# Menopause accelerates biological aging

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**Although epigenetic processes have been linked to aging and disease in other systems, it is not yet known whether they relate to reproductive aging. Recently, we developed a highly accurate epigenetic biomarker of age (known as the "epigenetic clock"), which is based on DNA methylation levels. Here we carry out an epigenetic clock analysis of blood, saliva, and buccal epithelium using data from four large studies: the Women's Health Initiative ($n$ = 1,864); Invecchiare nel Chianti ($n$ = 200); Parkinson's disease, Environment, and Genes ($n$ = 256); and the United Kingdom Medical Research Council National Survey of Health and Development ($n$ = 790). We find that increased epigenetic age acceleration in blood is significantly associated with earlier menopause ($P$ = 0.00091), bilateral oophorectomy ($P$ = 0.0018), and a longer time since menopause ($P$ = 0.017). Conversely, epigenetic age acceleration in buccal epithelium and saliva do not relate to age at menopause; however, a higher epigenetic age in saliva is exhibited in women who undergo bilateral oophorectomy ($P$ = 0.0079), while a lower epigenetic age in buccal epithelium was found for women who underwent menopausal hormone therapy ($P$ = 0.00078). Using genetic data, we find evidence of coheritability between age at menopause and epigenetic age acceleration in blood. Using Mendelian randomization analysis, we find that two SNPs that are highly associated with age at menopause exhibit a significant association with epigenetic age acceleration. Overall, our Mendelian randomization approach and other lines of evidence suggest that menopause accelerates epigenetic aging of blood, but mechanistic studies will be needed to dissect cause-and-effect relationships further.**

menopause | DNA methylation | aging | WHI | epigenetic clock

Reproductive senescence, concluding in menopause, is a feature of all female mammals (1), but humans are unique in that they experience exceptionally long postreproductive lifespans. Within human populations, the timing of menopause onset has been linked to susceptibility for age-related morbidity and mortality outcomes (1). For instance, observational studies have uncovered associations between a woman's age at menopause and her subsequent risk of mortality. Results based on 12,134 Dutch women showed that for every 1-y increase in the age of menopause, the age-adjusted mortality rate was decreased by 2% (2).

Although social/behavioral and developmental factors, such as smoking, lifetime socioeconomic circumstances, infant growth, breastfeeding, and childhood cognitive ability have been shown to influence reproductive aging, age at menopause is also considered to be highly heritable, with estimates from twin and sibling studies ranging from about 0.40–0.70 (3–10). A recent large-scale genome-wide association study identified 44 genomic loci with common variants that significantly related to age at menopause (11), and a case-control study comparing centenarian women and those with average lifespans found that individuals from families with a history of longevity also tend to exhibit delayed reproductive aging (12).

Although these and other studies suggest that there might be a relationship between age at menopause and the biological aging rate, it has been difficult to test this hypothesis because of the dearth of molecular biomarkers of aging. Several recent articles describe epigenetic biomarkers of aging based on methylation levels (13–16), drawing on the profound effect of chronological age on DNA methylation (DNAm) levels (17–26). Although previous articles describe epigenetic age measures that apply only to a single tissue, i.e., saliva (13) or blood (14), our recently developed "epigenetic clock" method (based on 353 CpGs) applies to the majority of human tissue and cell types that contain DNA, with the exception of sperm (15). Age acceleration (AgeAccel) effects can be estimated by contrasting DNAm age with an individual's chronological age. For instance, a woman whose blood has a higher DNAm age than expected based on her chronological age can be said to exhibit positive AgeAccel, i.e., to be aging faster than expected. AgeAccel also has been shown to have a strong

## Significance

**Within an evolutionary framework, aging and reproduction are intrinsically linked. Although both laboratory and epidemiological studies have observed associations between the timing of reproductive senescence and longevity, it is not yet known whether differences in the age of menopause are reflected in biomarkers of aging. Using our recently developed biomarker of aging, the "epigenetic clock," we examined whether age at menopause is associated with epigenetic age of blood, saliva, and buccal epithelium. This is a definitive study that shows an association between age of menopause and biological aging (measured using the epigenetic clock). Our results also indicate menopause may accelerate the epigenetic aging process in blood and that age at menopause and epigenetic age acceleration share a common genetic signature.**

