

Accelerated DNA methylation age and the use of antihypertensive medication among older adults

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ABSTRACT

The discrepancy of DNA methylation age (DNAmAge) with chronological age (termed as age acceleration, AA) has been identified to be associated with many aging-related health outcomes including hypertension. Since taking antihypertensive medication (AHM) could prevent aging-related diseases caused by hypertension, we hypothesized that using AHM could also reduce the AA. We examined this hypothesis among 546 males aged 55–85 years by exploring the associations of AHM use with AA and its change rate (Δ_{AA}) in two visits with a median follow-up of 3.86 years. Horvath DNAmAge was derived from DNA methylation profiles measured by Illumina HumanMethylation450 BeadChip and information on AHM use was collected by physician interview. A general decreasing pattern of AA was observed between the two visits. After the fully adjusting for potential covariates including hypertension, any AHM use showed a cross-sectional significant association with higher AA at each visit, as well as a longitudinal association with increased Δ_{AA} between visits. Particularly, relative to participants who never took any AHM, individuals with continuous AHM use had a higher Δ_{AA} of 0.6 year/chronological year. This finding underlines that DNAmAge and AA may not be able to capture the preventive effects of AHMs that reduce cardiovascular risks and mortality.

INTRODUCTION

DNA methylation, a major form of epigenetic modification, is known to play an important role in

aging and the development of age-related health outcomes [1, 2]. Recently, a DNA methylation-based biological age predictor, “DNA methylation age (DNAmAge)”, has been established and found to be

highly associated with chronological age [3]. The discrepancy between this epigenetic-based indicator and the chronological age has been termed age acceleration (AA), which was found to be heritable and has been used as an index of accelerated biological aging. Follow-up investigations have linked AA to lifestyle factors, environmental hazards, stressful life events, as well as all-cause mortality [4-13].

Several aging-related factors, including inflammation, neurohormonal disorder and endothelial dysfunction, have been found to play key mechanistic roles in the development of hypertension [14-16], the most common long-term medical condition among older adults that could lead to various forms of age-related health outcomes, such as cardiovascular diseases (CVD), kidney failure and dementia [17]. Relationships of hypertension and blood pressure with biological aging have also been studied since the introduction of DNAmAge. In 2016, Horvath et al. found that people with hypertension had a higher AA (0.5 – 1.2 years) in comparison to controls in the Bogalusa Heart study [7] and a more recent study from Quach et al. showed that elevated blood pressure was also correlated with higher extrinsic and intrinsic DNAmAge [10].

The use of antihypertension medication (AHM) reduces the risk of adverse age-related health outcomes caused by hypertension. Specifically, observational studies, clinical trials, and systematic reviews mostly suggested that effective antihypertensive therapy greatly reduces the risk of CVD in patients with hypertension [18, 19], and may also be associated with a decreased risk of cognitive decline and incident dementia [20]. As DNA methylation is a durable and reversible modification, we hypothesized that the use of AHMs might also be able to influence the biological aging reflected by the epigenetic AA. Therefore, we assessed the associations of AHM use with AA and further determined whether the change of AHM use could modify the change rate of AA (Δ_{AA}) during a median follow-up of 3.86 years. This investigation was carried out in the Normative Aging Study (NAS), which is an all-male longitudinal study of a cohort of older veterans living in the Greater Boston area.

RESULTS

Participant characteristics

Characteristics of the 546 participants at each visit are shown in Table 1. Overall, the average age at first and second visits was 72 and 75 years, respectively. More than 60% of the participants were former smokers and less than 5% were current smokers, and the majority of participants were overweight or obese, consumed no or

low amounts of alcohol, reported low physical activity and had less than 16 years of education. During a median follow-up of 3.86 years, the prevalence of hypertension increased from about 68% to 75%, stroke from nearly 6.0% to 8.6%, coronary heart disease (CHD) from 26% to 34%, diabetes from 12% to 16% and cancer from 50% to 59%. In particular, 313/374 and 383/411 participants with hypertension took the medication for elevated blood pressure at each visit, respectively. ACE inhibitors and beta blockers were the two most widely used medications. According to our definition of hypertension, the participants using AHM were considered to be have hypertension. Among the 546 participants, after the first visit, 13 stopped using any AHM and 83 started to use.

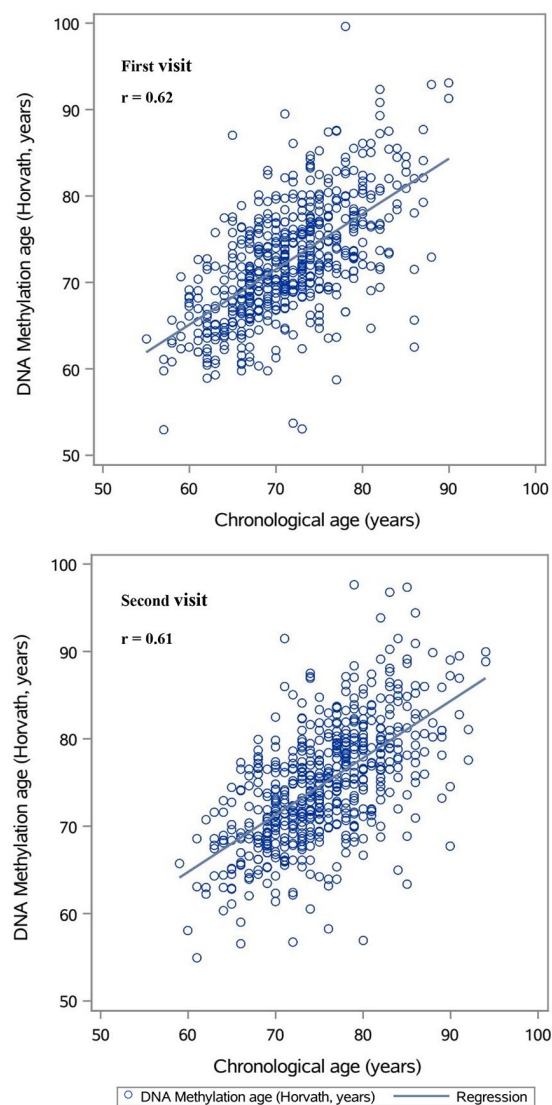


Figure 1. Plots of predicted DNA methylation ages against chronological age.

Table 1. Characteristics of participants from the Normative Aging Study (NAS), 1999–2013 (N = 546) ^a.

