# Supporting Information

## Levine et al. 10.1073/pnas.1604558113

### SI Methods

**Dataset 1: WHI Sample Description (Blood).** Participants were part of a subsample from the WHI, who were enrolled in an integrative genomics study with a primary aim of identifying novel genomic determinants of coronary heart disease. This sample included women age 50–79 y, with an overrepresentation of racial/ethnic minorities. The integrative genomics subsample used a case-control sampling design. All cases and controls were required to have undergone genome-wide genotyping at baseline and profiling of seven cardiovascular biomarkers, as dictated by the aims of other ancillary WHI studies. As shown in Table S1, the mean age at baseline for the 1,864 women in the WHI sample was 65.31 y (SD = 7.1 y). AgeAccel ranged from −22.6 to 42.9, with a mean of 0.08 and a SD of 5.3, and age at menopause ranged from 25–60 y, with a mean of 47.43 y (SD = 6.9 y). Overall, approximately half of our sample were non-Hispanic white (47.1%), one-third (32.2%) were African American, and about 20% were Hispanic. The majority of our sample reported never smoking (53.4%), 36.4% reported past smoking history, and about 10% reported they were current smokers at the time of blood draw.

**Dataset 2: InCHIANTI Sample Description (Blood).** The InCHIANTI study is a population-based prospective cohort study of residents age 30 y or older from two areas in the Chianti region of Tuscany, Italy. Sampling and data collection procedures have been described elsewhere (46). Briefly, participants were enrolled between 1998 and 2000 and were examined at 3-y intervals. Overall 1,326 participants donated a blood sample at baseline (1998–2000), 784 of whom also donated a blood sample at the 9-y follow-up (2007–2009). DNAm was assayed using the Illumina Infinium HumanMethylation450 platform for participants with sufficient DNA at both baseline and year 9 visits ($n = 499$). Our study focused only on women ($n = 200$). Ages for the 200 women in InCHIANTI ranged from 50–91 y, with a mean age of 70.64 y (Table S1); baseline AgeAccel ranged from −12.9 to 12.05, with a mean of 0. Age at menopause ranged from 26–60 y, with a mean of 49.1 y. Overall, ∼75.5% of the women in the InCHIANTI study had never smoked, 12.0% were former smokers, and about 12.5% were current smokers.

**Dataset 3: Women from the PEG Cohort (Blood and Saliva).** We used two types of tissues from the PEG study cohort: blood and saliva. The PEG study is a large, population-based, case-control study of PD in rural and township residents of California's central valley (47). Our blood data came from subjects from wave 1 (PEG1). PD status did not confound the relationship in blood, because it was not associated with age at menopause; however, we adjusted for it in multivariate analyses. The 256 women in the PEG study ranged in age from 35–91 y, with a mean age of 67.9 y (Table S1). Only three participants self-identified as non-Hispanic black, and 23 self-identified as Hispanic. Average age at menopause was 46.4 y. Overall, ∼4% of the women from PEG study were current smokers at the time of blood draw, and 38% were former smokers.

The saliva methylation data were collected at a later time point than the blood data. We had both blood and saliva methylation data for about half the women, but epigenetic AgeAccel of blood tissue was not correlated with AgeAccel in saliva.

**Dataset 4. NSHD (Buccal Epithelium).** The buccal samples came from a subsample of 790 women participants in a British birth cohort, the UK Medical Research Council NSHD as described in ref. 39. The women were all 53 y old at the time of sample collection in 1999. At that time, 419 women were postmenopausal, and 371 women were premenopausal. MHT status was coded as "yes" only if MHT started before the age of sample collection (i.e., 53 y). Our results regarding the relationship between age at menopause and epigenetic AgeAccel were largely unchanged after excluding women who experienced surgical menopause.

All women gave written informed consent for their samples to be used in genetic studies of health, and the Central Manchester Research Ethics Committee approved the use of these samples for epigenetic studies of health in 2012. Women were selected from those who provided a buccal and blood sample at age 53 y in 1999, who had not previously developed any cancer, and for whom there was complete information on epidemiological variables of interest. Smoking status did not confound the reported relationships, because smoking was not significantly associated with our measure of epigenetic age acceleration.

