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ORIGINAL RESEARCH

Associations Between Blood Pressure and Accelerated DNA Methylation Aging

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BACKGROUND: Individuals of the same chronological age may exhibit diverse susceptibilities to death. However, few studies have investigated the associations between blood pressure and the accelerated aging.

METHODS AND RESULTS: A cross-sectional study was conducted in 288 adults aged ≥50 years. We assessed the DNA methylation-based measures of biological age using CpG sites on the Illumina HumanMethylationEPIC BeadChip. Epigenetic age acceleration metrics were derived by regressing residuals (ΔAge) and ratios (aging rate) of DNA methylation age on chronological age. Dose-response relationships between blood pressure and epigenetic age acceleration were quantified using multiple linear regression and restricted cubic regression models. We found that each 10–mm Hg increase in systolic blood pressure was associated with 0.608 (95% CI, 0.231–0.984) years increase in ΔAge and 0.007 (95% CI, 0.002–0.012) increase in aging rate; meanwhile, for pulse pressure, the increase was 1.12 (95% CI, 0.625–1.61) years for ΔAge and 0.013 (95% CI, 0.007–0.020) for aging rate. Subgroup analysis showed that the significant associations of systolic blood pressure and pulse pressure with epigenetic age acceleration appeared to be limited to women, although interactions between blood pressure and sex were not significant (*P* values for interaction >0.05). The combination of women and hypertension was associated with a much higher increase in ΔAge (β [95% CI], 4.05 [1.07–7.02]) and aging rate (β [95% CI], 0.047 [0.008–0.087]), compared with male participants without hypertension.

CONCLUSIONS: Our findings suggested that high systolic blood pressure and pulse pressure were associated with the epigenetic age acceleration, providing important clues for relationships between blood pressure and epigenetic aging.

Key Words: blood pressure ■ DNA methylation age ■ epigenetic age acceleration

The aging population is increasing in both developed and developing parts of the world. With remarkable improvements in life expectancy, aging has become a critical public health priority.[1] Accumulating evidence indicated that aging is characterized by accumulation of chronic diseases, among which hypertension or hypotension represents one of the most prevalent and potentially modifiable risk factors.[2,3] Most observational studies have found that changes in systolic blood pressure (SBP) or diastolic blood pressure (DBP) were associated with a shortened life span.[4–6] However, whether blood pressure contributes significantly to the accelerated aging remains unclear.

It is well known that aging is an inevitable biological process, but individual decline in physical function is associated with accelerated aging, resulting in different lifespans between people.[7] Individuals of the same chronological age may exhibit different predisposition to age-related conditions or death, which is likely reflective of the discrepancy in biological age.[7] Because chronological age is an imperfect proxy of the aging process, mounting biomarkers have been reported as predictors of biological age, such as telomere length,

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CLINICAL PERSPECTIVE

What Is New?

- In the study, we assessed epigenetic age acceleration by DNA methylation-based measures and found that high systolic blood pressure and pulse pressure are significantly associated with increased epigenetic age acceleration with a dose-response trend.
- The associations of blood pressure with epigenetic age acceleration were influenced by sexual difference, which appeared to be limited to women, and the combination of women and hypertension was associated with a much higher increase in accelerated aging than the combined men and nonhypertension.

What Are the Clinical Implications?

- Our findings indicate significant relationships between blood pressure and DNA methylation-derived measures of epigenetic age acceleration in older adults, providing important clues for identifying effective antiaging interventions in controlling blood pressure.

Nonstandard Abbreviations and Acronyms

DBP	diastolic blood pressure
DMR	differentially methylated region
DNAm age	DNA methylation age
PP	pulse pressure
SBP	systolic blood pressure

mitochondrial damage, and epigenetic alterations.[8,9] Recent evidence indicated that DNA methylation-derived biomarkers exhibit statistically age-associated physiological decline.[10,11] Studies have observed that age-dependent changes in DNA methylation have been reported to be involved in millions of CpG methylations in the human genome.[12,13] With the availability of oligonucleotide arrays to vast CpG sites in the human genome, sets of CpGs coupled with a mathematical algorithm were imputed to assess DNA methylation age (DNAm age) referring to epigenetic clocks.[14,15] Pivotal aspects of age-related conditions are demonstrated to be captured by DNAm age, which has been reported to well predict lifespan of individuals.[13,16] The application of DNAm age can invariably reveal differences in physiological status among individuals with the same chronological age, where DNAm age exceeding chronological age is described as the epigenetic age acceleration.[13]

Because of the increasing mortality of cardiovascular events, it is crucial to explore the effects of blood pressure on the epigenetic age acceleration to emphasize the importance of blood pressure management. In the present study, we measured DNA methylation at ≈850 000 CpGs in a cross-sectional study and quantified the associations of blood pressure with epigenetic age acceleration by DNA methylation-based measures.

METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Population

This was a cross-sectional study (n=288), and participants originated from rural towns of Hongshuihe region, located in southern China, with a relatively high proportion of long-lived people.[17] Three towns (Wuzhuan, Sanshi, and Donglan) were selected using a stratified and cluster sampling approach. Individuals aged ≥50 years with residence in selected towns for >10 years were recruited to participate in the survey in appointed clinics with the assistance of local medical staff. Individuals were excluded if (1) they were aphasic, deaf, or blind; (2) they had psychiatric disturbances; or (3) they experienced severe organic diseases, such as stroke, cachexia, myocardial infarction, or cancers. Recruited participants received detailed standardized questionnaire; and information, such as age, sex, education, history of disease, and lifestyles, was collected by face-to face interview. Anthropometric metrics, such as height and weight, were conducted by qualified medical practitioners. With an exclusion of 8 individuals who refused to provide blood samples or failed to complete investigation survey, a total of 280 participants were included in the current study for subsequent analysis. The study was approved by the ethics committee of Guangxi Medical University. Each participant enrolled was informed of why and what the survey was performed and gave written informed consent before investigation.

