# **Reproduction predicts shorter telomeres and epigenetic age acceleration among young adult women**

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**Abstract**

Evolutionary theory predicts that energy invested into reproduction comes at the expense of somatic maintenance, thereby accelerating aging. Despite support from studies in human and non-human animals, mechanisms linking costs of reproduction (CoR) to senescence remains scarce. Pregnancy entails massive physiological and immunological shifts capable of accelerating aging through mitotic or non-mitotic cellular pathways - or both. Telomeres are repetitive DNA sequences that cap chromosomes and shorten with cell replication, and telomere length (TL) provides an index of mitotic age. DNA-methylation age (DNAmAge) is marker of non-mitotic cellular aging correlated with chronological age, but is accelerated in response to physiological and immunological stress. Shorter TL and accelerated DNAmAge both predict age-related morbidity and mortality. To elucidate mechanisms underpinning CoR, we examined the relationship between parity, TL (n=821), and DNAmAge (n=397) in young (20-22 year-old) Filipino women. TL decreased (p=0.016) and DNAmAge increased (p=0.011) with parity, an effect that was not contingent upon resource availability. Neither biomarker predicted subsequent fertility, supporting a causal effect of parity on aging (p>0.4 for both). Consistent with prior work, TL and DNAmAge were uncorrelated, but appear to represent distinct aspects of senescence, both exhibiting patterns of premature aging with reproduction among these young women.**Introduction**

Evolutionary theory predicts that energy expenditure in the form of reproductive effort comes at the expense of somatic maintenance and lifespan (1). Because resources are finite and selection favors early life fecundity over late life functional decline (2), reproduction is expected to carry a ‘cost’ in the form of mortality risk, senescence, and functional decline (3). Direct ‘costs of reproduction’ (CoR) have been demonstrated among animal models, whereby reproduction hastens senescence (4, 5). Conversely, selection for late life fecundity results in lifespan extension (6, 7). In humans, CoR has been predominantly studied through the use of historical datasets. This research suggests that increased reproductive effort is often associated with a shortening of lifespan (8–12, but see 13), and that these costs are more evident when resources are limited (14–16). However, most studies of CoR in humans are restricted to the use of mortality as the only outcome, and are unable to address the underlying biological processes through which CoR might lead to functional decline and mortality in humans.

Among women, CoR likely arise predominantly from pregnancy and lactation (41, 42). Human pregnancy is relatively ‘invasive’ and energetically demanding (ref.), and requires massive physiological and immunological modifications capable of accelerating senescence. Pregnancy induced cellular senescence could occur through either mitotic and non-mitotic pathways, or both (ref.). Mitotic senescence is commonly measured using telomere length (TL). Telomeres are non-coding DNA sequences that cap chromosomes, and are required for cell division and survival (17, 18). Telomere length shortens with cell division and chronological age, placing a limit on the number of cell divisions (19–21). Shorter TL, controlling for age, in turn predicts higher morbidity and mortality rates (22–25).

A powerful and emerging marker of non-mitotic senescence in human cells is epigenetic age (DNAmAge). DNAmAge in human (27) and non-human genomes (refs) is calculated from methylation at a species-specific subset of cytosine-guanine dyads (CpGs), and is strongly correlated with chronological age (26). Independent of a host of associated risk factors in humans, accelerated DNAmAge relative to chronological age is associated with elevated risks for morbidity and mortality (28–30). Vital to capitalizing on epigenetic age as a marker of non-mitotic senescence, DNAmAge predicts senescence and mortality independently of TL in living humans (31, 32), and independently of both TL and the DNA damage response in vitro (27, 31).

