Cross-sectional and longitudinal changes in gravidity are associated with accelerated epigenetic age in young and middle-aged women in the Philippines

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Abstract

A central prediction of evolutionary theory is that energy invested in reproduction comes at the expense of somatic maintenance and repair, accelerating biological aging. In women, such 'costs of reproduction' (CoR) are supported by evidence that high fertility predicts poor health and shorter lifespan later in life. Nevertheless, quantifying effects of reproduction on aging is complicated by social and environmental factors that influence both fertility and aging, and by limited tools for measuring biological aging in younger adults. Here, we examined relationships between the number of pregnancies and biological aging in 825 young (20-22 year old) women from the Cebu Longitudinal Health and Nutrition Survey, located in the Philippines. We quantified biological aging using six epigenetic measures reflecting several dimensions of cellular aging, health, and mortality risk (Horvath, Hannum, PhenoAge, GrimAge, DunedinPACE, and DNAmTL). In a subset of 331 women, we also tested whether longitudinal changes in gravidity between young (20-22 years old) and early-middle adulthood (25-31 years old) were associated with changes in epigenetic aging over that same time period. Sensitivity analyses were run to assess any role of changes in immune cell composition to findings. Cross-sectionally, gravidity was associated with all six measures of accelerated epigenetic aging. Furthermore, longitudinal increases in gravidity were associated with faster epigenetic aging using the Horvath and Hannum epigenetic clocks. These effects were robust to socioecological, environmental, and immunological factors, consistent with the hypothesis that reproduction accelerates biological aging, and that these effects can be detected in young women in a high-fertility context.

Introduction

A central evolutionary theory of aging posits that reproduction will occur at the expense of maintenance and repair, leading to accelerated biological decline (Williams, 1966). This idea has been supported across plant and animal taxa (Dijkstra et al., 1990; e.g. Obeso, 2002; Speakman, 2008), including in humans. Based upon analyses of historical records spanning the 8th to 19th centuries, women in the British Aristocracy who had more progeny also sustained shorter post-reproductive lifespans (Westendorp and Kirkwood, 1998). Similar findings have been reported for frontier women settlers of early Québec and the South-West United States (Gagnon et al., 2009). While improvements in nutrition and medical care have likely attenuated some of the costs of reproduction in women (Bolund et al., 2016), high parity is still associated with elevated morbidity (Guan et al., 2013; Li et al., 2019; Lv et al., 2015) and all-cause mortality (Grundy, 2009; Grundy and Tomassini, 2005; Tamakoshi et al., 2011; Zeng et al., 2016; Grundy and Kravdal, 2008), even in industrialized, economically developed contexts.

Although past work is generally consistent with the premise that reproduction carries costs that accelerate biological aging in women, quantifying these costs using only measures of health and mortality risk later in life has at least several obstacles. First, morbidity and mortality are generally only observable at more advanced chronological ages, which limits their utility in young populations (Belsky et al., 2015). Quantification of biological age in women while they are still young may be advantageous because social and environmental factors can contribute to both reproductive decisions and pace of aging (Lai et al., 2017; Schrempft et al., 2021), confounding that is compounded by the long human lifespan (Helle, 2017). Second, many measures of morbidity and mortality are unidirectional, making them less suitable for longitudinal studies of individual change in biological aging over time. Longitudinal studies of change in the rate of aging minimize individual heterogeneity in genetic background and access to resources or healthcare, which can result in positive phenotypic correlations between reproduction and health or lifespan (Bolund, 2020). Phenotypic correlations occur when individuals with the greatest reserves are able to invest more into both fecundity and somatic maintenance, effectively masking tradeoffs (Noordwijk and de Jong, 1986). Phenotypic correlations are minimized by studying individual change over time, and longitudinal measurements of the costs of reproduction are needed (Bolund, 2020).

A single gold standard for quantifying biological age across the lifespan has thus far been elusive. However, in recent years a collection of methods based on DNA methylation (DNAm) have shown great promise towards this end. These DNAm-based measures of aging have been shown to accurately predict chronological age, mortality risk, and physiological decline (Horvath and Raj, 2018; Ryan, 2020). They also appear to be sensitive to major life-history transitions, including pregnancy (Ryan et al., 2018) and menopause (Levine et al., 2016). Importantly, DNAm measures of aging can be used to study age acceleration decades before it becomes clinically apparent (Simpkin et al., 2016), providing an early indicator of biological aging in young adults. The predictive power of DNA measures of aging and the multitude of domains of aging they appear to capture have brought them to the forefront in the study of biological aging (Ferrucci et al., 2020), and point to their utility for quantifying possible tradeoffs between reproduction and aging in humans (Ryan, 2020).