Measurement of Blood Pressure

All the participants were asked not to exercise, smoke, and have stimulants for at least half an hour before the measurement. After sitting for at least 5 minutes, blood pressure was measured for most subjects in a sitting position with an electronic sphygmomanometer by placing the center of the cuff's rubber bag on the brachial artery and keeping the lower edge of the cuff 2 to 3 cm away from the elbow line. For centenarians unable to sit upright, blood pressure was measured in

a recumbent position. SBP and DBP were obtained on the basis of readings of the machine, and 2 or 3 measurements were taken to ensure the reading was accurate. Pulse pressure (PP) and mean arterial pressure (MAP) were calculated as follows: PP=SBP–DBP; MAP=DBP+PP÷3. Participants with measured SBP ≥140 mm Hg or DBP ≥90 mm Hg, or receipt of antihypertensive medications, were defined as having hypertension.

Assessment of DNA Methylation Age Acceleration

Peripheral venous blood samples were collected from each participant, and DNA from white blood cells was isolated as previously described.[18] Infinium HuamnMethylationEPIC BeadChip arrays (Illumina Inc) were used to measure DNA methylation at a single-nucleotide resolution at >850 000 CpG sites, according to manufacturer's instructions. Data of BeadChip were filtered if samples with 10% of CpG probes with detection P>0.01; sites of single-nucleotide polymorphisms rather than CpG probes; sites with bead counts <3 in 5% of samples or detection P>0.01; sites extended on single-nucleotide polymorphisms with mirror allele frequency >0.05; or sites blasted to multiple different chromosomal regions. After filtering quality control, normalized β values (proportions of methylated DNA) of selected CpGs were imputed for calculating DNAm age through the Horvath method (https://dnamage.genetics.ucla.edu/). The calculation of Horvath's DNAm age was derived from an elastic net penalized regression of 353 CpGs integrated with blood cell proportions.[15] The collective effects of 353 CpGs produce a composite multivariate biomarker, and the high accuracy of Horvath's DNAm age has been validated using 8000 publicly available microarray samples, including the whole blood.[19–21] Scatter plot for the correlation between chronological age and Horvath's DNAm age was presented in Figure S1. The correlation between chronological age and estimated methylation age was 0.88, with an root mean square error of 6.11 years, which showed a relatively higher accuracy and less error in our population. Epigenetic age acceleration was assessed by the measurement of ΔAge and aging rate.[14,15] ΔAge was derived by regressing DNAm age on chronological age and predicting the residuals from a linear model. Aging rate was assessed by ratios of DNAm age to chronological age.

Statistics Analysis

Associations of SBP, DBP, PP, and MAP with epigenetic age acceleration were tested by multiple linear regression models and quantified using estimated changes and 95% CIs of epigenetic age acceleration (ΔAge and aging rate) by each 10–mm Hg increase in blood pressure as continuous variables. Adjusting covariates were chosen on the basis of a priori knowledge and included sex (men/women), body mass index (BMI; kg/m^2), socioeconomic status (low/middle/high), cigarette smoking (yes/no), alcohol drinking (yes/no), physical activity (yes/no), sleep duration (hours), hyperlipidemia (yes/no), and diabetes (yes/no).[22,23] Restricted cubic spline models with 3 knots at 5th, 50th, and 95th percentiles were fitted to graphically visualize the dose-response trajectory between blood pressures metrics (SBP, DBP, PP, and MAP) and epigenetic age acceleration (ΔAge and aging rate). In addition, the epigenome-wide analysis for the differentially methylated region (DMR) associated with hypertension was conducted using Bumphunter function with adjustment for confounding factors and estimated cell type proportions by Houseman et al.[24,25] As a known risk factor for hypertension, BMI can be considered acceptable to discriminate individuals with hypertension from healthy subjects. Receiver-operating characteristic analyses were conducted to determine the area under the curves of ΔAge, aging rate, and BMI for hypertension.

Several additional sensitivity analyses were conducted in the present study to evaluate the robustness of our results. To assess whether associations of blood pressure with epigenetic age acceleration were affected by inclusion of individuals with receipt of antihypertensive medication, sensitivity analyses were conducted in participants without taking antihypertensive drugs. Other measures of DNAm age were also calculated on the basis of 71 CpGs using Hannum's clock to assess the epigenetic age acceleration.[14] To further explore differences in estimated changes varied by sexual difference, we assessed associations of epigenetic age acceleration with a 10–mm Hg increase in blood pressure among subgroups using multiple linear regression models with adjustment for potential confounders. We included a product term between each blood pressure measure and sex as a continuous variable in the multiple linear regression model to test the interaction between blood pressure and sex in association with epigenetic age acceleration. Statistic powers for the sample size (n=280) with multiple linear regression models were calculated using "pwr" packages. Assuming a significance level of 0.05 and r^2 of predictors varying from 0.16 to 0.23, the statistic power of the study could reach at least 90%. We performed all analyses using R software (version 4.0.2; R Core Team), and 2-tailed P<0.05 was defined as statistical significance in the present study.