Human pregnancy could generate costs to female health and lifespan by shortening TL (mitotic age), accelerating DNAmAge (non-mitotic age), or both. During pregnancy, blood cells proliferate to compensate for fluid volume expansion (43, 44), and the female body shifts towards a pro-inflammatory but immunocompromised state (45–47). Data from cell culture, rodent based experiments, and clinical studies show that inflammation and infection increase cell proliferation and DNA damage, both expected to accelerate the pace of telomere shortening (48–55). Accelerated DNAmAge relative to chronological age has been observed in other pro-inflammatory contexts (40, 56), and with menopause (57), an important physiological and life history transition in human females. DNAmAge acceleration arising from menopause, whether naturally-occurring or surgically-induced, was attenuated by hormone therapy (57),m, suggesting that physiological and hormonal changes like those accompanying pregnancy could have profound effects on DNAmAge. While two studies have recently examined TL and pregnancy with mixed results (refs), none have attempted to test for CoR in humans using mitotic and non-mitotic measures of senescence simultaneously. While numerous studies do support CoR on human aging (ref. Ziomewics, Jasienska), examining multiple pathways of senescence simultaneously may be necessary.

Here, we test for human CoR using mitotic (TL) and non-mitotic (DNAmAge) measures of cellular senescence. We test three inter-related hypotheses in a relatively young cohort (age 20-22) of women in the Philippines. First, we ask whether pregnancy history increases mitotic or non-mitotic measures of cellular senescence, or both (H1). We also ask whether any associations between pregnancy history and senescence are stronger among women for whom resources are constrained by socioeconomic status (H2). Finally, we test a more causal effect of TL and DNAm on pregnancy history by examining the effect of both measures on fecundity over the subsequent 4 years (H3).

**Results**

The women in our sample were relatively young (21.7 ± 0.4). Pregnancies were distributed as follows: nulliparous (61.7%), single (21.2%), two (12.4%), three (3.4%), four (0.8%), and five (0.4%) pregnancies (Table 1). The 397 individuals in this sample with available DNAmAge data showed 3.95 year higher average DNAmAge (25.6 ± 3.2) than their chronological age. In contrast, age-corrected TLs in this sample are longer than that of European and African American populations, but shorter than that of several African populations (details in Table S1). TL and DNAmAge showed no associations with each other (n=396, r=0.00, p=0.65), including when both measures had chronological age effects factored out (n=396, r=0.00, p=0.75).

**Table 1.** Descriptive statistics for key variables.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variable† | Mean | SD | Min | Max |
| Age (years) | 21.7 | 0.4 | 20.8 | 22.5 |
| Telomere length (relative units) | 0.79 | 0.16 | 0.03 | 1.45 |
| DNAmAge Acceleration‡ | 0.0 | 3.2 | -11.6 | 9.7 |
| Number of pregnancies | 0.6 | 0.9 | 0.0 | 5.0 |
| Maternal education (y) | 7.8 | 3.8 | 0.0 | 19.0 |
| Currently Pregnant (%) | 7.8 |  |  |  |
| †All variables n=811 aside from DNAm, for which n=397  ‡Residuals of DNAmAge on chronological age at blood draw | | | | |

Independently of chronological age, TL and DNAmAge showed the predicted (H1 & H2) evidence of accelerated aging with increasing number of pregnancies (Table 2; Figure 1; Table S2). After controlling for potential confounders, effect sizes of number of pregnancies on both TL and DNAmAge for most models increased (Table 2, Models 1 and 2 vs. 3 and 4 and Model 5 and 6 vs. 7 and 8). In contrast to total number of pregnancies, being currently pregnant was associated with a trend towards longer TL and significantly younger DNAmAge (Table 2, Models 3 & 7). However, these same models excluding current pregnancy slightly attenuated the associations of increasing numbers of pregnancies on TL and DNAmAge (Models 2 and 5). This suggests that controlling for what appears to be a transient protective effect of current pregnancy exposes more long-term costs of past pregnancies.