Characteristics	First visit	Second visit
Age (years)	71.6 (6.5)	75.4 (6.5)
DNA methylation age (Horvath, years)	72.6 (6.7)	74.9 (7.1)
Age acceleration (Horvath, years)	0.15 (5.3)	-0.05 (5.6)
Fasting glucose (mg/dL)	108.3 (29.2)	105.9 (21.6)
Total cholesterol (mg/dL)	198.8 (36.9)	180.4 (37.2)
Serum triglyceride (mg/dL)	139.5 (85.3)	125.8 (67.8)
HDL (mg/dL)	49.6 (13.4)	48.6 (13.1)
SBP (mm Hg)	131.5 (17.3)	124.6 (17.2)
Smoking status		
Current smoker	23 (4.2%)	23 (4.2%)
Former smoker	348 (63.7%)	349 (63.9%)
Never smoker	175 (32.1%)	174 (31.9%)
Body mass index		
Underweight or normal weight (<25.0)	103 (18.9%)	130 (23.8%)
Overweight (≥25 to <30)	299 (54.7%)	279 (51.1%)
Obese (≥30.0)	144 (26.4%)	137 (25.1%)
Alcohol consumption ^b		
Abstainer	114 (22.5%)	115 (25.1%)
Low (0 to <40 g/d)	355 (70.2%)	319 (69.5%)
Intermediate (40 to <60 g/d)	25 (4.9%)	18 (3.9%)
High (≥60 g/d)	12 (2.4%)	7 (1.5%)
Physical activity (MET-hours/week) ^c		
Low (≤12 kcal/kg hours/week)	321 (61.4%)	307 (64.2%)
Median (12–30 kcal/kg hours/week)	128 (24.5%)	110 (23.0%)
High (≥30 kcal/kg hours/week)	74 (14.1%)	61 (12.8%)
Major diseases		
Hypertension	374 (68.5%)	411 (75.3%)
Stroke	32 (5.9%)	47 (8.6%)
Coronary heart disease (CHD)	143 (26.2%)	184 (33.7%)
Diabetes	67 (12.3%)	89 (16.3%)
Cancer	271 (49.6%)	323 (59.2%)
Use of any antihypertensive medication		
Any	313 (57.3%)	383 (70.1%)
Calcium channel blockers	66 (12.1%)	84 (15.4%)
ACE inhibitors	143 (26.2%)	204 (37.4%)
Angiotensin receptor antagonists	21 (3.8%)	45 (8.2%)
Alpha blockers	64 (11.7%)	90 (16.5%)
Beta blockers	185 (33.9%)	222 (40.7%)
Diuretics	98 (17.9%)	141 (25.8%)
		Both visits
Years of education ^d		
≤12 years		233 (44.5%)
13 – 16 years		223 (42.6%)
>16 years		68 (12.9%)
Time between 1st and 2nd visits (years)		3.86 (1.6)
Change rate of age acceleration between visits (year/chronological year)		-0.03 (1.3)

a: Mean values (standard deviation) for continuous variables and n (%) for categorical variables;

b: Data missing for 40 and 87 participants at 1st and 2nd visits, respectively;

c: Data missing for 23 and 68 participants at 1st and 2nd visits, respectively;

d: Data missing for 22 participants.

Our main analyses based on the DNAmAge estimated by Horvath's algorithm. Figure 1 shows that the estimates were highly correlated with chronological age at each visit (Spearman coefficients >0.6). While DNAmAge increased between the first and second visit, the overall AA of the second visit was lower than that of the first visit (Table 1), showing declining trajectories with a crude average Δ_{AA} of about -0.03 year/chronological year. The trajectories of AA and distributions of Δ_{AA} were illustrated in Figure 2. Figure S1 showed strong correlations between the AAs at both visits among those with or without hypertension at both visits (Spearman coefficients ~ 0.7), while for those who had hypertension or hypertension was controlled after the first visit, their correlation was slightly attenuated (Spearman coefficients = 0.53).

Associations of hypertension with age acceleration and the change rate of age acceleration

We evaluated the relationship between hypertension and AA at each visit. Participants with hypertension had higher AA (years) than those without (first visit: 0.18 vs. 0.09; second visit: -0.01 vs. -0.18; p-value=0.016). The similar pattern was also observed for Δ_{AA} that

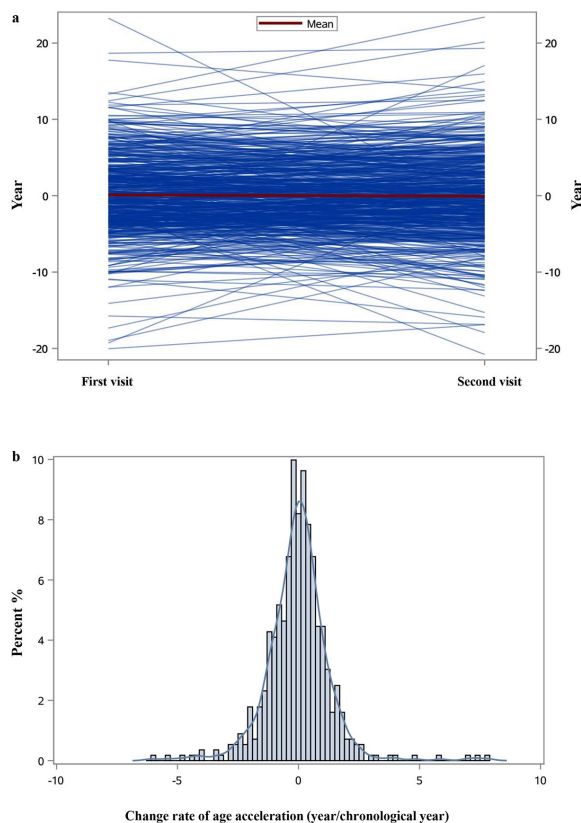


Figure 2. Individual trajectories of age acceleration (a) and the distribution of change rate of age acceleration (b).

participants with hypertension had a higher Δ_{AA} (year/chronological year) compared to those without (first visit: 0.03 vs. -0.14; second visit: 0.01 vs. -0.12; p-value=0.007). We further adjusted for other potential covariates at each visit in a mixed linear model to validate the observed patterns, including age, body mass index (BMI), smoking status, alcohol consumption, years of education, physical activity, leukocyte distribution and the batch of microarray experiments in DNA methylation measurement. As shown in Table S1, the correlations of hypertension with AA and Δ_{AA} remained positive, albeit not statistically significant. We also tested the associations of diabetes, metabolic syndrome and commonly measured clinical biomarkers with AA and Δ_{AA} (Tables S1 & S2). Despite the statistically significant association between diabetes and Δ_{AA} , neither metabolic syndrome nor any biomarkers were strongly associated with AA or Δ_{AA} .

Cross-sectional associations of antihypertensive medication with age acceleration and the change rate of age acceleration

We first investigated the cross-sectional associations of AHM use with AA and Δ_{AA} at each visit. Overall, taking any AHM was significantly associated with higher AA and Δ_{AA} (Table 2). After adjusting for potential covariates at each visit [age, BMI, smoking status, alcohol consumption, years of education, physical activity, leukocyte distribution, total cholesterol, high-density lipoprotein (HDL), triglycerides, fasting glucose, systolic blood pressure (SBP), hypertension, stroke, CHD, diabetes, cancer and the batch of microarray experiments in DNA methylation measurement], people who took any AHM at each visit were about 2.3 years older than the controls in terms of AA, and their AA increased significantly during follow-up at a rate of more than 0.4 year/chronological year compared to the people who did not take any AHMs. However, the effects of specific classes of medications varied. While taking diuretics was negatively associated with AA and Δ_{AA} , calcium channel blockers and alpha and beta blockers were positively correlated with AA and Δ_{AA} . Angiotensin-converting enzyme inhibitors (ACE inhibitors) and angiotensin receptor antagonists (ARBs) both had negative associations with Δ_{AA} , but their relationships with AA were in different directions. An additional subgroup analysis was carried out among people with hypertension at each visit (Table 3), and the use of any AHM remained robustly associated with increased AA and Δ_{AA} .