RESULTS

Characteristics of the Study Population

As summarized in Table 1, the overall population had an average chronological age of 78.5 years. The mean

Table 1. General Characteristics of the Study Population (n=280)

Characteristics	Values
Sex, women, n (%)	160 (57.1)
Chronological age, mean±SD, y	78.5±16.1
DNAm age, mean±SD, y	77.2±11.4
ΔAge, mean±SD, y*	−3.18±0.43
Aging rate, mean±SD†	0.952±0.11
BMI, mean±SD, kg/m^2	21.9±3.48
Socioeconomic status, n (%)	
Lower	110 (39.3)
Middle	87 (31.1)
Higher	83 (29.6)
Cigarette smoking, n (%)	36 (12.9)
Alcohol drinking, n (%)	67 (23.9)
Sleep, mean±SD, h	9.63±1.58
Physical activity, n (%)	130 (46.4)
Diabetes, n (%)	23 (8.2)
Hyperlipidemia, n (%)	101 (36.1)
Hypertension, n (%)	147 (52.5)
SBP, mean±SD, mm Hg	143±24.6
DBP, mean±SD, mm Hg	80.3±13.3
PP, mean±SD, mm Hg	62.7±18.5
MAP, mean±SD, mm Hg	101±15.6

Data are presented as number (percentage) for categorical variables and mean±SD for continuous variables. BMI indicates body mass index; DBP, diastolic blood pressure; DNAm age, DNA methylation age; MAP, mean arterial pressure; PP, pulse pressure; and SBP, systolic blood pressure.

*ΔAge was calculated as residual from regressing DNAm age on chronological age.

†Aging rate was calculated as the methylation age divided by chronological age.

(SD) values of estimated DNAm age, ΔAge, and aging rate were 77.2 (11.4), −3.18 (0.43), and 0.952 (0.11), respectively. Most participants were women (57.15%), and they were less likely to consume cigarettes (12.9%) or alcohol (23.9%). The prevalence rates of diabetes, hyperlipidemia, and hypertension were 8.2%, 36.1%, and 52.5%, respectively. Individuals with hypertension were more likely to be chronologically and biologically older and have a higher proportion of hyperlipidemia (shown in Table S1). The proportions of women and alcohol drinking in hypertensive participants were higher than those in nonhypertensive participants, although significant difference was not observed (P>0.05). The mean SBP, DBP, PP, and MAP were 143, 80.3, 62.7, and 101 mm Hg, relatively.

Associations of Blood Pressure With Epigenetic Age Acceleration

The univariate analysis showed that confounding factors, including sex, BMI, socioeconomic status, cigarette smoking, sleep duration, and physical activity, were significantly associated with epigenetic age acceleration (P<0.05; shown in Table S2). Both SBP and PP were found to be positively related to the accelerated DNA methylation aging, even with graded adjustments for related risk factors (shown in Table 2). After fully adjusted related covariates, each 10–mm Hg increment of SBP was associated with 0.608 (95% CI, 0.231–0.984) years increase in ΔAge and 0.007 (95% CI, 0.002–0.012) increase in aging rate; meanwhile, for PP, the increase was 1.120 (95% CI, 0.625–1.61) years in ΔAge and 0.013 (95% CI, 0.007–0.020) in aging rate. The results of restricted cubic splines in Figures 1 and 2 visually revealed significantly linear relationships of SBP and PP with ΔAge as well as of SBP and PP with aging rate (P overall <0.05 and P nonlinear >0.05). Increasing SBP and PP were significantly related to elevated ΔAge and aging rate in a likely linear dose-dependent manner.

In addition, we also performed a sensitivity analysis for relationships between blood pressure (SBP, DBP, PP, and MAP) and the epigenetic age acceleration using the DNA methylation age estimated with the Hannum's clock and found the results were consistent with primary analyses (shown in Table S3). The relations of blood pressure with ΔAge and aging rate in participants without taking antihypertensive medications were also consistent with those in the total population. Each 10–mm Hg increment of SBP was significantly related to 0.613 years increase in ΔAge and 0.009 increase in aging rate (P<0.05). For PP, each 10–mm Hg increment of PP was significantly related to 0.973 years increase in ΔAge and 0.012 increase in aging rate (P<0.05) (shown in Table S4).

Subgroup Analysis

Table 3 showed the relations of blood pressure with epigenetic age acceleration stratified by sex. We found that significant relations of blood pressure with epigenetic age acceleration were only observed in women (P<0.05). For female participants, estimated change of ΔAge and aging rate per 10–mm Hg increment of SBP were 0.810 (95% CI, 0.282–1.338) and 0.010 (95% CI, 0.003–0.017), relatively; meanwhile, estimated change of ΔAge and aging rate per 10–mm Hg increment of PP were 1.492 (95% CI, 0.751–2.233) and 0.018 (95% CI, 0.008–0.028), respectively. The interactions between blood pressure and sex in association with epigenetic age acceleration were not significant (all P values for interaction >0.05).