Here we use six DNAm measures of aging to test tradeoffs between reproduction and biological aging in a sample of 825 young women in the Philippines. Women are participants in the Cebu Longitudinal Health and Nutrition Survey, a long-running and well-characterized study of a birth cohort born in metropolitan Cebu, Philippines. We use detailed, prospectively-collected

reproductive records to focus on the relationship between the number of pregnancies and DNAm measures of aging. Records provide detailed measures of the social and physical environment, allowing us to account for individual differences in access to resources or healthcare that may independently affect the pace of aging. Third, we use DNAm-derived estimates of immune cell counts to test whether or not relationships between DNAm measures of aging are driven by the immunological changes that accompany pregnancy and breastfeeding (Miller, 2009; Ryan et al., 2022). Finally, we capitalize on the longitudinal nature of this study by following up on a subset of 331 women and asking if changes in DNAm measures of aging accompany changes in pregnancy number. This longitudinal approach eliminates residual individual variation in social and environmental exposures by comparing women at follow-up to themselves at baseline, providing a strong test of the tradeoff between reproduction and biological aging (Jasienska, 2020).

Results

Our baseline sample consisted of 825 young female participants in the Cebu Longitudinal Health and Nutrition Survey (CLHNS). The CLHNS is a prospective 1-year birth cohort study started in 1983 with the enrollment of 3327 pregnant women and their offspring in the Cebu Metropolitan Area in the Philippines. The women in our sample are members of the original birth cohort, and have been participating in the study since birth (see Adair et al., 2011; Kuzawa et al., 2020).

At baseline, women were on average 21.7 ± 0.36 years old (range 20.8-22.5y). Of 825 women in the study, 314 had a history of at least one pregnancy at baseline. Within this group, pregnancy number ranged from 1-5 (mean 1.61 ± 0.82). In a subset of 331 participants who became pregnant at least once during a longitudinal follow-up conducted from 2009-2014 (**Table 1**), we updated reproductive histories and collected additional blood samples for follow-up DNAm analysis. DNAm was collected for each woman's last pregnancy during the follow-up period, which ranged in time from 3.5-9 years after baseline measurement. During this time, women reported having been pregnant between 1-8 times (mean 2.31 ± 1.11).

Table 1. Summary of sociodemographic characteristics, reproductive histories, and DNAm measures of aging for women in the full baseline sample used in cross-sectional analyses (n = 825) and for a subsample of women at baseline and follow-up used in the longitudinal analyses (n = 331). Data come from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), a longitudinal study of health and development based in Metropolitan Cebu, Philippines and described in more detail in the methods and elsewhere (Adair et al., 2011; Kuzawa et al., 2020).

Characteristic	Full baseline sample N = 825[1]	Baseline N = 331[1]	Follow-up N = 331[1]
Age	21.67 (0.36)	21.66 (0.35)	27.85 (1.53)
Pregnancy status at sampling			
Not pregnant	761 (92%)	274 (83%)	0 (0%)
Pregnant	64 (7.8%)	57 (17%)	331 (100%)
Gravidity	0.61 (0.93)	0.89 (1.05)	3.11 (1.62)
Horvath	-0.7 (3.9)	-0.6 (4.0)	0.0 (3.6)
Hannum	-0.3 (3.4)	0.1 (3.3)	0.09 (2.63)
PhenoAge	0.3 (4.3)	0.9 (4.3)	0.1 (4.4)
GrimAge	-0.80 (2.35)	-0.56 (2.32)	-0.02 (2.03)
DunedinPACE	1.11 (0.12)	1.13 (0.12)	1.28 (0.10)
DNAmTL	0.04 (0.14)	0.02 (0.13)	0.00 (0.10)
Socioeconomic Score	0.00 (1.56)	-0.32 (1.36)	0.02 (1.42)
Current Smoker (Y)	23 (2.8%)	7 (2.1%)	15 (4.5%)

[1]Mean (SD); n (%)

Cross-sectional analysis of reproductive effort and biological aging

We first tested if women who had been pregnant by early adulthood were biologically older than women who had not been pregnant. Following our pre-registered analysis plan for dealing with

high-leverage outliers, we fit robust models as defined by Yohai (1987) with pregnancy (ever vs. never pregnant) as the exposure and six DNAm measures of aging as the outcomes of interest. DNAm measures included the Horvath and Hannum first generation clocks, the PhenoAge and GrimAge second-generation clocks, the DunedinPACE pace of aging measure, and DNAmTL, a DNAm surrogate measure of leukocyte telomere length. As covariates, we included: a composite measure of socioeconomic status that included all sources of household income, education, and assets that reflect population-relevant aspects of social class; a measure of urbanicity of the participants' primary residence; pregnancy status at the time of the blood sample; smoking status at the time of blood sample; and the top ten principal components of genome-wide genetic variation. Additional details on the derivation of these measures are provided in the **Supplementary Information**.