Table S3a showed sensitivity analyses for the cross-sectional associations of any AHM use with the AA and Δ_{AA} of Hannum DNAmAge and DNA methylation

Table 2. Cross-sectional associations of the antihypertensive medication use with age acceleration and change rate of age acceleration at first and second visits.

Visit	Medication	Medication use	N	Age acceleration				Change rate of age acceleration			
				Model 1 ^a		Model 2 ^b		Model 1		Model 2	
				Coefficients (SE)	p-value	Coefficients (SE)	p-value	Coefficients (SE)	p-value	Coefficients (SE)	p-value
First visit	Calcium channel blockers	No	480	Ref		Ref		Ref		Ref	
		Yes	66	0.059 (0.723)	0.935	0.381 (0.784)	0.627	0.222 (0.163)	0.175	0.263 (0.184)	0.154
	ACE inhibitors	No	403	Ref		Ref		Ref		Ref	
		Yes	143	0.241 (0.525)	0.646	0.281 (0.605)	0.643	-0.093 (0.119)	0.432	-0.133 (0.142)	0.350
	ARBs	No	525	Ref		Ref		Ref		Ref	
		Yes	21	-0.144 (1.169)	0.902	-0.792 (1.184)	0.504	-0.047 (0.265)	0.858	-0.042 (0.279)	0.881
	Alpha blockers	No	482	Ref		Ref		Ref		Ref	
		Yes	64	1.478 (0.721)	0.041	1.974 (0.769)	0.011	0.055 (0.164)	0.737	0.041 (0.184)	0.821
	Beta blockers	No	361	Ref		Ref		Ref		Ref	
		Yes	185	1.150 (0.499)	0.022	1.291 (0.647)	0.047	0.066 (0.114)	0.562	0.075 (0.153)	0.624
	Diuretics	No	448	Ref		Ref		Ref		Ref	
		Yes	98	-0.068 (0.614)	0.911	-0.100 (0.676)	0.882	-0.095 (0.138)	0.496	-0.001 (0.159)	0.973
	Any antihypertensive medication	No	233	Ref		Ref		Ref		Ref	
		Yes	313	0.935 (0.488)	0.056	2.274 (0.860)	0.009	0.195 (0.111)	0.079	0.433 (0.205)	0.035
Second visit	Calcium channel blockers	No	462	Ref		Ref		Ref		Ref	
		Yes	84	1.243 (0.681)	0.068	1.035 (0.783)	0.187	0.214 (0.144)	0.137	0.275 (0.163)	0.093
	ACE inhibitors	No	342	Ref		Ref		Ref		Ref	
		Yes	204	-0.293 (0.510)	0.565	-0.340 (0.648)	0.601	-0.045 (0.107)	0.671	-0.036 (0.135)	0.791
	ARBs	No	501	Ref		Ref		Ref		Ref	
		Yes	45	0.790 (0.923)	0.393	0.697 (1.092)	0.524	-0.247 (0.194)	0.203	-0.314 (0.227)	0.167
	Alpha blockers	No	456	Ref		Ref		Ref		Ref	
		Yes	90	0.612 (0.678)	0.367	0.875 (0.814)	0.283	0.034 (0.142)	0.809	0.073 (0.170)	0.668
	Beta blockers	No	324	Ref		Ref		Ref		Ref	
		Yes	222	1.213 (0.506)	0.017	1.406 (0.707)	0.047	0.059 (0.107)	0.581	0.155 (0.148)	0.296
	Diuretics	No	405	Ref		Ref		Ref		Ref	
		Yes	141	-0.136 (0.563)	0.809	-0.047 (0.683)	0.945	-0.029 (0.118)	0.809	-0.050 (0.142)	0.724
	Any antihypertensive medication	No	163	Ref		Ref		Ref		Ref	
		Yes	383	0.659 (0.543)	0.226	2.313 (1.096)	0.035	0.097 (0.114)	0.396	0.467 (0.229)	0.042

a: Model 1: Adjusted for covariates at each visit: age + leukocyte distribution (Houseman algorithm) + random effect (batch effect of DNA methylation measurement). Age acceleration was additionally adjusted for in the model for the change rate of age acceleration;

b: Model 2: Model 1 + BMI + smoking status + alcohol consumption + physical activity + years of education + total cholesterol + HDL + triglycerides + fasting glucose + SBP + hypertension + stroke + CHD + diabetes + cancer.

Table 3. Cross-sectional associations of antihypertensive medication use with age acceleration and change of age acceleration at first and second visits among the subpopulation who had hypertension.

Visit	Medication	Medication use	N	Age acceleration ^a		Change rate of age acceleration ^b	
				Coefficients (SE)	p-value	Coefficients (SE)	p-value
First visit	Calcium channel blockers	No	308	Ref		Ref	
		Yes	66	-0.390 (0.816)	0.633	0.346 (0.202)	0.089
	ACE inhibitors	No	231	Ref		Ref	
		Yes	143	-0.235 (0.628)	0.709	0.166 (0.156)	0.290
	ARBs	No	353	Ref		Ref	
		Yes	21	-0.144 (1.169)	0.902	0.012 (0.307)	0.968
	Alpha blockers	No	310	Ref		Ref	
		Yes	64	2.087 (0.795)	0.009	-0.048 (0.203)	0.813
	Beta blockers	No	189	Ref		Ref	
		Yes	185	1.410 (0.680)	0.039	0.107 (0.171)	0.531
	Diuretics	No	276	Ref		Ref	
		Yes	98	-0.010 (0.710)	0.989	0.020 (0.177)	0.909
	Any antihypertensive medication	No	61	Ref		Ref	
Yes		313	2.378 (0.898)	0.009	0.497 (0.228)	0.030	
Second visit	Calcium channel blockers	No	327	Ref		Ref	
		Yes	84	0.942 (0.794)	0.236	0.307 (0.153)	0.046
	ACE inhibitors	No	207	Ref		Ref	
		Yes	204	-0.215 (0.657)	0.743	0.016 (0.128)	0.899
	ARBs	No	366	Ref		Ref	
		Yes	45	0.687 (1.084)	0.527	-0.285 (0.215)	0.187
	Alpha blockers	No	329	Ref		Ref	
		Yes	82	0.584 (0.867)	0.501	0.032 (0.171)	0.851
	Beta blockers	No	189	Ref		Ref	
		Yes	222	1.287 (0.715)	0.073	0.123 (0.142)	0.388
	Diuretics	No	270	Ref		Ref	
		Yes	141	0.010 (0.685)	0.989	-0.031 (0.136)	0.818
	Any antihypertensive medication	No	36	Ref		Ref	
Yes		375	2.313 (1.214)	0.058	0.430 (0.235)	0.069	

a: Adjusted for covariates at each visit: age + leucocyte distribution (Houseman algorithm) + BMI + smoking status + alcohol consumption + physical activity + years of education + total cholesterol + HDL + triglycerides + fasting glucose + SBP + stroke + CHD + diabetes + cancer + random effect (batch effect of methylation measurement);

b: Additionally adjusted for age acceleration at each visit.