We observed that hypertension was significantly associated with epigenetic age acceleration (P<0.05; shown in Table S5). The adjusted estimated changes of ΔAge and aging rate were 3.10 (95% CI, 1.07–5.13) and 0.04 (95% CI, 0.015–0.065), respectively. Figure S2 shows that the area under the curves of ΔAge, aging

Table 2. Relationships Between Blood Pressure and Epigenetic Age Acceleration (n=280)

Variable	Estimated changes of ΔAge per 10–mm Hg increase in blood pressure		Estimated changes of aging rate per 10–mm Hg increase in blood pressure	
	β (95% CI)	*P* value	β (95% CI)	*P* value
SBP				
Model 1	0.640 (0.265 to 1.015)	<0.001	0.008 (0.003 to 0.013)	0.003
Model 2	0.623 (0.239 to 1.007)	0.002	0.008 (0.003 to 0.013)	0.003
Model 3	0.612 (0.222 to 1.001)	0.002	0.008 (0.002 to 0.013)	0.005
Model 4	0.608 (0.231 to 0.984)	0.002	0.007 (0.002 to 0.012)	0.004
DBP				
Model 1	−0.191 (−0.924 to 0.543)	0.611	−0.002 (−0.011 to 0.008)	0.727
Model 2	−0.122 (−0.859 to 0.615)	0.746	−0.001 (−0.01 to 0.009)	0.873
Model 3	−0.096 (−0.814 to 0.622)	0.794	−0.001 (−0.011 to 0.009)	0.843
Model 4	−0.087 (−0.809 to 0.635)	0.813	−0.001 (−0.010 to 0.009)	0.862
PP				
Model 1	1.186 (0.696 to 1.676)	<0.001	0.014 (0.007 to 0.021)	<0.001
Model 2	1.182 (0.674 to 1.69)	<0.001	0.014 (0.008 to 0.021)	<0.001
Model 3	1.154 (0.654 to 1.654)	<0.001	0.014 (0.007 to 0.021)	<0.001
Model 4	1.120 (0.625 to 1.61)	<0.001	0.013 (0.007 to 0.02)	<0.001
MAP				
Model 1	0.491 (−0.113 to 1.095)	0.112	0.007 (−0.002 to 0.015)	0.119
Model 2	0.468 (−0.152 to 1.088)	0.140	0.006 (−0.002 to 0.014)	0.142
Model 3	0.415 (−0.207 to 1.038)	0.192	0.005 (−0.003 to 0.014)	0.199
Model 4	0.470 (−0.137 to 1.08)	0.130	0.006 (−0.002 to 0.014)	0.163

Model 1 is the crude model, which did not adjust any covariates. Model 2 is the basically adjusted model, including sex and body mass index. Model 3 is the further adjusted model, including factors of model 2 plus socioeconomic status, smoking status, drinking status, physical activity, and sleep duration. Model 4 is the fully multivariate model, including factors of model 3 plus history of hyperlipidemia and diabetes. β indicates regression coefficient; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; and SBP, systolic blood pressure.

rate, and BMI for hypertension were 0.687, 0.690, and 0.687, respectively, indicating that measures of epigenetic age acceleration (ΔAge and aging rate) can also be considered as predictors of hypertension. Epigenome-wide analysis for DMR associated with hypertension showed that 11 independent DMRs were identified associated with hypertension at a false discovery rate <0.05. Most DMRs contained CpGs annotated to genes, spanning first exon, the body, TSS200, TSS1500, and 5' untranslated region (shown in Table S6). We further examined the joint effects of hypertension and sex on epigenetic age acceleration (shown in Table 4). Compared with men without hypertension, estimated changes of ΔAge and aging rate in women with hypertension were 4.045 (95% CI, 1.074–7.017) and 0.047 (95% CI, 0.008–0.087), respectively.

DISCUSSION

In the present study, we found that high SBP and PP are significantly associated with increased epigenetic age acceleration with a dose-response trend. The significant associations of SBP and PP with epigenetic age acceleration appeared to be limited to women, although interactions between blood pressure and sex were not significant. The combination of women and hypertension was associated with a much higher increase in accelerated aging than the combined men and nonhypertension.

Individuals of the same chronological age may exhibit different predisposition to age-related conditions or death, which is likely reflective of the discrepancy in biological age (ie, DNAm age). In the present study, the DNAm age of individuals was lower than chronological age. The possible reason may be that our study population originated from Hongshuihe region, which has been noted as a high-longevity area because it has leading centenarian ratios among the counties in China.[17] The study participants tended to have a prolonged lifespan with a relatively lower biological age.

Variation of interindividual difference exists in age-associated cardiometabolic dysregulation, especially for older adults. Higher risks of obesity and dyslipidemia were also found in participants with increasing epigenetic age acceleration.[26,27] Pottinger and his colleagues observed that poor cardiovascular health was associated with the epigenetic age acceleration in a