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**Table 1. Unadjusted metaanalysis of AgeAccel in blood versus age at menopause**

| Exclusion criteria | Metaanalysis Z statistics (*P* values) |
|---|---|
| Excluded because age at menopause ≤30 y | 3.3 ($P = 0.00091$) |
| Excluded because of surgical menopause | 2.6 ($P = 0.0083$) |
| None | 2.7 ($P = 0.0061$) |

The first and second rows correspond to analysis after excluding women who were 30 y or younger at the age of menopause and women with surgical menopause, respectively. The third row reports findings with no exclusions applied.

genetic basis, with heritability estimates of 40% for older subjects (15, 27). Although the epigenetic clock has been shown to relate to a number of aging-related outcomes (27–30), it is not yet known whether it relates to reproductive aging.

Using data from four large observational studies (described in Table S1)—the Women's Health Initiative (WHI), Invecchiare nel Chianti (InCHIANTI), Parkinson's disease, Environment, and Genes (PEG), and the United Kingdom Medical Research Council National Survey of Health and Development (NSHD)—we examined the associations between epigenetic age and three menopause-related phenotypes: age at menopause, bilateral oophorectomy, and the use of menopausal hormone therapies (MHT). Given the strong heritability of both age at menopause and epigenetic age, we also estimated the genetic correlation between age at menopause and epigenetic aging and carried out a Mendelian randomization analysis to examine causality using the top two SNPs shown previously to be strongly associated with age at menopause (31).

## Results

**AgeAccel of Blood Versus Age at Menopause.** In our primary analysis, we considered only women whose menopause occurred after age 30 y. As shown in Table 1, based on results from Pearson correlations in each of our three blood datasets (WHI, InCHIANTI, and PEG), metaanalysis showed that age at menopause was significantly associated with epigenetic AgeAccel ($P = 0.00091$). Similar metaanalytic *P* values were obtained in our secondary analysis, which excluded women with surgical menopause ($P = 0.0083$), and in our tertiary analysis, which used all women regardless of age or type (natural/surgical) of menopause ($P = 0.0061$). Pearson correlation results from individual studies and stratified by race/ethnicity can be found in Fig. S1.

**Multivariate Linear Models Linking AgeAccel with Age at Menopause.** Using blood methylation data from the WHI, InCHIANTI, and PEG studies, we conducted multivariate regression for women who experienced menopause after age 30 y and combined results using metaanalysis to determine whether covariates accounted for the association between epigenetic AgeAccel and age at menopause (Table 2). Models were adjusted for age, race/ethnicity (in the WHI and PEG cohorts), smoking status, age at menarche, and MHT use. Additionally, models run using PEG data were also adjusted for Parkinson's disease (PD). To retain the moderate number of women for whom data on MHT use were missing in the PEG study, those who were missing data were coded as "never," and a dummy variable for missing was added to the model. After adjusting for possible confounders, we found that higher epigenetic AgeAccel is associated with a younger age at menopause (Meta$P = 8.32 \times 10^{-4}$).

**Time Since Menopause and Surgical Menopause Are Associated with Epigenetic AgeAccel.** To examine whether menopause may contribute to accelerated aging, we tested the association between epigenetic aging and time since menopause using multivariate models that adjust for race/ethnicity (WHI and PEG studies) and smoking (Table S2). Results showed that the variable "time since menopause" was associated with AgeAccel ($\beta = 0.038$, $P = 0.007$) in the WHI and in our metaanalysis (AgeAccel, $P = 0.017$).

The menopause of a substantial number of women resulted from bilateral oophorectomy (i.e., $n = 127$ women in the WHI white, 112 in the WHI black, and 50 women in the WHI Hispanic cohorts and 48 women in the PEG study). We evaluated the effect of surgical menopause on epigenetic AgeAccel among women whose bilateral oophorectomy took place before age 50 y (Fig. 1). The association was highly consistent across the blood datasets (Fig. 1), leading to a significant metaanalysis *P* value for all three measures of AgeAccel: Stouffer's $Z = 3.2$; $P = 0.0014$.