Phenotypic age (DNAmPhenoAge). Taking any AHMs showed positive correlations with the AA of each biomarker and significantly associated with both forms of AA at the first visit. People who took medications were about 2.2 years/1.7 years older in people compared to the controls, respectively. Even though taking any AHMs was not robustly associated with the Δ_{AA} of neither biomarker, the effects were in the same direction as we identified for the Horvath DNAmAge.

Longitudinal associations of antihypertensive medication with age acceleration and the change rate of age acceleration

We further examined the longitudinal association between AHM use and Δ_{AA} (Table 4). People that with continuous medication use were older than that never used (76.6 vs. 73.7 years). After controlling for the potential covariates at the first visit, taking AHM showed positive correlations with the AA and Δ_{AA} . Compared to people who never took any AHM, individuals who started to take AHM after the first visit had an increased Δ_{AA} of about 0.3 year/chronological year, and individuals with continuous AHM use had an increased Δ_{AA} of about 0.6 year/chronological year. Consistent with this finding, stopping taking ACE inhibitors, ARBs, and alpha and beta blockers showed negative correlations with Δ_{AA} , compared to continuous use. A subgroup analysis among 313 participants with hypertension at first visit yielded a similar pattern (Table 5). After fully controlling for potential covariates, stopped taking AHM was correlated with a declining Δ_{AA} compared to the continued AHM use after the first visit albeit not statistically significant.

Same longitudinal tests were also performed for the Δ_{AA} of Hannum DNAmAge and DNAmPhenoAge as sensitivity analyses in Table S3b. The Δ_{AA} of neither biomarker showed significant associations with the change of AHM use, but an increasing pattern was still observed for the people with continuous AHMs use in comparison to the people who never used the AHM for each biomarker.

DISCUSSION

In the present study, we investigated the cross-sectional and longitudinal associations of AHM use with the AA of Horvath DNAmAge in a longitudinal study of older male participants examined over two visits. Even though a general decrease of AA was observed between the two visits, after fully adjusting for hypertension and other potential covariates, any AHM use showed a cross-sectional significant association with higher AA at each visit, as well as a longitudinal association with

increased Δ_{AA} between visits. Particularly, relative to participants who never took any AHM, individuals with continuous AHM use had a higher Δ_{AA} of 0.6 year/chronological year. Additional sensitivity analyses on another two DNA methylation-based biomarkers (Hannum DNAmAge & DNAmPhenoAge) showed the similar patterns with the use of AHM as the Horvath DNAmAge.

Recently, Marioni et al. found that DNAmAge increases at a slower rate than chronological age across the life course in five independent cohorts, especially in the older population [21], which suggested that, overall, AA declines as people get older. The global decreasing pattern of AA across the two visits observed in our study is in line with this finding. This pattern may also be explained by survival bias, due to the higher probability that healthier participants with relatively lower AA may stay longer in longitudinal studies. After adjusting for hypertension and other potential covariates that might affect the DNAmAge, we surprisingly found that taking AHMs was associated with faster AA. This finding is at variance with the declining pattern of DNAmAge across the lifespan and is not consistent with the preventive effects of AHMs against age-related health outcomes caused by hypertension [18, 19]. Several explanations may account for this discrepancy.

First, measurement bias or inaccuracies may affect the comparability of longitudinal AA and Δ_{AA} estimates. Nevertheless, we have restricted this technical bias to the greatest extent by adjusting for the batch of DNA methylation measurement as a random effect and normalizing the methylation profiles with Horvath's internal normalization method. Furthermore, selection bias due to differential survival rate may cause an underrepresentation of participants with higher AA [22], and may lead to underestimation or even contradicting results of the effect of AHM use on AA and Δ_{AA} , and distort their associations towards the opposite. In our analyses, we accounted for potential selection bias due to the loss of follow-up using inverse probability weighting (IPW) [23]. Point estimates were similar between models using and not using IPW, indicating that little selection bias was introduced due to the loss to follow-up. Additionally, since the use of AHMs is usually treated as the indicator of hypertension severity, our study might report a proxy outcome that indeed reflected the impact of hypertension on aging (confounding by indication). Due to this concern, despite the weak positive but not significant pattern we observed between hypertension and the AA (Δ_{AA}), we additionally adjusted for SBP and hypertension in the fully-adjusted analysis model and performed another sensitivity analysis by adding the interaction term of

Table 4. Associations between antihypertensive medication use and change rate of age acceleration from first to second visit.

Medication	Change of medication use	N	Change rate of age acceleration			
			Model 1 ^a		Model 2 ^b	
			Coefficients (SE)	p-value	Coefficients (SE)	p-value
Calcium channel blockers	Never used	440	Ref		Ref	
	Stopped use after first visit	22	0.103 (0.272)	0.706	0.264 (0.304)	0.386
	Started use after first visit	40	0.437 (0.201)	0.030	0.405 (0.216)	0.062
	Continuous use	44	0.338 (0.194)	0.083	0.338 (0.214)	0.116
ACE inhibitors	Never used	327	Ref		Ref	
	Stopped use after first visit	15	-0.121 (0.316)	0.701	-0.026 (0.348)	0.942
	Started use after first visit	76	-0.081 (0.159)	0.614	0.033 (0.185)	0.857
ARBs	Continuous use	128	0.103 (0.128)	0.419	0.163 (0.156)	0.297
	Never used	496	Ref		Ref	
	Stopped use after first visit	5	-0.223 (0.535)	0.677	-0.250 (0.556)	0.654
Alpha blockers	Started use after first visit	29	-0.216 (0.235)	0.359	-0.145 (0.276)	0.599
	Continuous use	16	-0.006 (0.303)	0.983	0.011(0.319)	0.972
	Never used	442	Ref		Ref	
Beta blockers	Stopped use after first visit	14	-0.422 (0.325)	0.195	-0.330 (0.371)	0.375
	Started use after first visit	40	0.105 (0.202)	0.605	0.269 (0.222)	0.226
	Continuous use	50	0.065 (0.185)	0.727	0.063 (0.201)	0.753
Diuretics	Never used	300	Ref		Ref	
	Stopped use after first visit	24	-0.586 (0.268)	0.029	-0.598 (0.300)	0.047
	Started use after first visit	61	0.180 (0.169)	0.288	0.290 (0.185)	0.117
Any antihypertensive medication	Continuous use	161	0.193 (0.122)	0.114	0.471 (0.164)	0.100
	Never used	385	Ref		Ref	
	Stopped use after first visit	20	0.183 (0.277)	0.509	0.010 (0.293)	0.972
Any antihypertensive medication	Started use after first visit	63	-0.017 (0.169)	0.921	-0.019 (0.186)	0.918
	Continuous use	78	0.068 (0.154)	0.658	-0.016 (0.181)	0.932
	Never used	150	Ref		Ref	
Any antihypertensive medication	Stopped use after first visit	13	-0.117 (0.374)	0.754	0.006 (0.433)	0.989
	Started use after first visit	83	0.135 (0.168)	0.422	0.285 (0.185)	0.124
	Continuous use	300	0.258 (0.127)	0.043	0.596 (0.220)	0.008