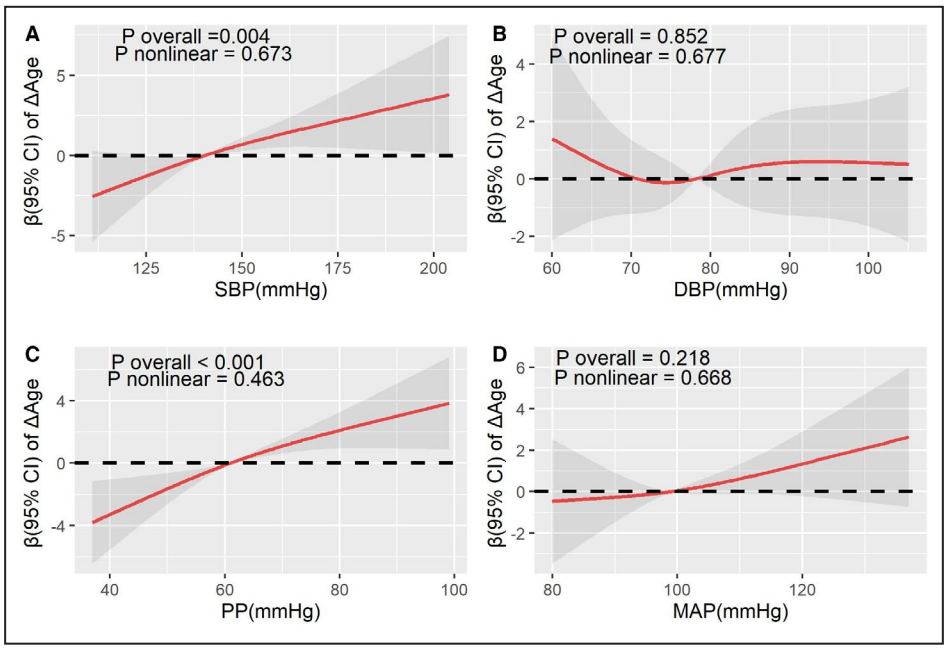

Figure 1. The restricted cubic splines for the associations between blood pressure and ΔAge (n=280).
The lines represent adjusted regression coefficient (β) (95% CI) based on restricted cubic spline for SBP (**A**), DBP (**B**), PP (**C**), and MAP (**D**) with knots at 5th, 50th, and 95th percentiles. The β was estimated with adjustment for sex, body mass index, socioeconomic status, smoking status, drinking status, physical activity, sleep duration, hyperlipidemia, and diabetes. ΔAge was calculated as residual from regressing DNA methylation age on chronological age. DBP indicates diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; and SBP, systolic blood pressure.

cross-sectional analysis of 2170 menopausal women.[28] In the present study, an increase in epigenetic age acceleration per 10–mm Hg of blood pressure was found to be <1 year. Several cardiometabolic risk factors pose threats to the premature decline of physical functioning attributable to accelerated aging, and blood pressure may play a small part in such declines.[29–31] Ammous and his colleagues found that epigenetic age acceleration may be attributed to a worse cardiometabolic file, such as higher SBP, PP, triglycerides, and fasting insulin, and lower low-density cholesterol.[29] A longitudinal study from the WHI (Women's Health Initiative) revealed that accelerated epigenetic aging has been associated with lifestyle factors, such as diet, physical activity, and alcohol consumption.[26] Ammous et al reported that a 10–mm Hg increase in epigenetic age acceleration was associated with an ≈1-year increase in SBP and PP.

A cross-sectional study conducted among 42 middle-aged adults reported that higher SBP and DBP is associated with shortened telomere length, but the sample size of the study limited the validity of their results.[32] Epigenetic age acceleration exhibits statistically age-associated physiological decline than telomere length. In the present study, SBP and PP were found to be positively related to the accelerated DNA methylation aging. Consistent with our study, increased epigenetic age acceleration was found to be associated with higher SBP and PP in a Black cohort with a high prevalence of hypertension.[29] The reason why significant associations were observed for SBP and PP may be that the 2 measures have better implicative power than DBP and MAP for coronary artery disease. Increased arterial stiffness promotes an increase in SBP and a decrease in DBP, leading to an increase in PP, but not MAP, which acts as an offsetting effect.[33,34] Kong et al found that SBP and PP had stronger relationships with coronary artery disease than DBP and MAP.[35] In a study of 1390 Black individuals, Smith and his colleagues found that epigenetic age acceleration may act as a subclinical biomarker for damage to peripheral vascular and heart, which may help to better characterize the functional mechanisms underlying organ damage from aging.[36]

Methylation of DNA plays an important role in regulation of the expression of individual genes. It is reported that 5-methylcytosine DNA levels have been reported to be lower in patients with essential hypertension compared with healthy controls.[37] Our findings of epigenome-wide analysis showed that

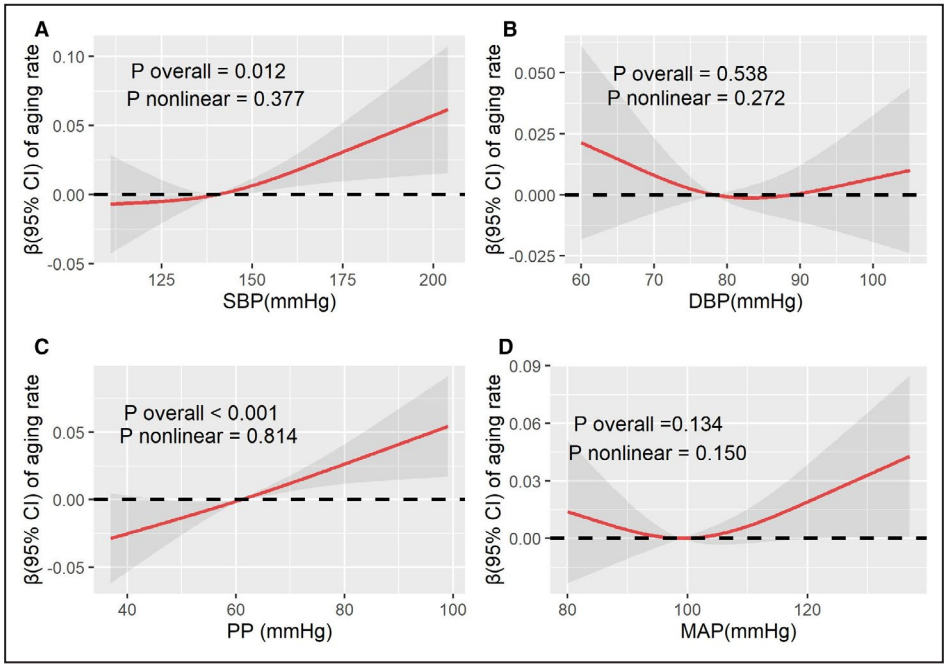

Figure 2. The restricted cubic splines for the associations between blood pressure and aging rate (n=280).
The lines represent adjusted regression coefficient (β) (95% CI) based on restricted cubic spline for SBP (**A**), DBP (**B**), PP (**C**), and MAP (**D**) with knots at 5th, 50th, and 95th percentiles. The β was estimated with adjustment for sex, body mass index, socioeconomic status, smoking status, drinking status, physical activity, sleep duration, hyperlipidemia, and diabetes. Aging rate was calculated as DNA methylation age divided by chronological age. DBP indicates diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; and SBP, systolic blood pressure.