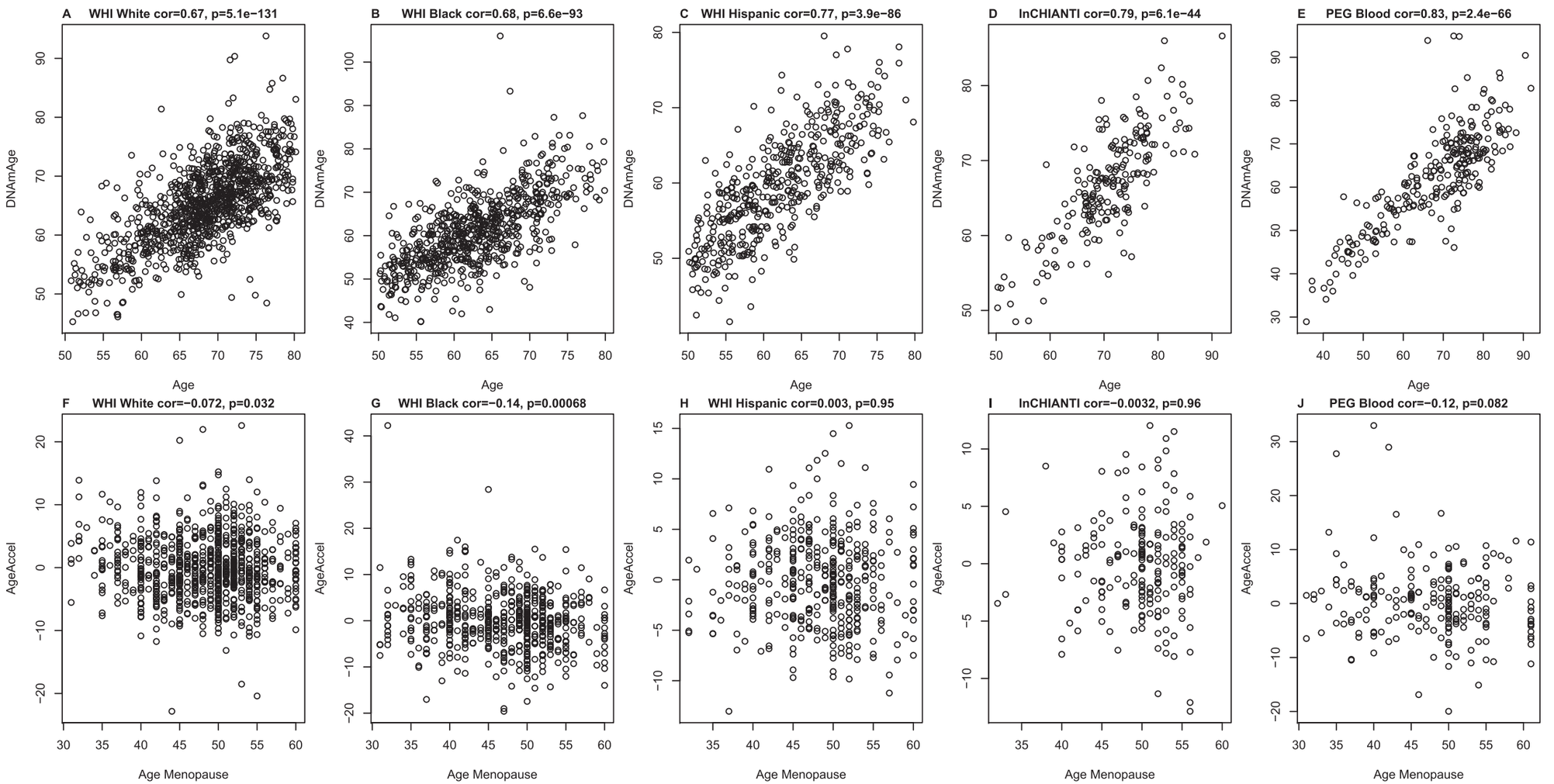


**Fig. S1.** Epigenetic age versus age at menopause. (*A–E*) DNA methylation age (*y* axis) versus chronological age (*x* axis) in the WHI (white) (*A*), WHI (black) (*B*), WHI (Hispanic) (*C*), InCHIANTI (*D*), and PEG (*E*) studies. (*F–J*) Correlations between epigenetic AgeAccel (*y* axis) and age at menopause (*x* axis) in the WHI (white) (*F*), WHI (black) (*G*), WHI (Hispanic) (*H*), InCHIANTI (*I*), and PEG (*J*) studies. Each panel reports a Pearson correlation coefficient and a corresponding *P* value. The analysis was restricted to women whose age at menopause was >30 y. For all five samples, DNAm age and chronological age are highly correlated. We also find significant inverse associations between epigenetic AgeAccel and age of menopause in the WHI white (*F*) and WHI black (*G*) cohorts and a marginally significant inverse association in the PEG cohort (*J*). Metaanalysis for these and other strata can be found in Table S2.

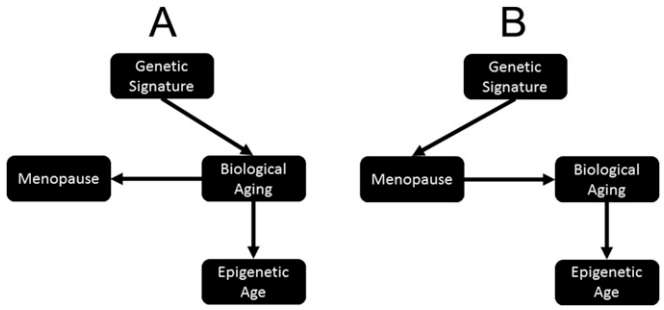