Each additional pregnancy was associated with a reduction in TL of 0.016 units (Table 2, Model 3), or an interpolated -50.6 bp/pregnancy based on previous southern blot measured TL in a subset of these samples (60). The age related decline in TL in 36-69 year old women in this population was previously found to be 0.0043 relative TL units/year (n=1,845, p=7.19 x 10-16) (61) or 13.6 bp/year, while the age related decline in TL within the younger and narrow age range in this sample is estimated between -0.027 and -0.047/year (Table 2, Models 1-4). In other populations the age-related decline in mid-late adulthood is approximately 25 bp/year (62). This implies that one pregnancy is equivalent to between 0.34 and 3.72 years of telomere aging depending on the comparison population. Consistent with this calculation, each additional pregnancy was associated with 0.44-year (0.27 to 0.61 years) increase in DNAmAge acceleration (Table 2, Model 7).

To test for reverse association, we checked whether TL/DNAmAge at the time of measurement (in 2005) predicted the number of pregnancies over the subsequent four years (from 2005-2009). Controlling for the time elapsed between the 2005 and 2009 surveys, we found no relationship of either TL (p = 0.835) or DNAmAge (p = 0.483) on subsequent parity, including after controlling for baseline parity in 2005 (Table S3).

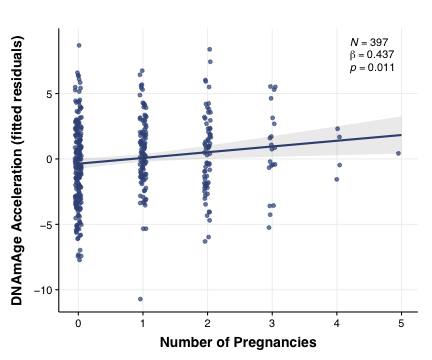
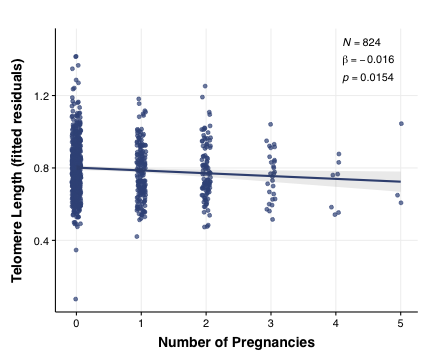
Contrary to expectations (H3), there were no significant interactions between number of pregnancies and SES in predicting either TL (p=0.35; Table 2, Model 4) or DNAmAge (p=0.37; Table 2, Model 8). However, the primary associations of number of pregnancies with TL and DNAmAge for these models increased slightly in magnitude with the inclusion of the interaction term.

**Table 2.** Regression models linking number of pregnancies to telomere length (models 1-4) and DNAmAge (models 5-8).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Telomere Length~** | | | | **DNAmAge~** | | | |
|  | (1) | (2)† | (3)† | (4)† | (5) | (6)† | (7)† | (8)† |
| **Chronological Age (yrs.)** | -0.047\*\* | -0.029+ | -0.028+ | -0.029+ | 0.485 | 0.667 | 0.656 | 0.645 |
| **No. Pregnancies** | -0.014\* | -0.013\* | -0.014\* | -0.016\* | 0.363\* | 0.326\* | 0.459\*\* | 0.510\*\* |
| **SES** |  | -0.006 | -0.006 | -0.004 |  | -0.180 | -0.214+ | -0.291+ |
| **Currently Pregnant** |  |  | 0.011 | 0.011 |  |  | -1.472\*\* | -1.460\*\* |
| **No. Pregnancies x SES** |  |  |  | -0.004 |  |  |  | 0.106 |
| **Intercept** | 1.826\*\* | 1.337\*\* | 1.332\*\* | 1.343\*\* | 14.818 | 10.319 | 10.611 | 10.850 |
| **Observations** | 821 | 821 | 821 | 821 | 397 | 397 | 397 | 397 |
| **Adjusted R2** | 0.015 | 0.063 | 0.062 | 0.062 | 0.011 | 0.041 | 0.067 | 0.067 |

†Includes controls for principal components of genetic variation to control for population structure, and average urbanicity (complete results shown in Table S2). +p<0.1;\*p<0.05;\*\*p<0.01;\*\*\*p<0.001

a) b)

  
**Figure 1**. a) TL association with number of pregnancies. Y-axis is residualized TL for all variables in Table 2, Model 3, and statistics from same model. b) DNAmAge association with number of pregnancies. Y-axis is residualized DNAmAge for all variables in Table 2, Model 7, and statistics from same model. Best fit lines are drawn with 95% CI of beta value.