For all DNAm measures of aging, women with a history of at least one pregnancy appeared biologically older than women who had never been pregnant (**Table 2, Panel A**). The effect sizes ranged from 0.13 standard deviations for the GrimAge clock to 0.27 for Horvath's clock (**Table 2, Panel A**). As expected, the effect of ever having been pregnant had the opposite effect on DNAmTL (i.e. pregnancy was associated with shorter DNAmTL, consistent with more advanced biological age). These effects are equivalent to between 3.7 and 13.1 months of accelerated aging for first- and second-generation clocks, an accelerated pace of aging of 2% per year according to DunedinPACE, and a shortening of 0.03 kilobases according to DNAmTL. Results for both robust models and ordinary least squares models including high leverage data points are provided in **Table S1**.

DNAm in blood can be affected by leukocyte composition at the time of sampling (Ziller et al., 2013), which may be reflected in DNAm measures of aging (Komaki et al., 2022; Zhang et al., 2019). To test for the robustness of our findings to differences in leukocyte composition between women, we refit the above models with the addition of estimates of CD4T, CD8T, Natural Killer cells, B cells, Monocytes, and Granulocytes (Houseman et al., 2012). As with models that did not include cell counts, women with a history of at least one pregnancy appeared biologically older than women who had never been pregnant for all DNAm measures of aging. Results for both robust models and ordinary least squares models including high leverage data points and controlling for cell counts are provided in **Table S2**.

Next, we tested whether women who experienced a greater number of pregnancies appeared biologically older than women with fewer or no pregnancies. We again fit robust models as defined by Yohai (1987), with gravidity (number of pregnancies) as the exposure of interest, and the same six DNAm measures of aging as the outcomes of interest. Socioeconomic status, urbanicity, pregnancy status, genetic variation, and smoking status were again included as covariates. For all DNAm measures of aging, women with a history of more pregnancies looked biologically older than women with fewer pregnancies (**Table 2, Panel B**). The per pregnancy effect sizes ranged from 0.05 standard deviations for the GrimAge clock to 0.12 standard deviations for DunedinPACE. These effects are equivalent to between 1.4 and 5.2 months per pregnancy for first- and second-generation clocks, an accelerated pace of aging of 1% per year per pregnancy according to DunedinPACE, and shortening of 0.013 kilobases per pregnancy according to DNAmTL. Results for both robust models and ordinary least squares models are provided in **Table S3**. Gradients in biological aging by number of pregnancies at study baseline are shown in **Figure 1**.

Our analysis of the effect of pregnancy number on DNAm measures of aging including immune cell composition yielded similar results. Results for both robust models and ordinary least squares models controlling for cell counts are provided in **Table S4**.

Table 2. Relationship between measures of reproductive effort and six DNAm measures of biological aging in 825 young women in the Philippines. Panel A shows the relationship between pregnancy (ever pregnant vs. never pregnant) and cross-sectional DNAm measures of aging. Panel B shows the relationship between pregnancy number and cross-sectional DNAm measures of aging. Estimates and 95% confidence intervals are in standard deviations, equivalent to Cohen's d, and p-values below alpha of 0.05 are bolded.

Panel A. Relationship between pregnancy (ever pregnant vs. never pregnant) and cross-sectional DNAm measures of biological aging

	Horvath		**Hannum**		**PhenoAge**		**GrimAge**		**Dunedin PACE**		**DNAmTL**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
Ever Pregnant (Yes)	0.27	**<0.001**	0.17	**0.019**	0.25	**0.001**	0.13	0.065	0.24	**0.001**	-0.2	**0.008**
	(0.14 – 0.40)		(0.03 – 0.32)		(0.10 – 0.39)		(-0.01 – 0.28)		(0.10 – 0.38)		(-0.35 – -0.05)	
Observations	825		825		825		825		825		825	
R^2 / R^2 adjusted	0.059 / 0.040		0.107 / 0.090		0.170 / 0.153		0.203 / 0.187		0.231 / 0.216		0.108 / 0.090	

Panel B. Relationship between pregnancy (number of times pregnant) and cross-sectional DNAm measures of biological aging