**Genetic Correlation and Mendelian Randomization Between Epigenetic AgeAccel and Age at Menopause.** Using the WHI data ($n = 1{,}940$), we conducted a bivariate restricted maximum likelihood (REML) analysis to examine the overlap in genetic variants that accounted for the heritability of both age at menopause and epigenetic AgeAccel. As shown in Table 3, the 10,769,392 autosomal SNPs or indel markers included in the analysis accounted for about 38% of the variance in age at menopause and 65% of the variance in AgeAccel. The genetic correlation (or pleiotropy) between AgeAccel and age of menopause was marginally significant with $rG = -0.256$ (one-sided $P = 0.054$).

Although we clearly demonstrate that age at menopause relates to epigenetic AgeAccel, our cross-sectional data make it difficult to dissect causal relationships. Mendelian randomization (reviewed in ref. 32)—the random assortment of genes from parents to offspring that occurs during gamete formation and conception—provides one method for assessing the causal nature of associations. We tested the hypothesis that menopause leads to an acceleration of epigenetic aging by leveraging the two most highly significant SNPs from a genome-wide association study for age at menopause, rs11668344 (replication *P* value $= 2.65 \times 10^{-18}$) and rs16991615 (replication *P* value $= 7.90 \times 10^{-21}$). If menopause accelerates

**Table 2. Multivariate metaanalysis of AgeAccel in blood versus age at menopause**

| | β-coefficient (*P* value) | | | |
|---|---|---|---|---|
| AgeAccel | WHI | InCHIANTI | PEG | Meta *P* value |
| Age at menopause | −0.063 (0.001) | −0.012 (0.772) | −0.060 (0.350) | $8.32 \times 10^{-4}$ |
| Chronological age | 0.012 (0.530) | −0.010 (0.825) | −0.051 (0.305) | 0.837 |
| Non-Hispanic black | 0.008 (0.980) | — | −5.113 (0.201) | 0.714 |
| Hispanic | −0.918 (0.008) | — | −1.905 (0. 321) | 0.005 |
| Former smoker | −0.312 (0.234) | 0.446 (0.648) | −1.177 (0.238) | 0.198 |
| Current smoker | −0.189 (0.666) | −0.870 (0.394) | −1.378 (0.613) | 0.427 |
| MHT | 0.041 (0.871) | 0.940 (0.368) | 2.864 (0.018) | 0.269 |
| Age at menarche | −0.055 (0.501) | 0.280 (0.179) | −0.020 (0.950) | 0.821 |
| PD status | — | — | 1.075 (0.282) | — |

For all models, women with age at menopause <30 y were excluded.