**Discussion**

We found that women with higher parity had shorter TL and accelerated DNAmAge. The effect sizes were dose-dependent and considerable, equivalent to between ~0.34 and 3.72 years of telomere aging and 0.27 to 0.61 years of DNAmAge aging per pregnancy. These findings were relatively robust to controlling for potential confounders, which is particularly important in light of selection effects that frequently confound observational studies of the costs of reproduction. Intriguingly, women who were currently pregnant exhibited trend towards more ‘youthful’ TL and significantly younger DNAmAge. These results could reflect the suite of physiological shifts that occur during pregnancy, such as the protective effects of estrogens on TL and DNAmAge (57, 63), or perhaps the presence of fetal DNA in maternal circulation during pregnancy (64, 65). Therefore, current pregnancy status may be an important confounder to include in models in future studies investigating the costs of reproduction.

Contrary to our prediction that the costs of reproduction would be greatest among individuals with limited resources (14–16), we found no evidence for an interaction between parity and SES in models predicting either TL or DNAmAge acceleration. It is unclear to what extent SES adequately captures resource limitations in this population, but given the relatively young age of the participants, a moderating effect of resource limitations might only emerge at more advanced ages. SES in this population may also index factors other than resource availability that contribute to accelerated aging, such as less healthful diets or decreases in physical activity.

Although consistent with theoretical predictions and non-human animal work, our findings contrast with two recent studies of TL and parity in women. The first, conducted among 75 Guatamalan Maya women, found a positive association between TL and number of surviving offspring over a 13-year time period (58). Unfortunately, TL in that study was determined using a combination of saliva- and buccal-derived DNA samples, which do not show a consistent relationship with chronological age (67–69). Unlike longitudinal assessments of blood TL (70), the two measures of TL in this study were also uncorrelated within individuals between the two timepoints, making comparisons between the findings of that study and our own blood-derived TL findings difficult. More recently, a study among 620 participants of the US-based CARDIA study found no evidence for an effect of parity on TL (59). The reasons for such contrasting findings are unclear, but may relate to pronounced differences in the age ranges and socio-ecological conditions in the different populations under investigation. TL attrition occurs more rapidly at younger ages (71), suggesting that any impacts of reproduction on TL shortening could be amplified among young women, especially if reproduction overlaps with late stages of somatic growth (1, 72). Our large sample of blood-measured TL among young, similarly-aged women overcomes many of these limitations, and has the advantage of being free of confounding by concurrent secular trends in parity or other factors that might affect TL among women across a broader age range.

Telomere length and DNAmAge appear to reflect different underlying biological pathways linking reproductive effort with somatic senescence. Telomere length and DNAmAge measured in the same individuals have been independently associated with aging and mortality in prior studies (32, 73), and appear to reflect distinct measures of cellular senescence (31). Accordingly, TL and DNAmAge acceleration were not associated with each other in this study. Also notable is our finding that DNAmAge was slightly elevated relative to chronological age at Cebu, while TLs were longer and show evidence for a slightly slower attrition rate relative to other populations (Table S1). These findings further support the idea that TL and DNAmAge are independent markers of aging and senescence (73). Accelerated TL attrition – a measure of ‘mitotic age’ that is modified directly by cellular division – could stem from factors that modify cellular proliferation rates, such as the elevated inflammation, blood cell production, and cell-turnover rates, all of which that characterize pregnancy in this and other samples (74).