**Fig. S2.** Potential causal relationships between age at menopause and epigenetic age. (*A*) One causal model assumes that both age at menopause and epigenetic AgeAccel reflect biological aging, which is a latent construct. (*B*) Another potential causal model assumes that menopause leads to an increase of epigenetic age via an acceleration of the biological aging process.

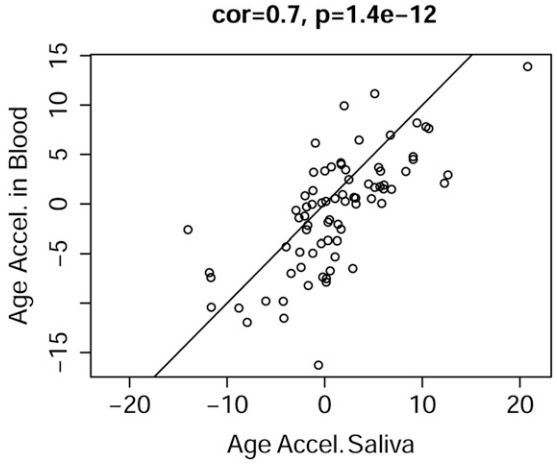


**Fig. S3.** Correlation between epigenetic AgeAccel in blood and saliva. Using data from women in the PEG study for whom blood and saliva measures were available, we examined the association between epigenetic AgeAccel in the two tissues. We found that epigenetic AgeAccel in saliva may be a good proxy for measures in blood, given that the two are correlated at $r = 0.70$ ($P = 1.4E\text{-}12$).