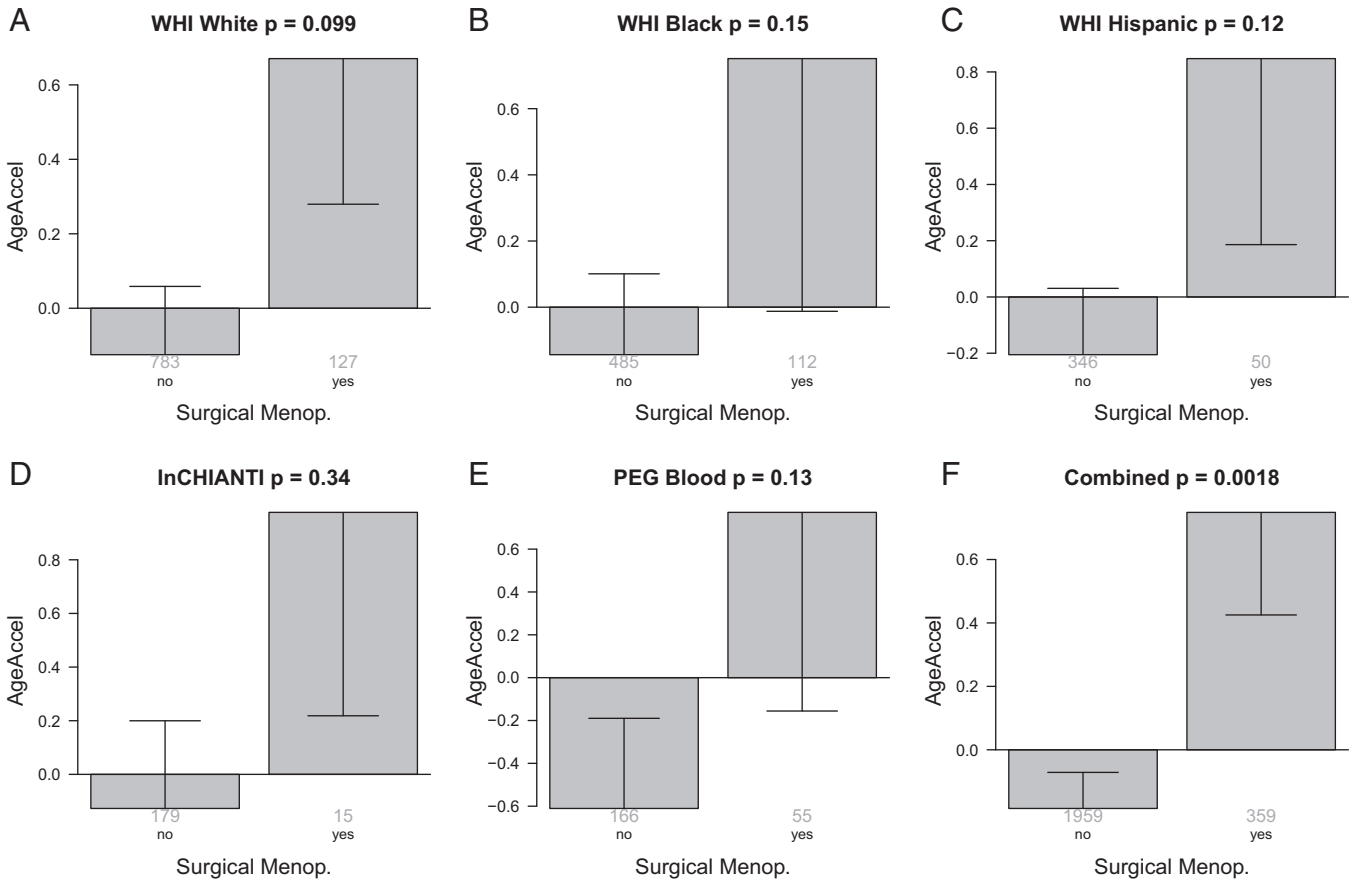


**Fig. 1.** Epigenetic AgeAccel versus surgical menopause status. The *x* axis of each plot reports surgical menopause status before age 50 y; i.e., "yes" denotes the group of women who experienced surgical menopause before age 50 y, and "no" corresponds to the group of women who did not undergo bilateral oophorectomy at any age before the blood draw. The bar plots report mean values of AgeAccel (± 1 SE) and the *P* value from a Student *t* test. The six panels show data from WHI (white) (*A*), WHI (black) (*B*), WHI (Hispanic) (*C*), InCHIANTI (*D*), and PEG (*E*) samples and all samples combined (*F*). A metaanalysis based on Stouffer's Z method indicates that AgeAccel is significantly positively associated with surgical menopause status ($P = 0.0018$).

epigenetic aging, then one would expect these two SNPs also to relate to epigenetic AgeAccel, as was found for rs11668344 ($P = 0.031$) (Table 4). Additionally, the directionality of the associations is consistent with the causal model SNP → age at menopause → AgeAccel, i.e., the minor allele is associated with earlier age at menopause and higher epigenetic AgeAccel. On the other hand, rs16991615 was not associated with epigenetic AgeAccel ($P = 0.763$).

**Analysis of Buccal Epithelium and Saliva.** We are not aware of any biological reason suggesting that blood tissue stands out when it comes to studying menopausal effects. Several previous datasets demonstrate that the epigenetic clock method also applies to buccal epithelium (33). Therefore we also examined epigenetic age measured in 790 buccal epithelium samples from women participating in the NSHD study from the UK Medical Research Council, a birth cohort of men and women born in Britain in March 1946.