hypermethylation or hypomethylation of DMRs was found independently associated with hypertension. Similarly, some studies reported that blood pressure was associated with hypermethylation or hypomethylation of several single genes.[38,39] Alexeeff and his colleagues found that SBP and DBP were negatively associated with methylation of interferon-γ gene and positively associated with methylation of inducible NO synthase and toll-like receptor 2 genes.[38] The results of Manuel and his colleagues showed that decreases in SBP and DBP were significantly associated with hypomethylation of Nuclear Factor Kappa B Subunit 1 gene.[39] However, comparing with the studies focusing on several genes, we used DNAm age with the status of multiple CpG sites to explore the associations between blood pressure and DNA methylation. The epigenetic clock of Horvath was established with 353 CpG sites, of which 160 CpGs were negatively related to aging while the remaining 193 CpGs had a negative association.[15] Of note, NADPH Oxidase 4 gene, a reactive oxygen species generating enzyme expressed in endothelium, was reported to exert potentially beneficial effects on vasodilator function and blood pressure in transgenic rats treated as overexpression of Nox4.[40] Intriguingly, CpG located in Nox4 was used in methylation age estimation of Horvath epigenetic clock, which was negatively associated with the age predictor.

Because of different hereditary and habitual diets, it has been shown that sex is associated with differences in epigenetic response.[41] In the present study, we found that significant associations of blood pressure with epigenetic aging were only observed in women. As has been previously reported, the blood pressure of women is typically higher than that of men.[42] Most observational studies showed that more women than men die of cardiovascular disease, indicating that the sex difference was associated with interindividual variability in the regulation of blood pressure.[43–45] In addition, hundreds of genes differentially expressed between sexes in an age-dependent manner result in sexual difference in blood pressure. For example, genes encoding angiotensin II type 2 receptor and angiotensin-converting enzyme 2 are located on the X chromosome and are associated with relative cardiovascular discrepancy for men and women.[46] Epigenetic age may take a role in sexual dimorphism relating to the regulation of blood pressure and cardiovascular function, and further studies with larger sample size were required to verify our findings.

Table 3. Associations of Blood Pressure With Epigenetic Age Acceleration Stratified by Sex (n=280)

Variables	Blood pressure, mean±SD, mm Hg	Estimated changes of ΔAge per 10–mm Hg increase in blood pressure			Estimated changes of aging rate per 10–mm Hg increase in blood pressure		
		β (95% CI)	*P* value*	*P* for interaction†	β (95% CI)	*P* value*	*P* for interaction†
SBP							
Men	135±15.6	0.277 (–0.214 to 0.768)	0.272	0.376	0.003 (–0.004 to 0.010)	0.403	0.507
Women	154±22.2	0.810 (0.282 to 1.338)	0.003		0.010 (0.003 to 0.017)	0.005	
DBP							
Men	73.1±7.59	–0.495 (–1.500 to 0.510)	0.336	0.223	–0.007 (–0.021 to 0.007)	0.353	0.229
Women	89.7±13.9	0.021 (–0.079 to 0.12)	0.682		0.003 (–0.010 to 0.016)	0.635	
PP							
Men	51.6±15.9	0.591 (–0.003 to 1.185)	0.054	0.297	0.007 (–0.002 to 0.015)	0.113	0.424
Women	77.2±18.3	1.492 (0.751 to 2.233)	<0.001		0.018 (0.008 to 0.028)	<0.001	
MAP							
Men	90.3±6.87	0.038 (–0.803 to 0.880)	0.929	0.331	0 (–0.012 to 0.012)	0.968	0.404
Women	115±14.7	0.074 (–0.009 to 0.158)	0.084		0.009 (–0.001 to 0.020)	0.092	

ΔAge was calculated as residual from regressing DNA methylation age on chronological age. Aging rate was calculated as DNA methylation age divided by chronological age. β indicates regression coefficient; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; and SBP, systolic blood pressure.

**P* values were estimated using multiple linear regression models, adjusted for sex, body mass index, socioeconomic status, smoking status, drinking status, physical activity, sleep duration, hyperlipidemia, and diabetes.

†*P* values for interaction were estimated by including a product term of each blood pressure measure and sex in multiple linear regression models.