In contrast to TL, DNAmAge is not considered a marker of mitotic age, as it is associated with chronological age even in immortal, non-dividing, and non-proliferative tissues and cells (27). Although the biological significance of DNAmAge is unknown, it is hypothesized to reflect the integrity of an epigenetic maintenance system, itself responsible for maintaining dynamic regulatory stability within cells (27). In light of the relationship between DNAmAge and epigenetic maintenance, our findings are consistent with the prediction that reproduction comes at the expense of ‘maintenance’ – in this case at the scale of cellular regulatory maintenance. Exactly how parity might lead to DNAmAge acceleration is unclear, but could involve tradeoffs between protein homeostasis and epigenetic control arising from immune activation or the buffering of oxidative stress (75–78). Indeed, cumulative changes in immune cell composition during pregnancy also likely contribute to DNAmAge acceleration with parity, although the measure of DNAmAge used here is remarkably robust across tissue types (27). Nevertheless, the fact that the functionally-distinct measures of TL and DNAmAge show similar associations with parity provides strong support for our prediction that reproduction accelerates senescence, at least among the young adult women represented by our sample.

The current study is not without limitations. While our models attempt to control for socio-ecological factors that could affect both parity and our biomarkers of aging, confounding arising from differences in health and/or resources remains a possibility. Although there was no evidence for powerful confounding effects in our data, confounding may explain the slight decrease in effect size of parity between models 1 and 2 (TL) and 4 and 5 (DNAmAge). Future studies employing a longitudinal measures of TL and DNAmAge acceleration would minimize the potential effects of such confounders (79), while modeling lactation and other indices for reproductive effort might provide a more accurate estimate of the CoR (66, 80). Finally, the women in this study all fell within a relatively narrow age range during young adulthood (20-22 years old). Because both TL and DNAmAge ‘age’ more rapidly early in adulthood (27, 71), it is possible that both measures are particularly sensitive to reproduction at this time. Multiple measures of TL and DNAmAge for each individual that capture the ‘pace’ of aging over time will again prove important to resolving such effects.

In sum, we find evidence that parity is associated with shorter telomeres and epigenetic among the young women in our sample. The consistent relationships between parity and aging in two distinct pathways—one reflecting cellular turnover and genomic stability, and the second a marker of epigenomic stability—supports a cost of reproduction in humans.

**Materials and methods**

*Data collection.* Data came from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), a birth cohort study in Metropolitan Cebu, Philippines that began with enrollment of 3,327 pregnant mothers in 1983-1984 (81). Longitudinal data are available for download at https://dataverse.unc.edu/dataverse/cebu. In 2005 blood samples from overnight fasted subjects were collected into EDTA-coated vacutainer tubes. Automated and manual DNA extraction (Puregene, Gentra) was conducted on blood samples. Informed consent was obtained from all participants and data collection was conducted with approval and oversight from the Institutional Review Boards of the University of North Carolina at Chapel Hill and Northwestern University.

*Telomere length.* TLs were measured using a modified form of the monochrome multiplex quantitative polymerase chain reaction assay that was externally validated. Details of the protocol and external validity can be found in (60) and since the coefficient of variation (CV) has recently been recognized to be an invalid statistic to assess TL measurement reliability (82, 83), intraclass correlation coefficient (ICC) statistics of measurement error can be found in (84).

*Epigenetic age (DNAmAge).* 160ng of sodium bisulfite converted DNA (Zymo AZDNA methylation kit, Zymo Research, Irvine, CA, USA) was applied to the Illumina HumanMethylation450 Bead Chip using manufacturer’s standard conditions. Standard methods for background subtraction and color correction were carried out using default parameters in Illumina Genome Studio and exported into R for further analyses. Quality control involved first confirming participant sex and replicate status. This was followed by quantile normalization using *lumi* (85) on all probes including SNP-associated and XY multiple binding probes. To maximize the number of sites available for the epigenetic age calculator, probes with detection p-values above 0.01 were called NA for poor performing samples only, and were otherwise retained. DNAmAge was calculated using an online calculator (http://labs.genetics.ucla.edu/ horvath/dnamage/), designed to be generally robust to cell-type differences associated with age (27). Background-corrected beta values were pre-processesed using the calculator’s internal normalization algorithms.