	Horvath		**Hannum**		**PhenoAge**		**GrimAge**		**Dunedin PACE**		**DNAmTL**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
Gravidity	0.11	**0.002**	0.08	**0.030**	0.08	**0.041**	0.05	0.194	0.12	**0.002**	-0.09	**0.031**
	(0.04, 0.18)		(0.01, 0.15)		(0.00, 0.16)		(-0.03, 0.13)		(0.05, 0.20)		(-0.17, -0.01)	
Observations	825		825		825		825		825		825	
R^2 / R^2 adjusted	0.051 / 0.032		0.106 / 0.088		0.162 / 0.146		0.201 / 0.185		0.230 / 0.214		0.105 / 0.087	

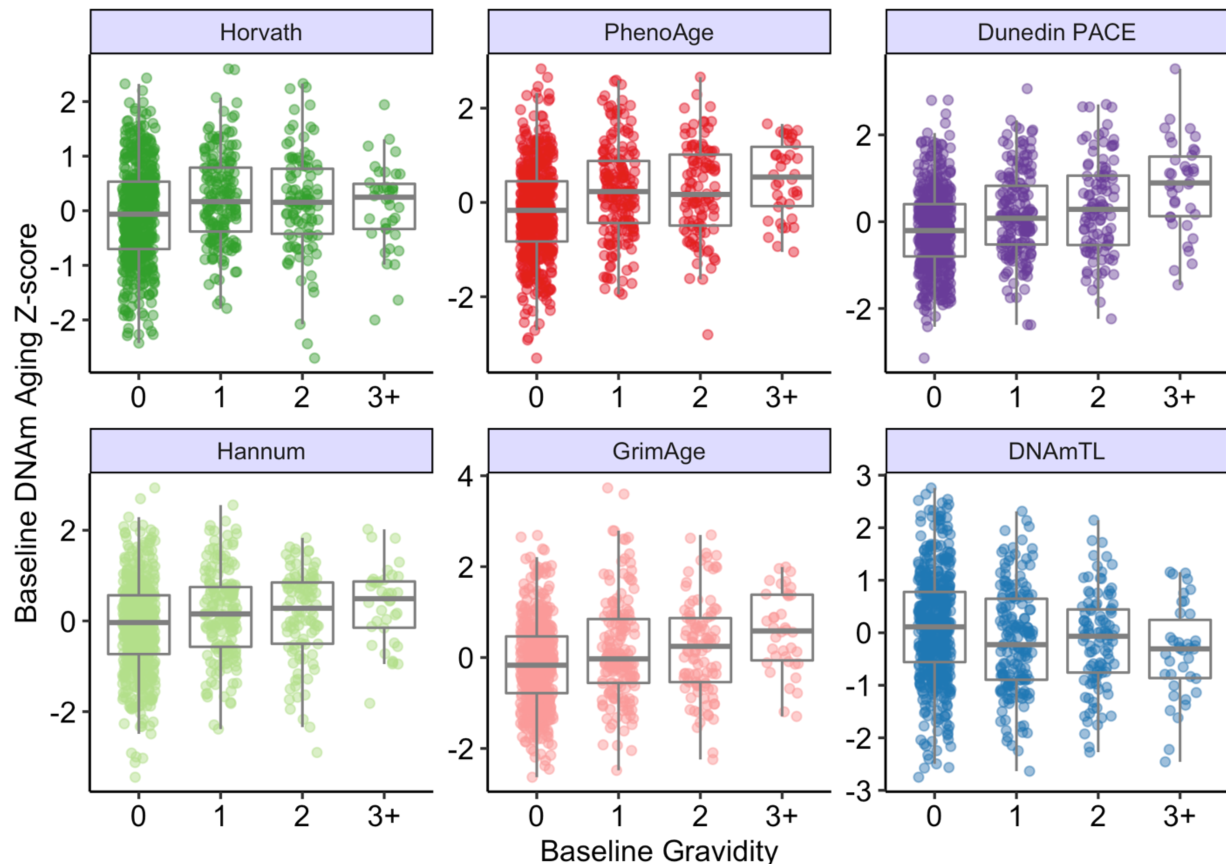

Figure 1. Cross-sectional relationship between number of pregnancies and first (Horvath, Hannum) and second (PhenoAge, GrimAge) generation epigenetic clocks, DunedinPACE pace of aging, and a DNAm surrogate measure for leukocyte telomere length (DNAmTL). Higher values for all clocks correspond to accelerated biological aging, except for DNAmTL, where lower values correspond to shorter telomere length and accelerated aging. Four high leverage data points identified during model diagnostics are excluded from the figure, and models were run using robust regression. Results for both robust and ordinary least squares models are provided in **Table S2**.

