavorable) vs. 1st quintile	---	---	-9.60% (-17.44% to -1.75%); p=0.016	-20.55% (-36.17% to -4.94%); p=0.015	-3.17% (-13.17% to 6.83%); p=0.534

Table 2. Telomere Length, Associations with Clinical Variables, Pre-ART and On-Suppressive-ART Time Periods, and Polygenic Risk Score Quintiles

[a] all HIV RNA values <50 copies/mL**.** [b] These same results are illustrated in **Figure 4**. **Abbreviations.** F, female; M, male.

FIGURE 1

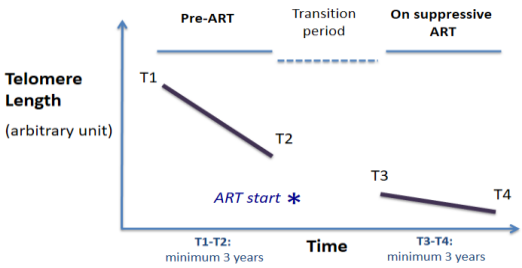

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Figure 2

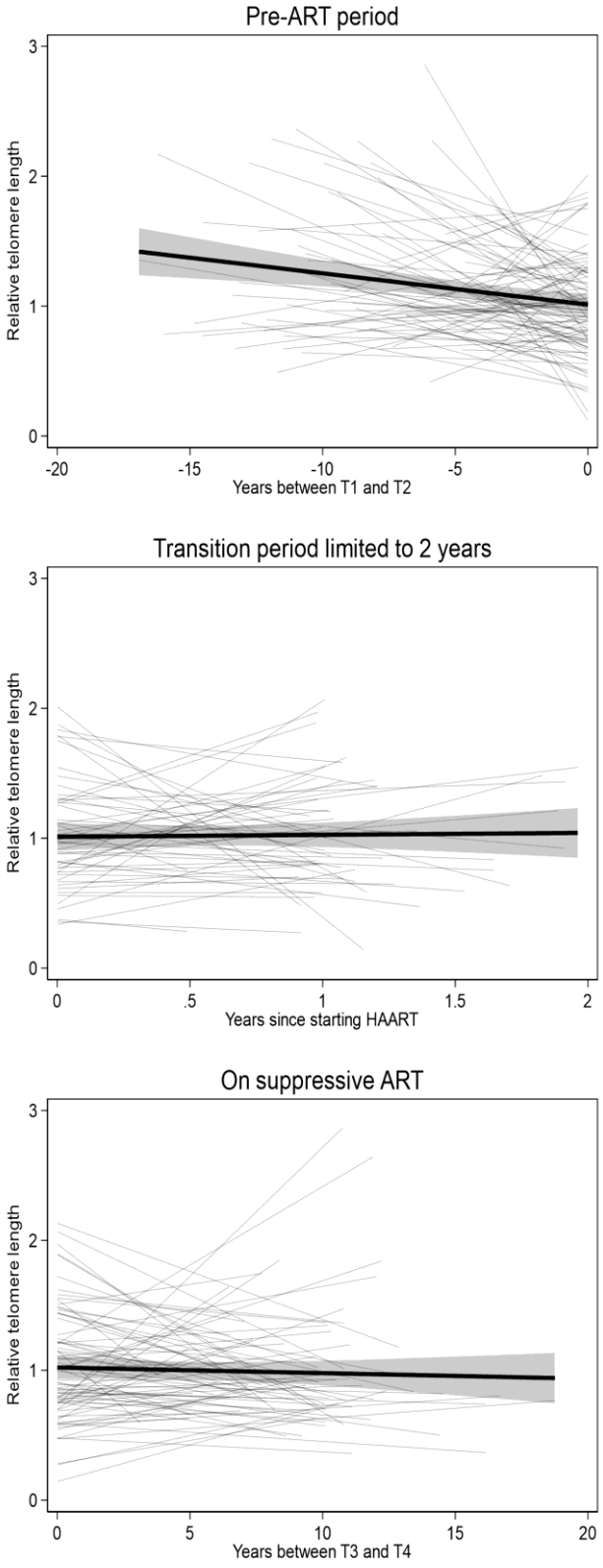

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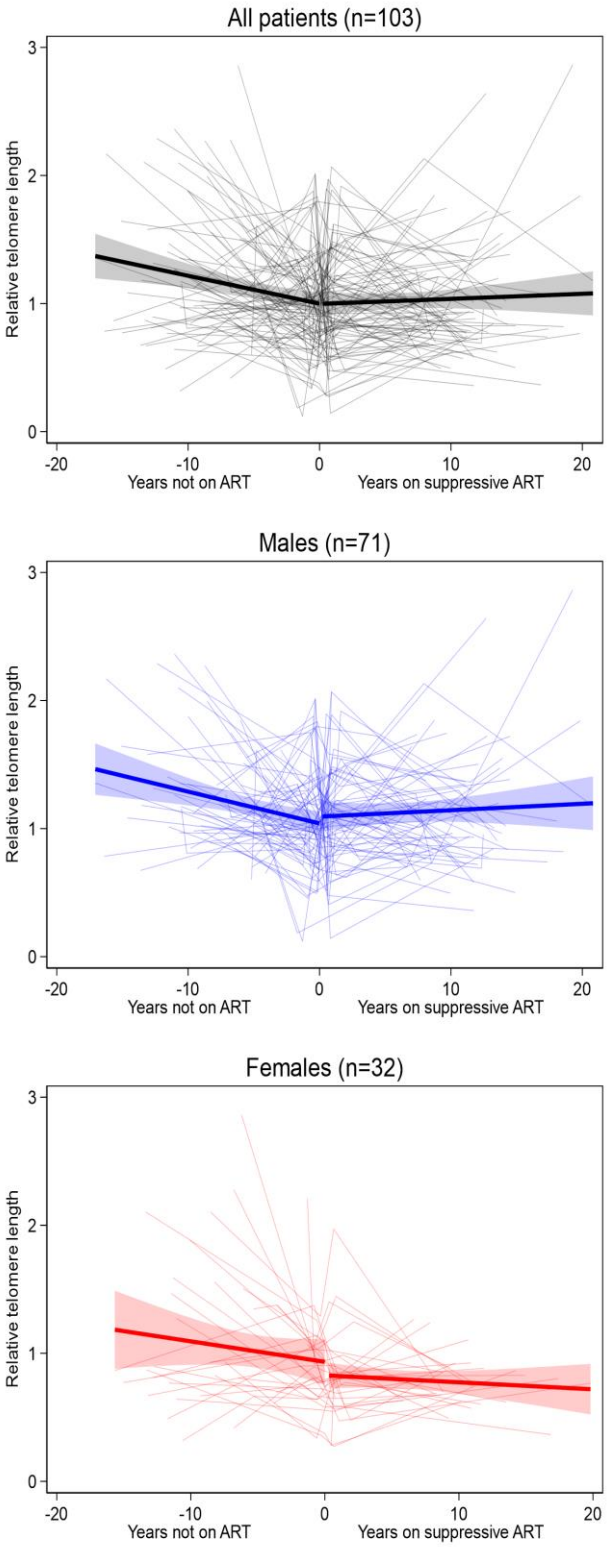
Figure 3



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87 Figure 4

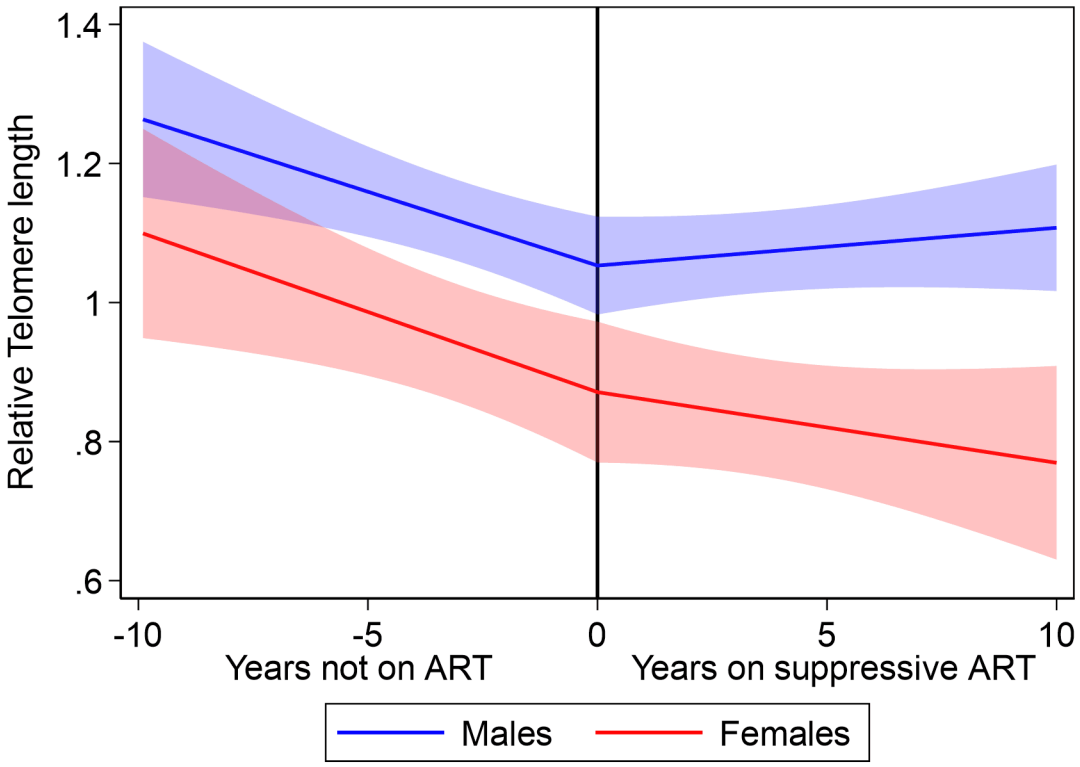

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Figure 5

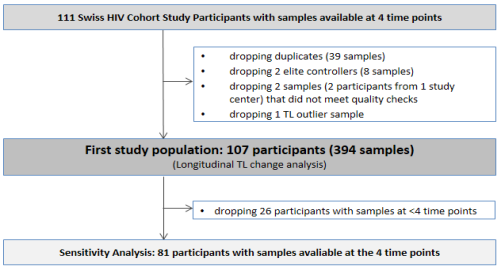

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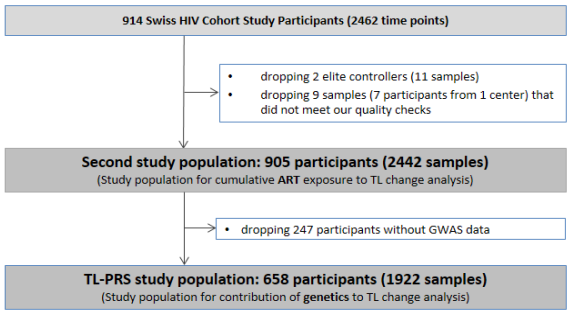
Figure 6



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