*Socioeconomic status (SES).* SES is measured as a combination of income, education, and assets. Participants reported their annual income from all sources, including in-kind services, and the sale of livestock or other products by household members during the prior year, which were summed to determine total household income. Incomes were deflated to 1983 levels, and log-transformed. Maternal education (in years) was also reported. Participants also reported on nine assets (coded 0, 1) that were selected to capture population-relevant aspects of social class, including electricity, televisions, refrigerators, air conditioners, tape recorder, electric fans, jeepneys, cars, and their residence. In addition, house construction type (i.e., light, mixed, permanent structure) was coded as 0,1, and 2, respectively. Thus, asset scores ranged from 0 to 11. A principal components analysis was run on log income and assets at birth (1983) and at sample collection (2005) along with maternal education in Stata (v. 14.1). The first component of variation accounted for 49% of the variation and individual scores for this component of variation were used as our measure of SES.

*Statistical methods.* The key predictor variable was the number of pregnancies (including stillbirths, miscarriages and live births, but not current pregnancies) the respondent reported having had in 2005 (at the time of blood sampling). Control variables included chronological age in 2005 (the time of blood collection), the measure of socioeconomic status (SES) described above, average urbanicity score between 1983 and 2005 (86), and whether the respondent was pregnant at the time of blood collection. DNAmAge acceleration refers to DNAmAge residualized on chronological age. Principal components (PCs) of genome-wide genetic variation were considered to control for potential population structure effects. The derivation of these principal components have been described previously (87–89). As in previous analyses (84, 90), the bivariate association between the first ten principal components and TL were tested. The top principal components up to and including the last one showing a significant bivariate association with TL (10 total) were retained as control variables, with the same 10 principal components used for DNAmAge models. Linear regression was used for analyses predicting TL and DNAmAge, while generalized linear models with a poisson family and log-link were used to test for reverse association – that TL/DNAmAge would predict parity over the subsequent 4 years. The absence of collinearity in predictor variables was confirmed with variance inflation factors (VIFs) for all models falling below 1.1, while Poisson GLMs showed no signs of under- or over-dispersion (91). All models were two-tailed with α = 0.05 and were run in R (92) with ggplot2 (93) and stargazer (94) for figures and tables.

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**Supplementary Information**

**Table S1. Comparison of TL across ethnicities.** qPCR measured TL does not permit comparisons across populations, but 190 samples from the population for which the samples from this paper come from have had southern blot TL measured (Eisenberg et al. 2015). The average age of this sample is 35.96 (± 15.83, range 21.02-68.33), with an average TL of 7.86 kb (± 0.76), and an estimated age related decline in TL of 21.98 bp/year (95% CI 15.81-28.15). This age related decline is non-significantly less than that found in other populations (African=27.7 bp/year; African Americans = 25.6 bp/year; Europeans = 27.3 bp/year) (Hansen et al. 2016). These three populations from Hansen et al. (2016) have an average age of 43.25. Adjusting the Cebu population TL for the 7.29 years younger they are by assuming the observed 21.98 bp/year attrition rates yields an interpolated TL of 7.70 kb if the mean age was 43.25, the same as the samples in Hansen et al. (2016). This suggests that the TL in Cebu is longer than that of European and African Americans, but slightly shorter than that of Africans (Table S1).

|  |  |  |  |
| --- | --- | --- | --- |
| **Ethnicity (N)** | **% Female** | **Age (years)** | **LTL (kb)** |
| Europeans (90)\* | 63.3 | 43.9 (18-78) | 7.21 (5.39-9.42) |
| African Americans (97)\* | 67.1 | 42.9 (21-79) | 7.45 (5.55-9.16) |
| Africans (100)\* | 68.0 | 43.0 (18-79) | 7.85 (5.64-10.13) |
| Cebu, Philippines (190) | 50.3 | 43.3~ | 7.70 (6.10-9.21) |

\* from Hansen MEB, et al. (2016) Shorter telomere length in Europeans than in Africans due to polygenetic adaptation. *Human Molecular Genetics*.