Longitudinal changes in reproductive effort and epigenetic aging

We next asked whether the pace of biological aging that women experienced during the follow-up period was related to their number of new pregnancies during that period. This approach minimizes individual variability in health and access to resources that may confound cross-sectional findings, and allowed us to test whether greater increases in reproductive effort between baseline and follow-up were associated with accelerated biological aging.

Women who had more pregnancies between baseline and follow-up showed greater changes in both Horvath and Hannum first-generation clocks, but no significant changes according to any of the other DNAm measures of aging (**Table 3**). Horvath and Hannum effect sizes were similar to those of our cross-sectional analysis. Horvath epigenetic age increased 0.06 standard deviations – equivalent to 2.9 months – for each additional pregnancy (95% CI β = 0.01-0.11, $p = 0.024$). Hannum's epigenetic clock increased 0.06 standard deviations – equivalent to 2.4 months – for each additional pregnancy (95% CI β = 0.01-0.11, $p = 0.026$). Results for both robust models and ordinary least squares models are provided in **Table S5**. Gradients in changes in DNAm measures of aging by change in pregnancy number between baseline and follow-up are shown in **Figure 2**.

Our sensitivity analysis that included changes in immune cell composition yielded similar results. Results for both robust models and ordinary least squares models controlling for cell counts are provided in **Table S6**.

Table 3. Relationship between longitudinal changes in reproductive effort and changes in six DNAm measures of biological aging in 331 young women in the Philippines. Estimates and 95% confidence intervals are in standard deviations, equivalent to Cohen's d, and p-values below alpha of 0.05 are bolded.

Relationship between longitudinal changes in pregnancy number and longitudinal changes in DNAm measures of biological aging

	ΔHorvath		**ΔHannum**		**ΔPhenoAge**		**ΔGrimAge**		**ΔDunedin PACE**		**ΔDNAmTL**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
ΔGravidity	0.06 (0.01, 0.11)	**0.024**	0.06 (0.01, 0.11)	**0.026**	-0.03 (-0.12, 0.05)	0.419	-0.03 (-0.12, 0.05)	0.441	-0.03 (-0.10, 0.03)	0.329	-0.01 (-0.07, 0.06)	0.884
Observations	331		331		331		331		331		331	
R^2 / R^2 adjusted	0.485 / 0.457		0.711 / 0.695		0.438 / 0.408		0.522 / 0.496		0.513 / 0.487		0.513 / 0.486	