Our research has the following strengths. First, SBP, DBP, PP, and MAP were adopted as the metrics of blood pressure, which provided a comprehensive reflection of cardiovascular function than just using SBP and DBP. It was reported that PP and MAP were more sensitive to aortic regurgitation or aortic stiffness, but the same level of attention to SBP and DBP has not given to PP and MAP in previous studies.[47,48] Second, we assessed epigenetic age acceleration (ΔAge and aging rate) of individuals to further explore the relationships between blood pressure and aging pace, which provides important clues for relationships between blood pressure and epigenetic aging in an Asian population. Finally, linear and nonlinear models were performed in the study with adjustment for potential confounding variables, making our findings more convincing. However, there are some limitations worth noting. First, the cross-sectional nature of the study cannot allow us to infer the causality between blood pressure and epigenetic age acceleration. Therefore, our findings should be confirmed in further prospective cohort studies. Second, our study was restricted in the population aged ≥50 years, which might limit the generalizability of our findings to younger populations. On the other hand, we minimize the confounding effects by age variation. Finally, adjusted covariates, such as cigarette smoking and alcohol drinking, were self-reported, which may result in potential recall bias. However, we conducted questionnaire investigation through face-to-face interviews, and completed questionnaires were logically checked by another trained investigator.

CONCLUSIONS

We found that both SBP and PP were positively related to the elevated ΔAge and aging rate, which were influenced

Table 4. Joint Associations of Hypertension and Sex in Relation to Epigenetic Age Acceleration (n=280)

Groups	Proportions of hypertension, n (%)	Estimated changes of ΔAge β (95% CI)	*P* value	Estimated changes of aging rate β (95% CI)	*P* value
Nonhypertension and men	…	0 (Reference)	…	0 (Reference)	…
Nonhypertension and women	…	–0.288 (–3.09 to 2.51)	0.322	–0.006 (–0.041 to 0.029)	0.423
Hypertension and men	58 (48.3)	0.132 (–2.61 to 2.88)	0.295	0.001 (–0.036 to 0.038)	0.254
Hypertension and women	89 (55.6)	4.05 (1.07 to 7.02)	<0.001	0.047 (0.008 to 0.087)	<0.001

ΔAge was calculated as residual from regressing DNA methylation age on chronological age. Aging rate was calculated as DNA methylation age divided by chronological age. The β was estimated with adjustment for sex, body mass index, socioeconomic status, cigarette smoking, alcohol drinking, physical activity, sleep duration, hyperlipidemia, and diabetes. β indicates regression coefficient.

by sexual difference. Our findings suggested that high SBP and PP were associated with the epigenetic age acceleration, providing important clues for relationships between blood pressure and epigenetic aging.

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Disclosures

None.

Supplementary Material

Tables S1–S6
Figures S1–S2

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Supplemental Material

Table S1. Differences of general characteristics between hypertensive and non-hypertensive participants (N=280).

Characteristics	Hypertension (n=147)	Non-hypertension (n=133)	P value
Sex, Female, n (%)	89 (60.5)	71 (53.4)	0.276
Chronological age (years, mean ±SD)	81.3±16.2	75.5±16.1	0.003
DNAm age (years, mean ± SD)	79.9±12.4	74.2±9.96	<0.001
ΔAge† (years, mean ± SD)	-2.54±0.61	-3.83±0.56	<0.001
Aging rate*(mean ± SD)	0.965±0.10	0.931±0.09	0.001
BMI (kg/m^2, mean ±SD)	22.3 ±4.15	21.5±3.06	0.057
Socioeconomic status, n (%)			
Lower	56 (38.1)	54 (40.6)	0.690
Middle	49 (33.3)	38 (28.6)	
Higher	42 (28.6)	41 (30.8)	
Cigarette smoking, n (%)	19 (12.9)	17 (12.8)	0.990
Alcohol drinking, n (%)	42 (28.6)	25 (18.8)	0.080
Sleep duration (hours, mean ± SD)	9.76±1.68	9.46±1.54	0.121
Physical activity, n (%)	70 (47.6)	60 (45.1)	0.764
Diabetes, n (%)	13 (8.8)	10 (7.5)	0.853
Hyperlipidemia, n (%)	62 (42.2)	39 (29.3)	0.035

Abbreviations: BMI, body mass index; SD, standard deviance; DNAm age, DNA methylation age. †ΔAge was calculated as residual from regressing DNAm on chronological age. *Aging rate was calculated as the methylation age divided by chronological age. Data are presented as n (%) for categorical variables, and mean ± SD for continuous variables.

Table S2. Univariate analysis of related factors with epigenetic age acceleration (N=280).

Factors	β (95% CI) of ΔAge	P value	β (95% CI) of aging rate	P value
Sex (Female vs. Male)	2.08 (0.65, 3.51)	<0.001	0.016 (0.005, 0.027)	<0.001
BMI (kg/m^2)	0.492(0.161, 0.823)	0.004	0.007(0.002, 0.011)	0.003
Socioeconomic status				
Middle vs. Lower	-3.95(-6.84, -1.06)	0.008	-0.052(-0.088, -0.016)	0.005
Upper vs. Lower	-6.00 (-8.92, -3.07)	<0.001	-0.078(-0.115, -0.042)	<0.001
Cigarette smoking (yes vs. no)	4.57(0.916, 8.23)	0.015	0.071(0.023, 0.12)	0.004
Alcohol drinking (yes vs. no)	1.36(-1.54, 4.26)	0.358	0.024(-0.015, 0.062)	0.234
Sleep (hours)	-2.80 (-3.49, -2.11)	<0.001	-0.039(-0.048, -0.03)	<0.001
Physical activity (yes vs. no)	-2.36 (-4.56, -0.164)	0.010	0.046(0.016, 0.076)	<0.001
Diabetes (yes vs. no)	2.99 (-1.51, 7.48)	0.194	0.038(-0.022, 0.098)	0.213
Hyperlipidemia (yes vs. no)	1.01(-1.56, 3.59）	0.442	0.009(-0.024, 0.041)	0.603

Abbreviations: BMI, body mass index. ΔAge was calculated as residual from regressing DNAm on chronological age. Aging rate was calculated as the methylation age divided by chronological age.