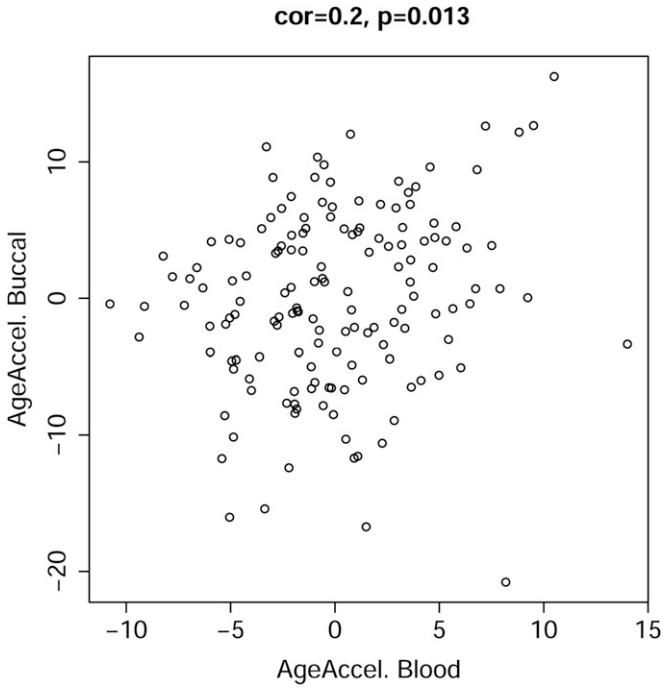


**Fig. S4.** Correlation between epigenetic AgeAccel in blood and buccal cells. Using data from women in the NSHD study for whom data from both blood and buccal epithelium were available, we found that the correlation of epigenetic AgeAccel in these two tissues is relatively weak ($r = 0.20$, $P = 0.013$), suggesting they may capture distinct phenomena.

**Table S1. Study population characteristics**

| | Study | | | |
|---|---|---|---|---|
| Characteristic | WHI, $n$ = 1,864 | InCHIANTI, $n$ = 200 | PEG, $n$ = 256 | NSHD, $n$ = 790 |
| Sample tested | Blood | Blood | Blood/saliva | Buccal |
| Chronological age in years, mean (SD) | 65.3 (7.1) | 70.6 (7.6) | 67.9 (12.0) | 53 (0) |
| Non-Hispanic black, frequency | 0.32 | 0 | 0.01 | 0 |
| Hispanic, frequency | 0.21 | 0 | 0.09 | 0 |
| Current smoker, frequency | 0.102 | 0.125 | 0.04 | 0.21 |
| Former smoker, frequency | 0.364 | 0.120 | 0.38 | 0.30 |
| Age at menopause in years, mean (SD) | 47.4 (6.9) | 49.1 (5.9) | 46.4 (9.0) | 50.3 (3.9) |
| MHT use ever, frequency | 0.39 | 0.12 | 0.71 | 0.57 |

**Table S2. Multivariate metaanalysis of AgeAccel in blood versus years since menopause**

| | β-coefficient (*P* value) | | | |
|---|---|---|---|---|
| AgeAccel | WHI | InCHIANTI | PEG | Meta *P* value |
| Years since menopause | 0.038 (0.007) | −0.001 (0.994) | −0.013 (0.766) | 0.017 |
| Non-Hispanic black | 0.185 (0.519) | — | −4.731 (0.237) | |
| Hispanic | −0.771 (0.022) | — | −1.417 (0. 456) | |
| Former smoker | −0.303 (0.249) | 0.409 (0.674) | −0.952 (0.335) | |
| Current smoker | −0.066 (0.879) | −0.793 (0.424) | −1.120 (0.681) | |
| MHT | 0.111 (0.660) | 1.019 (0.316) | 3.080 (0.010) | |
| Age at menarche | −0.068 (0.408) | −0.278 (0.182) | −0.006 (0.989) | |
| Parkinson's disease status | — | — | 0.751 (0.443) | |

**Table S3. Reported causes of hysterectomy for women in the NSHD**

| Reason for hysterectomy, from hospital records and self-reports | Hysterectomy and oophorectomy status, prior to 2009 | | | |
|---|---|---|---|---|
| | No operation | Oophorectomy, bilateral or unilateral | Hysterectomy only | Total |
| Fibroids | | 35 | 29 | 64 |
| Endometriosis | | 5 | 4 | 9 |
| Fibroids and endometriosis | | 3 | 0 | 3 |
| Menstrual disorders | | 24 | 35 | 59 |
| Disorders of the uterus | | 0 | 2 | 2 |
| Prolapse | | 4 | 12 | 16 |
| Cancer | | 7 | 7 | 14 |
| Neoplasm unspecified | | 1 | 1 | 2 |
| Benign neoplasms | | 1 | 0 | 1 |
| Inflammatory disease | | 2 | 0 | 2 |
| Noninflammatory disorders | | 4 | 2 | 6 |
| Other | | 2 | 0 | 2 |
| Had operation but reason unknown, pre-2009 | | 3 | 5 | 8 |
| Subtotal | | 91 | 97 | |
| Unknown (operation after 2009) | | 24 | 7 | 609 |
| Total | 578 | 115 | 104 | 797 |