a: Model 1: Adjusted for: age (first visit) + age acceleration (first visit) + leukocyte distribution (first visit, Houseman algorithm) + random effect (batch effect of methylation measurement at first visit);

b: Model 2: Model 1 + BMI (first visit) + smoking status (first visit) + alcohol consumption (first visit) + physical activity (first visit) + years of education (first visit) + total cholesterol (first visit) + HDL (first visit) + triglycerides (first visit) + fasting glucose (first visit) + SBP (first visit) + hypertension (first visit) + stroke (first visit) + CHD (first visit) + diabetes (first visit) + cancer (first visit).

Table 5. Associations between stopping antihypertensive medication use and change rate of age acceleration among the subpopulation that used the antihypertensive medications at first visit.

Medication	Change of medication use	N	Changes of age acceleration			
			Model 1 ^a		Model 2 ^b	
			Coefficients (SE)	p-value	Coefficients (SE)	p-value
Calcium channel blockers	Stopped use after first visit	22	-0.415 (0.316)	0.196	-0.143 (0.442)	0.751
	Continuous use	44	Ref		Ref	
ACE inhibitors	Stopped use after first visit	15	-0.308 (0.322)	0.342	-0.212 (0.369)	0.567
	Continuous use	128	Ref		Ref	
ARBs	Stopped use after first visit	5	-1.724 (0.831)	0.077	-0.050 (3.331)	0.985
	Continuous use	16	Ref		Ref	
Alpha blockers	Stopped use after first visit	14	-0.482 (0.294)	0.109	-0.040 (0.569)	0.945
	Continuous use	50	Ref		Ref	
Beta blockers	Stopped use after first visit	24	-0.731 (0.333)	0.030	-0.824 (0.401)	0.042
	Continuous use	161	Ref		Ref	
Diuretics	Stopped use after first visit	20	0.156 (0.335)	0.642	-0.263 (0.407)	0.521
	Continuous use	78	Ref		Ref	
Any antihypertensive medication	Stopped use after first visit	13	-0.317 (0.391)	0.418	-0.585 (0.434)	0.180
	Continuous use	300	Ref		Ref	

a: Model 1: Adjusted for age (first visit) + age acceleration (first visit) + leukocyte distribution (first visit, Houseman algorithm) + random effect (batch effect of methylation measurement at first visit);

b: Model 2: Model 1 + BMI (first visit) + smoking status (first visit) + alcohol consumption (first visit) + physical activity (first visit) + years of education (first visit) + total cholesterol (first visit) + HDL (first visit) + triglycerides (first visit) + fasting glucose (first visit) + SBP (first visit) + hypertension (first visit) + stroke (first visit) + CHD (first visit) + diabetes (first visit) + cancer (first visit).

hypertension and AHMs (data not shown). Neither of the two adjustments altered the patterns of taking AHMs with AA and Δ_{AA} in any relevant manner. In the meanwhile, people might stop taking AHMs after reducing the blood pressure through changing lifestyles and exercises, two factors that are likely to decrease the Δ_{AA} . After controlling for the factors, we still observed a declining pattern between stopping taking any AHM and Δ_{AA} , albeit not significant due to the limited sample size.

One biologically plausible explanation for this inconsistent observation is a potential causal connection among aging, epigenetic biomarkers of age, hypertension and AHM use. We speculate that in these four-corner relationships, aging independently leads to the change of epigenetic biomarkers of age (e.g., DNAmAge, DNAmPhenoAge) and hypertension via two separate pathways. Aging could cause the change of epigenetic biomarkers of age by altering the methylation levels of age-related CpG sites. In parallel, aging could prompt hypertension via a “vicious cycle”, which consists of inflammation, oxidative stress and endothelial dysfunction, and might not be closely related to age-related DNA methylation changes [16, 17]. Taking AHMs could control blood pressure and reduce the risks of aging-related diseases, such as CVD, kidney failure and dementia, which are caused by hypertension. Nevertheless, as the causation between aging and hypertension is one-way, reducing blood pressure using AHM might not reversely affect biological aging by bringing the aberrant changes of age-related CpG sites back to normal levels. On the contrary, the AHMs’ potential side effects, such as abnormal glucose and lipid metabolism [24] and psychological/cognitive disorders [25, 26], might have the potential to accelerate epigenetic biomarkers of age as suggested by previous studies [6, 10, 27]. However, this hypothesis needs to be evaluated by further research with larger populations and multiple follow-ups along with corresponding causal inferences and functional tests.

It is worth noting that the effects of specific medications on AA and Δ_{AA} were not all unfavorable. In our study, ACE inhibitors, ARBs, and diuretics showed weak negative correlation with AA and Δ_{AA} , while beta blockers showed a strong aging accelerating effect. Beyond the effects from specific medications, we should not ignore the adverse drug reactions (ADRs) from combined and inappropriate medication use [28], given most of the participants with hypertension took multiple medications at the same time. ADRs could become more severe and frequent in the elderly due to age-dependent pharmacodynamics and pharmacokinetic changes promoting drug-drug or drug-disease interactions, and such reactions could directly (or

indirectly) facilitate the development of aging-related health outcomes including frailty and all-cause mortality [29, 30]. This effect might play another key role in accelerating DNAmAge if the ADRs are not identified and treated with further changes in prescriptions.

Major strengths of the present study include the relatively large sample size and repeated measurements with detailed information on a broad range of covariates in a large cohort study. We also acknowledge several limitations in the interpretation of results. First, shifts of leukocyte distribution might affect the DNA methylation changes in whole blood samples [31]. Hence, we adjusted for leukocyte distribution by the Houseman algorithm to restrict potential confounding from differential blood counts to the greatest possible extent [32]. Nevertheless, residual confounding by this and other factors cannot be excluded entirely for the longitudinal analysis. Furthermore, the selected study participants were Caucasians and all male, which limits the generalizability of our results to other racial/ethnic groups and women. Finally, although our overall sample size was relatively large, some of the nonsignificant results may have been due to the lack of statistical power with the relatively smaller size of subsamples for specific AHMs. And given those subgroup analyses did not appear as robust as the whole population and showed contradicted patterns, there might be a risk of getting false positive findings, further research with a bigger sample size and longer multiple follow-ups are warranted to validate our results by eliminating the possibility of false positives.