†from Eisenberg DT, Kuzawa CW, Hayes MG (2015) Improving qPCR telomere length assays: Controlling for well position effects increases statistical power. *Am J Hum Biol* 27(4):570–5.

~ interpolated to match mean age of other populations - see above text

**Table S2. Full model estimates for parity and telomere length and epigenetic age (DNAmAge) among young women in the Philippines.**

|  | **Telomere Length~** | | | | **DNAmAge~** | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
| **Age** | -0.047 | -0.028 | -0.027 | -0.028 | 0.485 | 0.667 | 0.629 | 0.618 |
|  | p = 0.004\*\* | p = 0.080+ | p = 0.086+ | p = 0.079+ | p = 0.293 | p = 0.157 | p = 0.179 | p = 0.188 |
| **No.Pregnancies** | -0.013 | -0.012 | -0.016 | -0.018 | 0.363 | 0.326 | 0.437 | 0.491 |
|  | p = 0.030\* | p = 0.044\* | p = 0.016\* | p = 0.010\*\* | p = 0.026\* | p = 0.049\* | p = 0.011\* | p = 0.007\*\* |
| **PC1** |  | -0.426 | -0.437 | -0.438 |  | -11.623 | -10.481 | -10.457 |
|  |  | p = 0.060+ | p = 0.054+ | p = 0.054+ |  | p = 0.084+ | p = 0.117 | p = 0.118 |
| **PC2** |  | -0.155 | -0.144 | -0.131 |  | 5.098 | 4.679 | 4.207 |
|  |  | p = 0.499 | p = 0.530 | p = 0.568 |  | p = 0.458 | p = 0.494 | p = 0.540 |
| **PC3** |  | 0.013 | 0.002 | 0.008 |  | 8.510 | 9.075 | 8.853 |
|  |  | p = 0.955 | p = 0.992 | p = 0.972 |  | p = 0.202 | p = 0.172 | p = 0.183 |
| **PC4** |  | -0.024 | -0.015 | -0.022 |  | 11.991 | 11.495 | 11.551 |
|  |  | p = 0.917 | p = 0.949 | p = 0.922 |  | p = 0.069+ | p = 0.080+ | p = 0.078+ |
| **PC5** |  | -0.252 | -0.251 | -0.244 |  | -14.123 | -14.441 | -14.157 |
|  |  | p = 0.277 | p = 0.279 | p = 0.291 |  | p = 0.023\* | p = 0.020\* | p = 0.022\* |
| **PC6** |  | -0.293 | -0.304 | -0.294 |  | -4.002 | -3.125 | -3.748 |
|  |  | p = 0.205 | p = 0.189 | p = 0.204 |  | p = 0.548 | p = 0.637 | p = 0.574 |
| **PC7** |  | -0.497 | -0.529 | -0.531 |  | 18.513 | 19.744 | 20.019 |
|  |  | p = 0.034\* | p = 0.025\* | p = 0.024\* |  | p = 0.008\*\* | p = 0.005\*\* | p = 0.004\*\* |
| **PC8** |  | 0.426 | 0.428 | 0.433 |  | 3.987 | 3.796 | 3.691 |
|  |  | p = 0.062+ | p = 0.061+ | p = 0.058+ |  | p = 0.534 | p = 0.552 | p = 0.563 |
| **PC9** |  | -0.457 | -0.476 | -0.481 |  | -1.730 | -1.521 | -1.534 |
|  |  | p = 0.042\* | p = 0.035\* | p = 0.033\* |  | p = 0.789 | p = 0.813 | p = 0.812 |
| **PC10** |  | 0.559 | 0.548 | 0.541 |  | -2.701 | -1.976 | -1.672 |
|  |  | p = 0.020\* | p = 0.022\* | p = 0.024\* |  | p = 0.690 | p = 0.769 | p = 0.804 |
| **SES-score** |  | -0.006 | -0.006 | -0.004 |  | -0.180 | -0.190 | -0.271 |
|  |  | p = 0.140 | p = 0.158 | p = 0.395 |  | p = 0.146 | p = 0.123 | p = 0.075+ |
| **Urbanicity-score** |  | 0.002 | 0.002 | 0.003 |  | 0.015 | 0.016 | 0.015 |
|  |  | p = 0.00000\*\* | p = 0.00000\*\* | p = 0.00000\*\* |  | p = 0.270 | p = 0.260 | p = 0.275 |
| **Currently Pregnancy** |  |  | 0.038 | 0.038 |  |  | -1.223 | -1.211 |
|  |  |  | p = 0.080+ | p = 0.079+ |  |  | p = 0.018\* | p = 0.019\* |
| **No.Pregnancies\*SES-score** |  |  |  | -0.004 |  |  |  | 0.111 |
|  |  |  |  | p = 0.354 |  |  |  | p = 0.365 |
| **Intercept** | 1.816 | 1.315 | 1.303 | 1.314 | 14.818 | 10.319 | 11.177 | 11.422 |
|  | p = 0.00000\*\* | p = 0.0002\*\* | p = 0.0002\*\* | p = 0.0002\*\* | p = 0.138 | p = 0.318 | p = 0.276 | p = 0.266 |
| Observations | 824 | 824 | 824 | 824 | 397 | 397 | 397 | 397 |
| R2 | 0.017 | 0.080 | 0.083 | 0.084 | 0.016 | 0.075 | 0.089 | 0.091 |
| Adjusted R2 | 0.015 | 0.064 | 0.066 | 0.066 | 0.011 | 0.041 | 0.053 | 0.052 |
| Residual Std. Error | 0.161 (df = 821) | 0.157 (df = 809) | 0.157 (df = 808) | 0.157 (df = 807) | 3.165 (df = 394) | 3.117 (df = 382) | 3.098 (df = 381) | 3.098 (df = 380) |
| F Statistic | 7.089\*\* (df = 2; 821) | 5.007\*\* (df = 14; 809) | 4.892\*\* (df = 15; 808) | 4.639\*\* (df = 16; 807) | 3.220\* (df = 2; 394) | 2.214\*\* (df = 14; 382) | 2.475\*\* (df = 15; 381) | 2.371\*\* (df = 16; 380) |