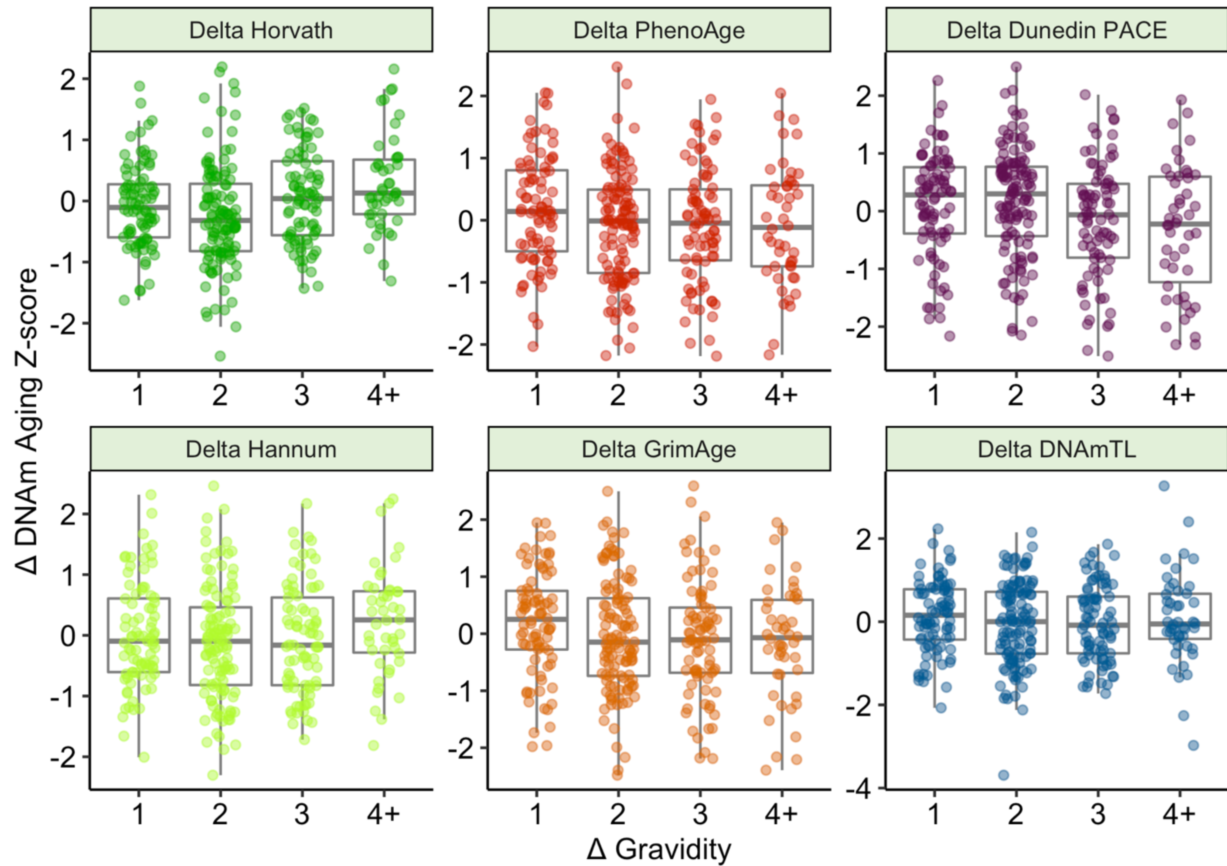

Figure 2. Longitudinal association between change in pregnancy number and change in biological aging measured using and first (Horvath, Hannum) and second (PhenoAge, GrimAge) generation epigenetic clocks, DunedinPACE pace of aging, and a DNAm surrogate measure for leukocyte telomere length (DNAmTL). Higher values for all clocks correspond to accelerated biological aging, except for DNAmTL, where lower values correspond to shorter telomere length and accelerated aging. Four high leverage data points identified during model diagnostics are excluded from the figure, and models were run using robust regression. Results for both robust and ordinary least squares models are provided in **Table S5**.

Discussion

Using six DNAm measures shown to predict mortality risk, physiological dysregulation, and biological decline, we provide evidence that women with a history of at least one pregnancy exhibited faster biological aging compared to those without a history of pregnancy. We also show that women who had experienced a greater number of pregnancies by early adulthood exhibited faster biological aging. Longitudinally, women who experienced a greater number of pregnancies between baseline and follow-up exhibited faster rates of biological aging using the Horvath and Hannum clocks. These relationships were robust to potential social, environmental, and genetic confounding in the form of socioeconomic status and measures of urbanicity, smoking, and genetic variation, and were not substantively modified by adjustment for differences in estimated cell composition. Taken together, these findings provide evidence that pregnancy accelerates molecular aging in a healthy, young adult population.

Our findings are consistent with our preregistered hypotheses based on evolutionary theory, and are in broad agreement with previous cross-sectional work documenting relationships between reproductive effort and DNAm measures of biological age. In a pilot sample from this population, we previously reported that gravidity was associated with accelerated aging using Horvath's clock as well as leukocyte telomere length, another measure of molecular aging (Ryan et al., 2018). Similarly, Kresovich and colleagues found evidence that parity was associated with faster Horvath, Hannum, and PhenoAge clocks in a large sample of American women participating in the Sister Study (Kresovich et al., 2019). Notably, the standardized effect sizes reported by Kresovich et al. for both Horvath and Hannum clocks are comparable to those reported here (Horvath β=0.10 vs. 0.11; Hannum β=0.12 vs. 0.08). These findings contrast with a recent cross-sectional study among young Finnish women, which reported no relationship between nationally-registered births and four measures of epigenetic aging (Harville et al., 2021). To the extent that individual differences in access to resources and factors like healthcare might obscure tradeoffs at the population level, social and economic differences between countries may partly explain these divergent findings. The women in our study come mostly from low or middle-income households, have limited state-level social support, and variable access to high-quality healthcare (Adair et al., 2011). Furthermore, our study is characterized by comparatively high fertility and early age at reproductive debut. Support for costs of reproduction on biological aging in the CLHNS is consistent with the expectation that tradeoffs between reproduction and somatic maintenance will be greatest when resources are limited, reproductive effort is high, and when pregnancy overlaps temporally with late adolescent growth (Jasienska, 2020).