In summary, we observed cross-sectional and longitudinal associations of any AHM use with increased AA and Δ_{AA} . Our findings suggest that controlling blood pressure by taking medications might not be able to reduce the accelerated epigenetic aging, on the contrary, was associated with accelerated DNAmAge. This study partly reveals the relationship between AHMs and biological aging, and also underlines that DNAmAge and AA may not be able to capture the preventive effects of AHMs that reduce cardiovascular risks and mortality. Future investigations are required to confirm our findings and to elucidate the causal relationship between AHM use and DNAmAge, as well as the underlying pathophysiological mechanisms.

MATERIALS AND METHODS

Study design and population

The NAS study is an ongoing longitudinal study of aging, established by the U.S. Department of Veterans Affairs in 1963. Details of this study have been

published previously [33]. Briefly, the NAS is a closed cohort of 2,280 male veterans from the Greater Boston area. They were enrolled after an initial health screening that determined that they were free of known chronic medical conditions. Blood samples were collected from 657 participants, most of whom were visited up to 4 times between 1999 and 2013. Participants have been reevaluated every 3–5 years on a continuous rolling basis using detailed on-site physical examinations and questionnaires. We restricted the current analysis to the data of the first two visits of 546 Caucasian participants (aged 55–85 years) who had been visited twice at least, in order to control for the heterogeneity of race and to analyze the change of AA longitudinally. The NAS study was approved by the Department of Veterans Affairs Boston Healthcare System, and written informed consent was obtained from each subject prior to participation.

Data collection

As previously described [34], at each visit, participants were asked to provide detailed information about their lifestyles, dietary habits, activity levels, and demographic factors. Height and weight were measured and were used to calculate body mass index (BMI, in kg/m²). Blood samples were collected for assessing blood-based biomarkers. Participants' status of major diseases was assessed based on the medical history and physicians' diagnosis. In particular, hypertension was defined as a measured SBP of ≥ 140 mmHg, a measured diastolic blood pressure (DBP) of ≥ 90 mm Hg, or participants' use of AHMs [35]. SBP and DBP were measured by a physician, and the AHMs included calcium channel blockers, ACE inhibitors, ARBs, alpha blockers, beta blockers and diuretics.

DNA methylation data

DNA of whole blood samples were collected between 1999 and 2013. As previously described [36, 37], we used the QIAamp DNA Blood Kit (Qiagen, CA, USA) to extract DNA from buffy coat, and performed bisulfite conversion with the EZ-96 DNA Methylation Kit (Zymo Research, CA, USA). To minimize batch effects, we randomized chips across plates and randomized samples based on a 2-stage age-stratified algorithm so that age distributed similarly across chips and plates. We measured DNA methylation of CpG probes using Illumina HumanMethylation450 BeadChip for all the samples from both visits. After quality control, the remaining samples were preprocessed using the Illumina-type background correction, dye-bias adjustment and BMIQ normalization [38], which were used to generate methylation status. The methylation

status of a specific CpG site was quantified as a β value ranging from 0 (no methylation) to 1 (full methylation).

A total of 353 CpG sites were retrieved from the methylation profiles for the estimation of DNAmAge for each participant based on the algorithm proposed by Horvath [3]. This algorithm was derived from a range of tissues and cell types using 353 probes targeted in the Illumina 27k and 450k methylation arrays. In this study, we performed the estimation by using the online calculator (<http://labs.genetics.ucla.edu/horvath/dnamage/>), where background-corrected beta values were pre-processed by the calculator's internal normalization method [3]. AA was determined as discrepancies between methylation and chronological age in the form of residuals, which have a mean of 0 and represent positive and negative deviations from chronological age in years. The residuals were calculated by a linear regression procedure in which DNA methylation age was the outcome and chronological age was the independent variable. The Δ_{AA} for each participant between the two visits was determined as:

$$\Delta_{AA} = \frac{AA \text{ of second visit (year)} - AA \text{ of first visit (year)}}{\text{Time between first and second visits (year)}} \quad (1)$$

with a unit of year/chronological year.

Statistical analysis

Study population of each visit was described with respect to major socio-demographic characteristics, lifestyle factors and detailed AHM use. The correlation between estimated DNAmAge and chronological age at each visit was evaluated by Spearman correlation coefficient.

We first tested the cross-sectional association of hypertension with AA at each visit and the Δ_{AA} between the two visits. A linear mixed model with AA (Δ_{AA}) as outcome was employed, controlling for covariates that have been reported to be associated with DNA methylation changes or with the use of AHM, including age (years), BMI [kg/m², underweight (<18.5, < 1% of the study population) or normal weight (18.5 to <25), overweight (25 to <30), obese (≥ 30)], alcohol consumption [abstainer, low (0 to <40 g/d), intermediate (40 to <60 g/d), high (≥ 60 g/d)], smoking (current/ former/ never smoker), education (≤ 12 years, 13 – 16 years, >16 years), physical activity [metabolic equivalent of task (MET), low (≤ 12 kcal/kg hours/week), median (12–30 kcal/kg hours/week), high (≥ 30 kcal/kg hours/week)] and leukocyte distribution (Houseman algorithm [32]). The batch of microarray experiments in DNA methylation measurement was controlled for as the random effect. Corresponding AA

was additionally adjusted for in the analyses of Δ_{AA} at each visit.

We then investigated the cross-sectional associations of AHM use with AA and Δ_{AA} at each visit in two mixed linear regression models. Model 1 adjusted for age, leukocyte distribution and the random batch effect, and Model 2 further adjusted for BMI, smoking status, alcohol consumption, physical activity, education, total cholesterol (mg/dL), HDL (mg/dL), triglycerides (mg/dL), fasting glucose (mg/dL), SBP (mm Hg), hypertension, stroke, coronary heart disease, diabetes and cancer (yes/no). AA was additionally adjusted for in the analyses of Δ_{AA} at each visit.

Finally, we examined the longitudinal association between AHM use and the Δ_{AA} . AHM use in the longitudinal analysis was classified as: never used, stopped use after first visit, started use after first visit and continuous use according to the reports of AHM use between the two visits, and was treated as a predictor in the analysis models. Two mixed linear regression models were used, adjusting for the same covariates described above.

Sensitivity analyses

We further performed sensitivity analyses for the main results by using another two DNA methylation based aging biomarkers: DNAmAge estimated by Hanum et al.'s algorithm [39], and DNAmPhenoAge recently developed by Morgan et al. as an "update" of the Horvath DNAmAge for the lifespan [40].

Hanum DNAmAge was estimated based on 71 age-related CpG sites reported in 2013 [39] and determined as the sum of the methylation beta values multiplied by the reported effect sizes of the predictors. AA of Hanum's DNAmAge was also determined as discrepancies between DNA methylation and chronological age in the form of residuals.

Another batch of 513 CpG sites was retrieved for the estimation of DNAmPhenoAge for each participant based on the algorithm proposed by Levine et al. [40]. With the coefficient and intercept values provided by the authors, we estimated the DNAmPhenoAge as:

$$\text{DNAmPhenoAge} = CpG_1 \times \beta_1 + CpG_2 \times \beta_2 + \dots + CpG_{513} \times \beta_{513} + \text{intercept}.$$

As defined by the authors [40], difference between Phenotypic and chronological age (DNAmPhenoAge – chronological age) was defined as the AA of DNAmPhenoAge. The Δ_{AA} for the two aging biomar-

kers between the two visits was also determined by the formula (1).