**Table S3. Predictive effect of Telomere Length (TL) or Epigenetic Age (DNAmAge) on parity over the subsequent 4 years in young women in the Philippines.** Models derived from Poisson generalized linear regression meeting assumptions of equidispersion.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Parity 2005-2009** | | | |
|  | **Age Adjusted TL~** | | **Age Adjusted DNAmAge**~ | |
|  | (1) | (2) | (3) | (4) |
| Measurement time bt. 2005-2009 (Days) | -0.003 | -0.003 | -0.002 | -0.002 |
|  | p = 0.004\*\* | p = 0.009\*\* | p = 0.058+ | p = 0.068+ |
| Parity in 2005 |  | 0.252 |  | 0.123 |
|  |  | p = 0.000\*\* |  | p = 0.016\* |
| Age Adjusted Telomere Length in 2005 | 0.059 | 0.148 |  |  |
|  | p = 0.835 | p = 0.601 |  |  |
| Age Adjusted DNAmAge in 2005 |  |  | -0.011 | -0.016 |
|  |  |  | p = 0.483 | p = 0.325 |
| Intercept | 4.457 | 3.777 | 3.460 | 3.265 |
|  | p = 0.006\*\* | p = 0.022\* | p = 0.062+ | p = 0.082+ |
| Observations | 743 | 743 | 397 | 397 |
| Log Likelihood | -836.740 | -818.205 | -485.276 | -482.433 |
| Akaike Inf. Crit. | 1,679.481 | 1,644.411 | 976.552 | 972.866 |
| *Note:* | +p<0.1;\*p<0.05;\*\*p<0.01;\*\*\*p<0.001 | | | |