Our cross-sectional analyses are consistent with an effect of gravidity on the pace of biological aging. Although we control for potential social, environmental, genetic, and immunological confounders, estimates of the effect of pregnancy on biological aging using cross-sectional data could be sensitive to residual confounding by variation not captured using these measures. Longitudinal approaches that model the predictors of change in the outcome over time minimize the impact of factors that vary across individuals but are stable within individuals over time (e.g. birth weight, early growth and development, family socioeconomic stratum, parental education)(Singer et al., 2003). Using this longitudinal approach, we found that women who had more pregnancies between baseline and follow-up aged more quickly according to both Horvath and Hannum clocks. To our knowledge, this is the first study connecting longitudinal changes in pregnancy number to longitudinal changes in DNAm measures of aging, thus providing a stronger basis for causal inference.

It is unclear why the effect of gravidity on longitudinal changes in epigenetic age were present for Horvath and Hannum clocks, but not the other DNAm measures of aging we examined. One potential explanatory factor is the underlying construction of the various DNAm measures of aging, which differ in the predictive targets and data used in their development. Both Horvath and Hannum were trained using machine-learning algorithms to predict chronological age. In contrast, PhenoAge, GrimAge, and DunedinPACE were trained using measures of blood chemistry, physiology, and organ-system integrity, and were built using samples from high-income, Western settings (Belsky et al., 2021; Levine et al., 2018; Lu et al., 2019). The metabolic, immunological, and inflammatory profiles prevalent in high-income, Western settings often differ from those observed in the CLHNS and other non-Western contexts (McDade et al., 2010, 2009). More importantly, all of our follow-up samples were taken from pregnant women, whose metabolic, physiological and immunological profiles – as well as methylomes – are quite different from the largely non-pregnant population used in the training datasets (Gruzieva et al., 2019; Miller, 2009; Ryan et al., 2022). As a result, PhenoAge, GrimAge, and DunedinPACE may be less well-suited to detect mortality and physiological decline in our study.

First-generation Horvath and Hannum clocks trained on chronological age may therefore be less sensitive to differences between populations and to reproductive status. Alternatively, the fact that we detected a longitudinal relationship between gravidity and DNAm age for Horvath and Hannum clocks alone could reflect an artifact of the clocks themselves. Both of these clocks were trained to predict chronological age blind to other age-correlated variables, including reproductive effort (Hannum et al., 2013; Horvath, 2013). It is possible, therefore, that some portion of the chronological age-associated signal in Horvath and Hannum clocks reflects gravidity in the training dataset, which by necessity is positively correlated with chronological age. While it is difficult to test this theory in the original data used to develop Horvath and Hannum clocks, which lack information on reproductive history, other approaches, such as simulations generating chronological age clocks with and without reproductive history as a covariate in the training data, may help to resolve this question.

Our findings should be interpreted in the context of several limitations. First, we used gravidity – the number of pregnancies – as a proxy for reproductive effort, and did not include other forms of reproductive investment, such as breastfeeding and child-rearing (Jasienska, 2020). Pregnancy number provides an unambiguous measure of reproductive effort compared to breastfeeding or child-rearing, which are highly heterogeneous and often socially-stratified (Ekholuenetale et al., 2021). However, tallying the number of pregnancies is unlikely to fully reflect reproductive investment, thus underestimating the costs of reproduction in these women (Jasienska, 2020). A second limitation is the use of relatively young women in both cross-sectional and longitudinal analyses. If the costs of reproduction are cumulative, becoming most evident at older ages and higher parity, our analysis may not capture the full impact of pregnancy on DNAm measures of biological aging. Work in at least one large representative sample of American women suggests that the effect of reproductive effort on biological aging may not be fully apparent until later in life (Shirazi et al., 2020). If this is the case in the Philippines, our focus on young women would tend to underestimate the effect of gravidity on DNAm measures of biological aging. Third, we are not yet able to link these DNAm measures of biological to morbidity and mortality in later life in this population. While such links are now well-established among older individuals in the USA and Europe, the connection between faster biological aging and morbidity and mortality in young people, and in individuals living in non-Western contexts – where physiological and molecular aging follows different trajectories (e.g. Horvath et al., 2016) – is still unclear. Future work validating the predictive ability of DNAm measures of aging among younger individuals, and in more socioecologically diverse global samples like the Philippines, is urgently needed.

Conclusions

This is the first pre-registered, large-scale study to examine a suite of DNAm measures of aging in young women in a high fertility context while controlling for a range of social, environmental, genetic, and immunological confounders. We find support for an effect of gravidity on DNAm measures of aging, consistent with theorized tradeoffs between reproduction and aging and supported by epidemiological findings that high reproductive effort may increase the risk for a range of diseases and early mortality. These findings suggest that gravidity accelerates biological aging early in women's reproductive careers, and that these effects may be detectable starting at a relatively young age.