Unlike our results in blood, MHT was associated with a significantly lower epigenetic AgeAccel of buccal epithelium ($P = 0.00078$) (Fig. 2*A*), whereas age at natural or surgical menopause did not correlate with epigenetic AgeAccel across the postmenopausal buccal samples ($n = 419$) (Fig. 2 *B* and *D*), and no significant association was found between AgeAccel and menopausal status at age 53 y ($n = 469$ postmenopausal and 321 premenopausal samples) (Fig. 2*C*).

We also analyzed saliva samples from 113 women from the PEG study, 16 of whom had undergone surgical menopause. As in buccal epithelium, age at menopause and time since menopause were not associated with epigenetic AgeAccel in saliva. However, the directionality and size of the effect were consistent with results in blood ($r = -0.11$, $P = 0.3$), suggesting that the null association could reflect the low sample size. However, surgical menopause was associated with increased AgeAccel of saliva ($P = 0.0079$) (Fig. 3).

## Discussion

To the best of our knowledge, ours is the first study showing that (*i*) the epigenetic age of blood has a negative correlation with age at menopause; (*ii*) surgical menopause is associated with an increased epigenetic age of blood and saliva; (*iii*) MHT is inversely associated with epigenetic AgeAccel of buccal epithelium; and (*iv*) an SNP that relates to age at menopause also relates to epigenetic AgeAccel. Although our study demonstrates that postmenopausal

**Table 3. GCTA bivariate REML results**

| Statistic | Value |
|---|---|
| Heritability of AgeAccel (SE) | 0.651 (0.13) |
| Heritability of Age at menopause (SE) | 0.384 (0.14) |
| Genetic correlation with age at menopause (*P* value) | −0.256 (0.054) |
| Genetic variance (SE) | 18.71 (3.90) |
| Genetic covariance) (SE) | −4.70 (3.69) |
| Residual variance (SE) | 10.03 (3.61) |
| Residual covariance (SE) | 1.69 (3.47) |
| Log likelihood | −9017.31 |

**Table 4. Mendelian randomization**

| Chromosome | SNP | Base pair position | Minor/major alleles | β-coefficient (*P* value) |
|---|---|---|---|---|
| 19 | rs11668344 | 55833664 | G/A | 0.506 (0.031) |
| 20 | rs16991615 | 5948227 | A/G | 0.151 (0.763) |

women with a late onset of menopause are epigenetically younger than women with an early onset of menopause, dissecting cause-and-effect relationships is challenging. Here we discuss several causal scenarios that could explain the reported findings (Fig. S2).

The first causal model, age at menopause ← biological age → epigenetic AgeAccel, assumes that both age at menopause and epigenetic AgeAccel are indicator variables of a latent variable, which can be thought of as true biological age. There is evidence that risk factors for heart disease, which arguably accelerate biological age, likely contribute to an earlier age of menopause, rather than the commonly held notion that early menopause is a risk factor for heart disease (34). Consistent with evolutionary theories of aging, a genetic predisposition to later menopause may coincide with an innate protection against early mortality (35). In this study, we observe a suggestive genetic correlation between age at menopause and epigenetic AgeAccel which may suggest that increased age at menopause is genetically linked to decreased epigenetic AgeAccel. This observation advocates for a common genetic etiology and is consistent with both variables being indirect measures of biological age; genetic variants of these variables influence biological aging therefore may lead to both slower reproductive aging and lower epigenetic age.

Another causal model assumes that menopause leads to an increase of epigenetic age. This model is supported by the following lines of evidence. First, we find that longer time since menopause (irrespective of age at menopause) is associated with increased epigenetic AgeAccel. Second, bilateral oophorectomy is associated with increased epigenetic AgeAccel in both blood and saliva. This finding is congruent with a growing body of evidence suggesting that the premature loss of ovarian function caused by bilateral oophorectomy performed before natural menopause contributes to increased susceptibility to premature death, cardiovascular disease, dementia, parkinsonism, osteoporosis, and bone fractures (33). This model is also consistent with findings that transplantation of young ovaries into old mice significantly increases lifespan (36). Third, our study demonstrated that MHT, which arguably counters some of the effects of menopause, is associated with decreased epigenetic AgeAccel of buccal epithelium (but not of blood). Fourth, our Mendelian randomization analysis provided evidence of a causal pathway in which menopause accelerates epigenetic aging in blood: One of the most significant SNPs for age of menopause (rs11668344) also relates to epigenetic AgeAccel in blood. Although we acknowledge that each of these arguments in support of the causal model has pitfalls, in aggregate our results strongly support the causal model: menopause → epigenetic AgeAccel.