We tested the cross-sectional and longitudinal associations of the AA and Δ_{AA} of the two biomarkers with the use of any AHM using the same analysis models employed in the main analyses for Horvath DNAmAge.

Data cleaning and all aforementioned analyses were performed by SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), and all statistical tests were two-sided with p-values of <0.05.

Abbreviations

DNAmAge: DNA methylation age; AA: age acceleration; Δ_{AA} : change rate of age acceleration; AHM: antihypertensive medication; CVD: cardiovascular disease; CpG: cytosine-phosphate-guanine; BMI: body mass index; HDL: high-density lipoprotein; NAS: Normative Aging Study; SBP: systolic blood pressure; DBP: diastolic blood pressure; CHD: Coronary heart disease; IPW: inverse probability weighting; ADRs: adverse drug reactions; DNAmPhenoAge: DNA methylation Phenotypic age.

AUTHOR CONTRIBUTIONS

XG contributed to the data analysis and drafting. Other authors contributed to critical revision of the manuscript. All authors read and approved the final manuscript.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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SUPPLEMENTARY MATERIAL

Table S1. Associations of hypertension, diabetes and metabolic syndrome with DNA methylation age, age acceleration and change rate of age acceleration ^a.

	Health outcomes	Status at first visit	N	Coefficients (SE)	p-value	Status at second visit	N	Coefficients (SE)	p-value
Age acceleration	Hypertension	No	172	Ref		No	135	Ref	
		Yes	374	0.041 (0.543)	0.940	Yes	411	0.326 (0.703)	0.644
	Diabetes	No	479	Ref		No	457	Ref	
		Yes	67	0.783 (0.728)	0.283	Yes	89	0.431 (0.804)	0.592
	Metabolic syndrome	No	265	Ref		No	249	Ref	
		Yes	281	0.867 (0.527)	0.101	Yes	297	0.847 (0.644)	0.683
Change rate of age acceleration	Hypertension	No	172	Ref		No	135	Ref	
		Yes	374	0.144 (0.129)	0.267	Yes	411	0.219 (0.137)	0.111
	Diabetes	No	479	Ref		No	457	Ref	
		Yes	67	0.419 (0.174)	0.016	Yes	89	0.361 (0.156)	0.021
	Metabolic syndrome	No	265	Ref		No	249	Ref	
		Yes	281	0.171 (0.126)	0.176	Yes	297	0.343 (0.124)	0.061

a: Model adjusted for corresponding covariates at each visit: age, BMI, alcohol consumption, smoking, years of education, physical activity, leucocyte distribution (Houseman algorithm) and the random batch effect of DNA methylation measurement. Age acceleration was additionally adjusted for in the model for the change rate of age acceleration.

Table S2. Associations of clinical biomarkers with DNA methylation age, age accelerations and change rate of age acceleration^a.

	Biomarkers	First visit		Second visit	
		Coefficients (SE)	p-value	Coefficients (SE)	p-value
Age acceleration	SBP	9.5 e-3 (0.014)	0.498	-2.6 e-3 (0.016)	0.874
	DBP	-0.011 (0.027)	0.690	0.012 (0.031)	0.706
	Fasting glucose	0.017(0.009)	0.058	0.005 (0.014)	0.713
	Total cholesterol	-2.6 e-3(6.6 e-3)	0.698	-8.8 e-3 (7.5 e-3)	0.243
	HDL	-0.020 (0.019)	0.287	-0.037 (0.023)	0.100
	Triglyceride	4.1 e-4 (3.4 e-3)	0.904	-6.2 e-3 (5.1 e-3)	0.227
	Uric acid	0.084 (0.168)	0.617	-0.113 (0.205)	0.584
	Calcium	0.665 (0.648)	0.306	-0.273 (0.823)	0.740
	Phosphorus	-0.251 (0.545)	0.645	-0.159 (0.625)	0.799
	Potassium	2.1 e-4 (2.2 e-4)	0.338	-1.0 e-4 (2.4 e-4)	0.684
	Zinc	-0.024 (0.014)	0.100	0.010 (0.016)	0.527
	Magnesium	8.1 e-5 (1.9 e-3)	0.966	-7.9 e-4 (2.1 e-3)	0.706
	Sodium	7.2 e-5 (1.3 e-4)	0.587	-1.4 e-4 (1.6 e-4)	0.364
Change rate of age acceleration	SBP	2.4 e-3 (3.4 e-3)	0.477	4.1 e-3 (3.6 e-3)	0.260
	DBP	1.9 e-3 (6.6 e-3)	0.773	0.011 (0.007)	0.102
	Fasting glucose	-4.0 e-3 (2.2 e-3)	0.067	-9.8 e-4 (3.0 e-3)	0.746
	Total cholesterol	2.4 e-3 (1.6 e-3)	0.146	2.3 e-3 (1.7 e-3)	0.175
	HDL	1.5 e-3 (4.6 e-3)	0.745	2.9 e-3 (5.0 e-3)	0.566
	Triglyceride	5.0 e-4 (8.3 e-4)	0.552	-9.1 e-4 (1.1 e-3)	0.423
	Uric acid	-4.2 e-3 (0.041)	0.919	-0.049 (0.048)	0.307
	Calcium	0.116 (0.160)	0.469	-0.078 (0.190)	0.684
	Phosphorus	-0.181 (0.134)	0.178	-0.106 (0.144)	0.462
	Potassium	1.1 e-5 (5.4 e-5)	0.845	-5.0 e-5 (5.2 e-5)	0.353
	Zinc	6.0 e-3 (3.6 e-3)	0.092	3.8 e-3 (3.6 e-3)	0.286
	Magnesium	1.7 e-4 (4.7 e-4)	0.715	-6.1 e-4 (4.6 e-4)	0.184
	Sodium	-9.5 e-6 (3.3 e-5)	0.771	-7.0 e-5 (3.4 e-5)	0.055

a: Model adjusted for corresponding covariates at each visit: age, BMI, alcohol, smoking, years of education, physical activity and leucocyte distribution (Houseman algorithm). Age acceleration was additionally adjusted for in the model for the change rate of age acceleration.

Table S3. Sensitivity analyses: associations of any antihypertensive medication use with age acceleration and change rate of age acceleration for Hannum DNA methylation age and DNA methylation Phenotypic age.

a. Cross-sectional associations with age acceleration and change rate of age acceleration.