Methods

Population and Study Context

Data are derived from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), a birth cohort study started in 1983-84 in Metropolitan Cebu, Philippines, and are available for download at: https://dataverse.unc.edu/dataverse/cebu. The current study focuses on surveys conducted in 2005 (baseline) and 2009-2014 (follow-up). Descriptive statistics of both baseline and follow-up samples are provided in **Table 1**. Surveys were conducted in-home by a trained interviewer, and included questions about mental and physical health, behavior, socioecological context. A subset of questions focused on reproductive history, including number of known pregnancies, their duration, and outcomes. Pregnancy status in 2005 was reported at the time of sampling, and through back-calculation from subsequent surveys based on parturition within 9 months of the original interview (all past pregnancies and their outcomes are recorded as part of ongoing tracking process). Household income, parental education, and assets were used to create a composite score of socioeconomic status, described in more detail in Ryan et al. (2018) and in the **Supplementary Information**. Follow-up samples focused on a subset of participants enrolled in additional surveys tracking new pregnancies between 2009-2014. Informed consent was obtained from all participants and data collection was conducted with approval and in accordance with the Institutional Review Boards of the University of North Carolina at Chapel Hill, the Office of Population Studies Foundation, and Northwestern University

DNA methylation and Epigenetic Clocks

Blood samples for DNA methylation were collected concurrent with in-home interviews. Baseline blood samples were collected in EDTA-coated vacutainer tubes from overnight fasted subjects. Follow-up blood samples were collected using capillary whole blood collected on filter paper. DNA was extracted using a standard protocol; 750ng of genomic DNA was treated with sodium bisulfite (Zyme EZDNA, Zymo Research, Irvine, CA, USA) and 160ng of converted DNA was applied to the Illumina Infinium MethylationEPIC BeadChip under standard conditions (Illumina Inc., San Diego, CA, USA). Technicians were blind to information regarding participant characteristics, and samples were randomly assigned to plate, chip, and row. Background subtraction and color correction were performed using Illumina Genome Studio with default parameters. Data were then exported into R for further analysis. Quality control for baseline was performed as part of a larger sample to confirm participant sex and replicate status. This was followed by quantile normalization 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 (Ryan 2020). The same quality control steps were followed for follow-up samples. DNAmAge for DNAmHorvath, DNAmHannum, DNAmPheno, DNAmGrim, and DNAmTL clocks were calculated using the online calculator (http://labs.genetics.ucla.edu/horvath/dnamage/). Background-corrected beta values were processed further using the calculator's internal normalization algorithms. The DNAmPACE clock was generated using the DunedinPACE calculator, available at (https://github.com/danbelsky/DunedinPACE).

Statistical Methods

We first examined cross-sectional DNAm measures of aging for women at baseline who had never been pregnant compared to those who had. We next examined the relationship between cross-sectional DNAm measures of aging and gravidity as a continuous variable. In both cases, DNAm measures of aging were the outcome of interest, with age, gravidity, pregnancy status at the time of blood sampling, composite score of socioeconomic status, an urbanicity score (Dahly and Adair,

2007), smoking status, and the top-10 principal components of genetic variation as covariates. Exploratory data analysis and quality checks revealed potential outliers with values greater than 3 standard deviations from the mean for several epigenetic clocks. In accordance with our pre-registration analysis plan (https://osf.io/mqb37), we fit ordinary least squares models including these values and examined diagnostics plots. These extreme observations had large Cook's distances and were high leverage, with influential effects on model estimates (**Figures S1** and **S2**). Again, in accordance with our preregistration protocol, we fit all models using a robust regression method defined by Yohai (1987). Results shown in the manuscript are for models fit with robust regression, but both approaches yielded qualitatively similar results. Results for ordinary least squares regression including high-leverage outliers are provided in the **Supplementary Information**. Next, we fit modelled longitudinal effects of reproduction by examining whether changes in DNAm measures of aging were associated with changes in gravidity. Here, change in DNAm measures of aging were the outcome of interest, changes in gravidity was the predictor of interest, and change in age, baseline pregnancy status at the time of blood sampling, composite score of socioeconomic status, urbanicity score, smoking status, and the top-10 principal components of genetic variation were included as covariates. Finally, we ran sensitivity analyses on all models that included bioinformatically estimated proportions of CD4T, CD8T, Natural Killer (NK), B cell, Monocytes, and Granulocytes (Houseman et al., 2012). Cross-sectional analyses at baseline included baseline measures of immune cell counts, while longitudinal models included changes in cell counts between baseline and follow-up.

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