In moving forward, it will be important to examine longitudinal change in epigenetic age of blood before and after women transition from pre- to postmenopausal status. Additionally, it will be important to examine how long it takes accelerated epigenetic aging effects to become apparent following surgical menopause and whether they were apparent before surgical menopause. For instance, if accelerated aging is triggered by menopause, then one would expect AgeAccel to increase as a function of time since bilateral oophorectomy. However, if the condition that led to a surgical removal of both ovaries (e.g., fibroids, menstrual disorders, or endometriosis) is driving AgeAccel, one would not see a linear increase in epigenetic AgeAccel over time. In the NSHD study, it was reported that women who underwent bilateral oophorectomy tended to do so as a result of reported fibroids, menstrual disorders, or endometriosis (or some combination of these) (Table S3).

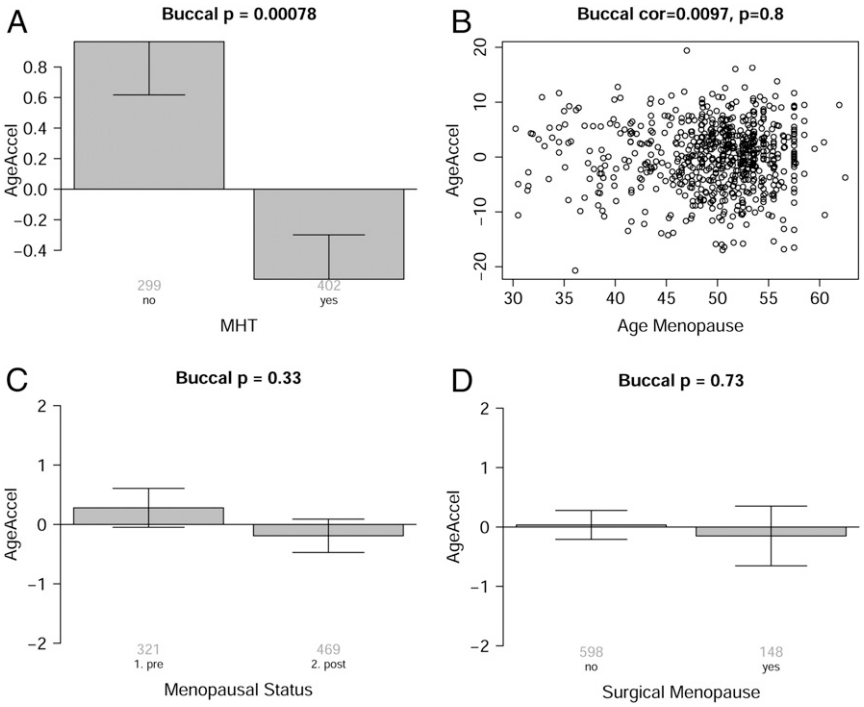


**Fig. 2.** Epigenetic age analysis of buccal samples from the NSHD. The measure of AgeAccel was defined as the difference between DNAm age and the mean DNAm age in this birth cohort. The scatter plot (*B*) reports Pearson correlation coefficients and corresponding *P* values, and the bar plots (*A*, *C*, and *D*) report the *P* value (±1 SE) from a nonparametric group comparison test (Kruskal–Wallis test). Epigenetic AgeAccel (*y* axis) is associated with MHT ($P = 0.00078$) (*A*) but not with age at menopause (*B*), menopausal status (*C*), or surgical menopause (*D*).