Visit	Types of aging biomarkers	Use of any antihypertension medication	N	Age acceleration				Change rate of age acceleration			
				Model 1 ^a		Model 2 ^b		Model 1		Model 2	
				Coefficients (SE)	p-value	Coefficients (SE)	p-value	Coefficients (SE)	p-value	Coefficients (SE)	p-value
First visit	DNA methylation age (Hannum)	No	233	Ref		Ref		Ref		Ref	
		Yes	313	0.899 (0.406)	0.014	2.150 (0.727)	0.003	0.050 (0.073)	0.489	0.137 (0.112)	0.383
	DNA methylation Phenotypic age	No	233	Ref		Ref		Ref		Ref	
		Yes	313	1.406 (0.568)	0.0001	1.687 (0.999)	0.002	0.038 (0.108)	0.731	0.037 (0.198)	0.854
Second visit	DNA methylation age (Hannum)	No	163	Ref		Ref		Ref		Ref	
		Yes	383	0.925 (0.445)	0.113	1.801 (0.992)	0.153	0.016 (0.075)	0.832	0.015 (0.153)	0.920
	DNA methylation Phenotypic age	No	163	Ref		Ref		Ref		Ref	
		Yes	383	1.732 (0.573)	0.003	1.692 (1.340)	0.087	0.091 (0.116)	0.435	0.221 (0.238)	0.355

a: Model 1: Adjusted for covariates at each visit: age + leukocyte distribution (Houseman algorithm) + random effect (batch effect of DNA methylation measurement). Age acceleration was additionally adjusted for in the model for the change rate of age acceleration;

b: Model 2: Model 1 + BMI + smoking status + alcohol consumption + physical activity + years of education + total cholesterol + HDL + triglycerides + fasting glucose + SBP + hypertension + stroke + CHD + diabetes + cancer.

b. Longitudinal associations with change rate of age acceleration.

Types of aging biomarkers	Change of any antihypertension medication use	N	Change rate of age acceleration			
			Model 1 ^a		Model 2 ^b	
			Coefficients (SE)	p-value	Coefficients (SE)	p-value
DNA methylation age (Hannum)	Never used	150	Ref		Ref	
	Stopped use after first visit	13	-0.789 (0.241)	0.001	-0.861 (0.287)	0.003
	Started use after first visit	83	0.055 (0.109)	0.614	0.039 (0.123)	0.753
	Continuous use	300	0.139 (0.112)	0.187	0.151 (0.148)	0.199
DNA methylation Phenotypic age	Never used	150	Ref		Ref	
	Stopped use after first visit	13	-0.516 (0.355)	0.147	-0.540 (0.412)	0.191
	Started use after first visit	83	0.159 (0.162)	0.325	0.116 (0.178)	0.517
	Continuous use	300	0.187 (0.124)	0.227	0.146 (0.215)	0.130

a: Model 1: Adjusted for: age (first visit) + age acceleration (first visit) + leukocyte distribution (first visit, Houseman algorithm) + random effect (batch effect of methylation measurement at first visit);

b: Model 2: Model 1 + BMI (first visit) + smoking status (first visit) + alcohol consumption (first visit) + physical activity (first visit) + years of education (first visit) + total cholesterol (first visit) + HDL (first visit) + triglycerides (first visit) + fasting glucose (first visit) + SBP (first visit) + hypertension (first visit) + stroke (first visit) + CHD (first visit) + diabetes (first visit) + cancer (first visit).

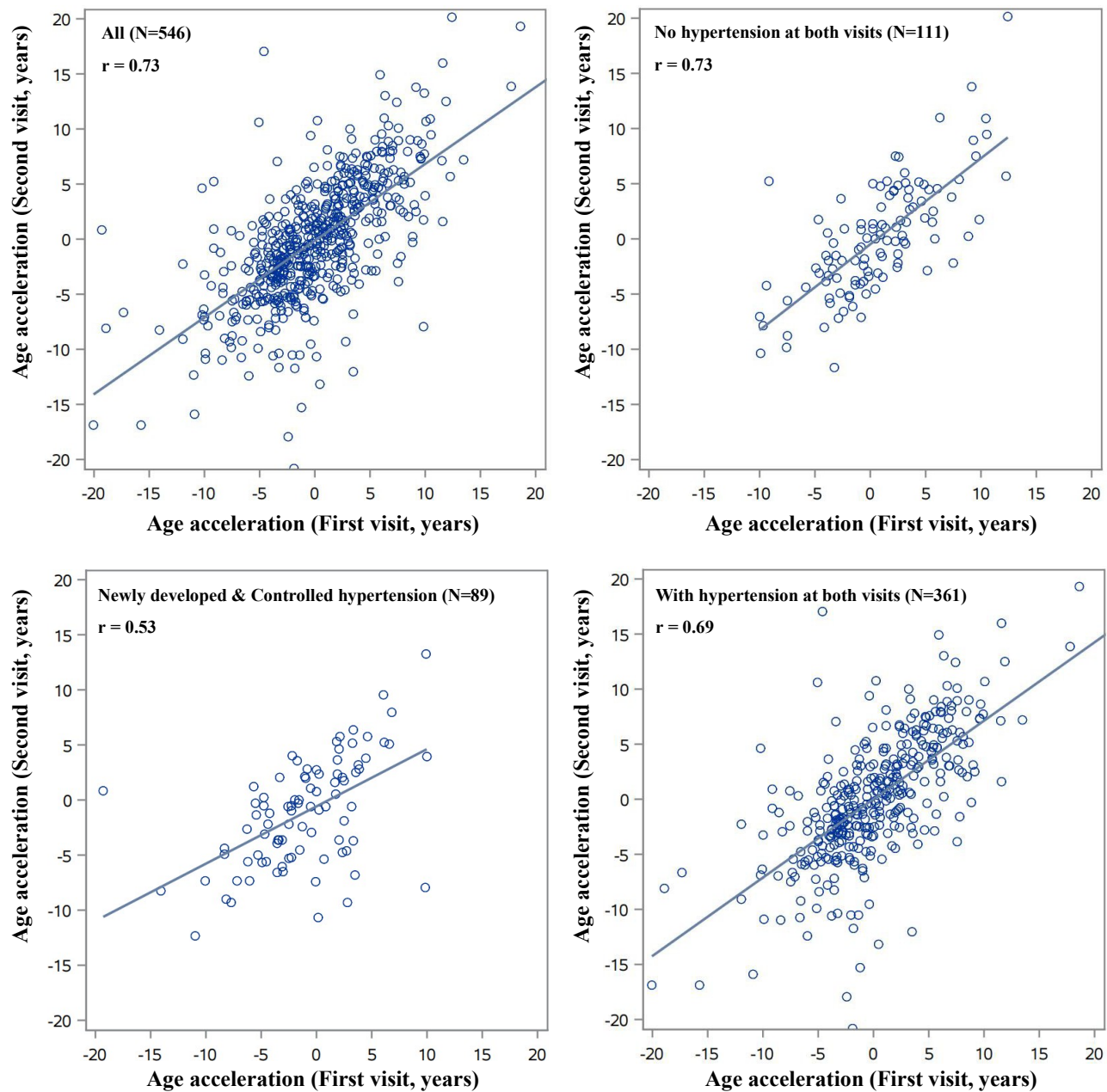


Figure S1. Correlations of age accelerations between the first and second visit based on the status of hypertension at each visit.