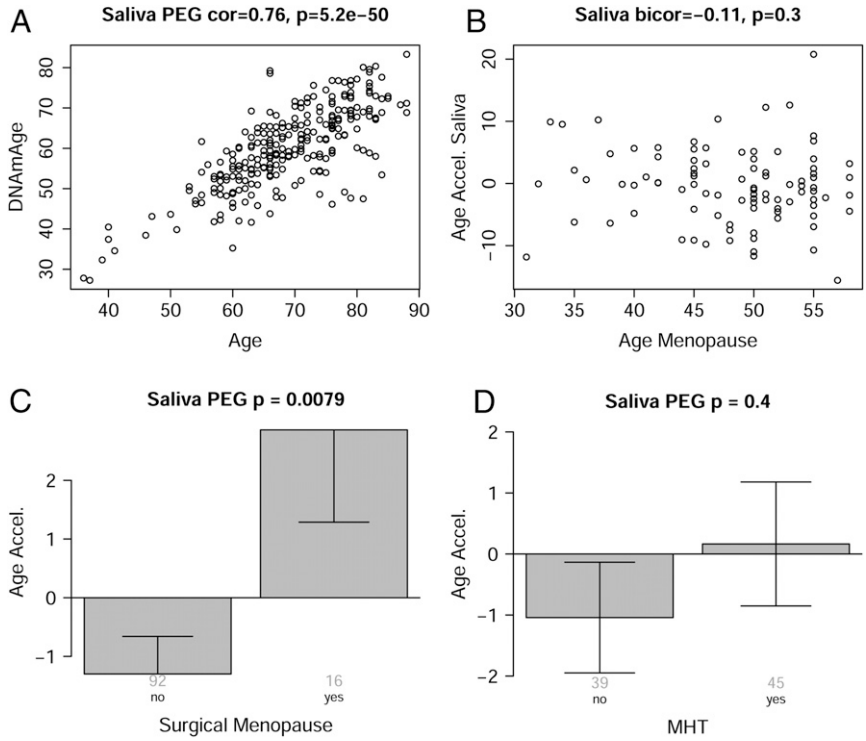


**Fig. 3.** Epigenetic age analysis of saliva samples from PEG. The measure of AgeAccel in saliva was defined in the same way as in blood. The scatter plots (*A* and *B*) report biweight midcorrelation coefficients and corresponding *P* values, and the bar plots (*C* and *D*) report the *P* value (±1 SE) from a nonparametric group comparison test (Kruskal–Wallis test). (*A*) There is a strong correlation between epigenetic age (*y* axis) and chronological age. (*B*) Although the correlation between epigenetic AgeAccel and age at menopause in saliva is about twice that of the association in blood, the finding is not significant. (*C*) However, we do observe an association between epigenetic AgeAccel in saliva and surgical menopause ($P = 0.0079$). (*D*) No association was observed between epigenetic AgeAccel and MHT ($P = 0.4$).

Although our study detected an association between epigenetic age in blood and age at menopause, similar associations were not found for epigenetic age estimates from buccal epithelium. One potential explanation is that aging measures in the various tissues capture different phenomena. Although we found a robust correlation between AgeAccel in blood and saliva ($r = 0.70$, $P = 1.4\text{E-}12$) (Fig. S3), when comparing the measures of AgeAccel in women with data from both blood and buccal epithelium, we found that the correlation is relatively weak ($r = 0.20$, $P = 0.013$) (Fig. S4). At first sight, the low correlation of AgeAccel between tissues is surprising. However, we interpret it as follows. The epigenetic clock is a strong predictor of age in multiple tissues in comparisons across individuals but is not necessarily correlated across tissues within a single individual. Stress factors and other perturbations act in a largely tissue-specific manner (37). Therefore one would not expect all tissue within an individual to age at the same rate, and these differences in rate potentially account for the low/moderate correlation between epigenetic AgeAccel in blood and buccal epithelium.

To the best of our knowledge, our study is the first to demonstrate that reproductive age, bilateral oophorectomy, and MHT relate to measures of epigenetic AgeAccel, but the reported associations are specific to blood, buccal epithelium, or saliva. Future studies are warranted to examine these effects in other tissues. Because epigenetic age captures aspects of biological age (27–30), our study strongly suggests that the hormonal changes that accompany menopause accelerate biological aging in women.

## Methods

Information on sample characteristics and recruitment for the WHI, PEG, InCHIANTI, and NSHD studies can be found in *SI Methods*.

**DNAm Data.** All DNAm datasets used the Illumina Infinium 450K platform. These data were generated by following the standard protocol of Illumina methylation assays, which quantifies methylation levels by the β value using the ratio of intensities between methylated and unmethylated alleles. Specifically, the β value is calculated from the intensity of the methylated (M, corresponding to signal A) and unmethylated (U, corresponding to signal B) alleles as the ratio of fluorescent signals: $\beta = \text{Max}(M,0)/[\text{Max}(M,0)+\text{Max}(U,0)+100]$. Thus, β values range from 0 (completely unmethylated) to 1 (completely methylated). For our blood datasets (the WHI and PEG cohorts) we used background-corrected β values. Buccal cells were normalized with the same method from the original publications (38, 39). The correlation between DNAm age and chronological age is highly robust with respect to different normalization methods because (*i*) the epigenetic clock implements a custom normalization method, and (*ii*) it was constructed using training data that were normalized in different ways.

**Epigenetic AgeAccel.** We used the DNAm age-based biomarker of aging from ref. 15 because (*i*) its accurate measurement of age across tissues is unprecedented (and it applies to both blood and buccal epithelium); (*ii*) it is prognostic for all-cause mortality (27, 28); (*iii*) it correlates with measures of cognitive and physical fitness in the elderly (27, 40); and (*iv*) it has been found useful for studying aging effects in Down syndrome (41), PD (42), neuropathological variables (40), obesity (37), and HIV infection (43). DNAm age was defined using the 353 CpGs and coefficient values reported in ref. 15. These CpGs and coefficient values were chosen in independent data using the Elastic Net penalized regression model to regress age on CpGs, resulting in DNAm age measures defined as predicted age, in years. Our measure of AgeAccel, which applies to all sources of DNA, was defined as residual resulting from a linear model that regressed DNAm age on chronological age. Thus, a positive value for AgeAccel indicates that the observed DNAm age is higher than expected. AgeAccel has only a weak correlation with blood cell counts (43).

**Genome-Wide SNP Data from the WHI Study.** Genotyping was performed for all participants on Affymetrix 6.0, Illumina HumanOmni1-Quad v1.0, or Illumina HumanOmniExpressExome-8v1.0. Imputation was performed using MaCH with haplotypes phased in Beagle or Minimac (44, 45). The reference panel for imputation was based on the 1000 Genome haplotypes (released in June 2011). The quality of imputed markers was assessed by MaCH $R^2 > 0.3$. Additional quality-control filters that were used included Hardy–Weinberg

Equilibrium $P$ values $<10^{-3}$ and minor allele frequency (MAF) >0.01. Finally, to account for population structure, principal component analysis (PCA) was performed to generate sample eigenvectors, the first two of which were included as covariates in all genome-wide complex trait (GCTA) analysis.

We related the two highly significant SNPs from the recent large-scale metaanalysis of age at menopause (31) to AgeAccel using the WHI data. Association analysis was conducted in the two subsets of individuals stratified by platform. All women were of European ancestry, identified by multidimensional scaling analysis in PLINK. We combined the results into a single estimate by fixed-effects models weighted by inverse variance, as implemented in R metaphor (Meta-Analysis Package for R). For association analysis, we regressed each of the three AgeAccel trait values on expected genotype dosage, adjusted for the first two principal components when necessary.

**Ethics.** All participants of the WHI, InCHIANTI, PEG, and NSHD gave written informed consent for their samples to be used in genetic studies of health. This study was reviewed by the Institutional Review Board of the University of California, Los Angeles (IRB nos. 13-000671 and 14-000061) and the Central Manchester Research Ethics Committee approved the use of the NSHD samples for epigenetic studies of health in 2012.

**Software Code and Data Availability.** We used the online version of the epigenetic clock software, which is freely available at https://dnamage.genetics.ucla.edu/. The R source code is publicly available from additional file 20 in ref. 15. The WHI and inCHIANTI data are available through the National Heart, Lung, and Blood Institute (NHLBI) (https://biolincc.nhlbi.nih.gov/studies/bhs/). The PEG data are available from Gene Expression Omnibus (accession no. GSE72775).

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