Table S3. Sensitivity analysis for associations of blood pressure with epigenetic age acceleration in participants without taking antihypertensive medications (n=241)

Variables	Estimated changes of ΔAge (years) per 10 mmHg increase in blood pressure		Estimated changes of ageing rate per 10 mmHg increase in blood pressure	
	β (95%CI)	*P*	β (95%CI)	*P*
SBP	0.613 (0.019, 1.21)	0.045	0.009(0.001, 0.017)	0.033
DBP	-0.17(-1.14, 0.80)	0.731	-0.002(-0.015, 0.011)	0.769
PP	0.973 (0.27, 1.68)	0.008	0.012(0.003, 0.022)	0.012
MAP	0.37(-0.542, 1.28)	0.428	0.005(-0.007, 0.017)	0.440

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; β, regression coefficients; CI, confidence interval. ΔAge was calculated as residual from regressing DNAm age on chronological age. Ageing rate was calculated as DNAm age divided by chronological age. The β was estimated with adjustment for sex, BMI, socioeconomic status, cigarette smoking, alcohol drinking, physical activity, sleep duration, hyperlipidemia, and diabetes

Table S4. Sensitivity analysis for associations between blood pressure and epigenetic age acceleration based on Hannum's clock (n=280)

Variables	Estimated changes in ΔAge (years) by tertiles of blood pressure parameters			Per 10 mmHg increase in blood pressure	*P* value for trend
	T1 β (95%CI)	T2 β (95%CI)	T3 β (95%CI)		
SBP	≤129 mmHg	129 to 147 mmHg	>147 mmHg		
ΔAge	0(referent)	1.298(-1.179, 3.775)	4.021(1.598, 6.445)	0.682(0.281, 1.083)	0.001
Ageing rate	0(referent)	0.014(-0.02, 0.049)	0.052(0.018, 0.085)	0.009(0.003, 0.014)	0.003
DBP	≤74 mmHg	74 to 83 mmHg	>83 mmHg		
ΔAge	0(referent)	-0.172(-2.711, 2.367)	0.001(-2.546, 2.548)	-0.029(-0.799, 0.741)	0.993
Ageing rate	0(referent)	-0.001(-0.036, 0.034)	0.001(-0.034, 0.035)	-0.001(-0.01, 0.011)	0.966
PP	≤53 mmHg	53 to 66 mmHg	>66 mmHg		
ΔAge	0(referent)	1.283(-1.138, 3.705)	4.864(2.472, 7.256)	1.221(0.696, 1.745)	<0.001
Ageing rate	0(referent)	0.011(-0.022, 0.045)	0.061(0.028, 0.094)	0.016(0.008, 0.023)	<0.001
MAP	≤93 mmHg	93 to 105 mmHg	>105 mmHg		
ΔAge	0(referent)	0.335(-2.215, 2.886)	2.464(-0.082, 5.009)	0.561(-0.086, 1.207)	0.054

Ageing rate	0(referent)	0.006(-0.03, 0.041)	0.031(-0.004, 0.066)	0.008(-0.001, 0.016)	0.075

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; β, regression coefficients; CI, confidence interval. ΔAge was calculated as residual from regressing DNAm age on chronological age. Ageing rate was calculated as DNAm age divided by chronological age. All models were adjusted for sex, BMI, socioeconomic status, cigarette smoking, alcohol drinking, physical activity, sleep duration, hyperlipidemia, and diabetes.

Table S5. The associations of hypertension with epigenetic age acceleration (N=280).

Models	β (95% CI) of ΔAge	β (95% CI) of aging rate
Crude model for hypertension		
No	0 (referent)	0 (referent)
Yes	3.96 (1.53, 6.40)	0.051(0.02-0.082)
P value	0.002	0.001
Adjusted model for hypertension		
No	0 (referent)	0 (referent)
Yes	3.10 (1.07-5.13)	0.04(0.015-0.065)
P value	0.003	0.002

Abbreviations: ΔAge was calculated as residual from regressing DNAm on chronological age. Aging rate was calculated as the methylation age divided by chronological age. Adjusted models were adjusted for sex, BMI, socioeconomic status, cigarette smoking, alcohol drinking, physical activity, sleep duration, hyperlipidemia, and diabetes.

Table S6. Summary of EWAS-derived differentially methylated region analysis in relation to hypertension (N=280).

DMR location (hg 19)	No. (CpGs)	Mean effect*	P	Nearest gene
chr1:203320223-203320732	10	0.255	0.004	FMOD
chr1:55267046-55267293	8	-0.205	0.019	TTC22
chr2:37423361-37424104	11	-0.187	0.009	CEBPZOS
chr4:57547347-57548290	9	0.222	0.009	HOPX
chr5:68628240-68628856	8	-0.211	0.016	CCDC125
chr5:135416029-135416613	9	-0.18	0.023	MIR886
chr6:32551749-32552453	10	-0.24	0.005	HLA-DRB1
chr6:31650735-31651070	11	0.161	0.014	-
chr7:27183133-27183990	16	0.186	0.002	HOXA5
chr10:81967195-81967666	7	0.255	0.007	LINC00857
chr12:75784855-75785295	9	-0.19	0.018	GLIPR1L2

*Mean effect was estimated from limma models of Beta-values. P values were false discovery rate (FDR) values.

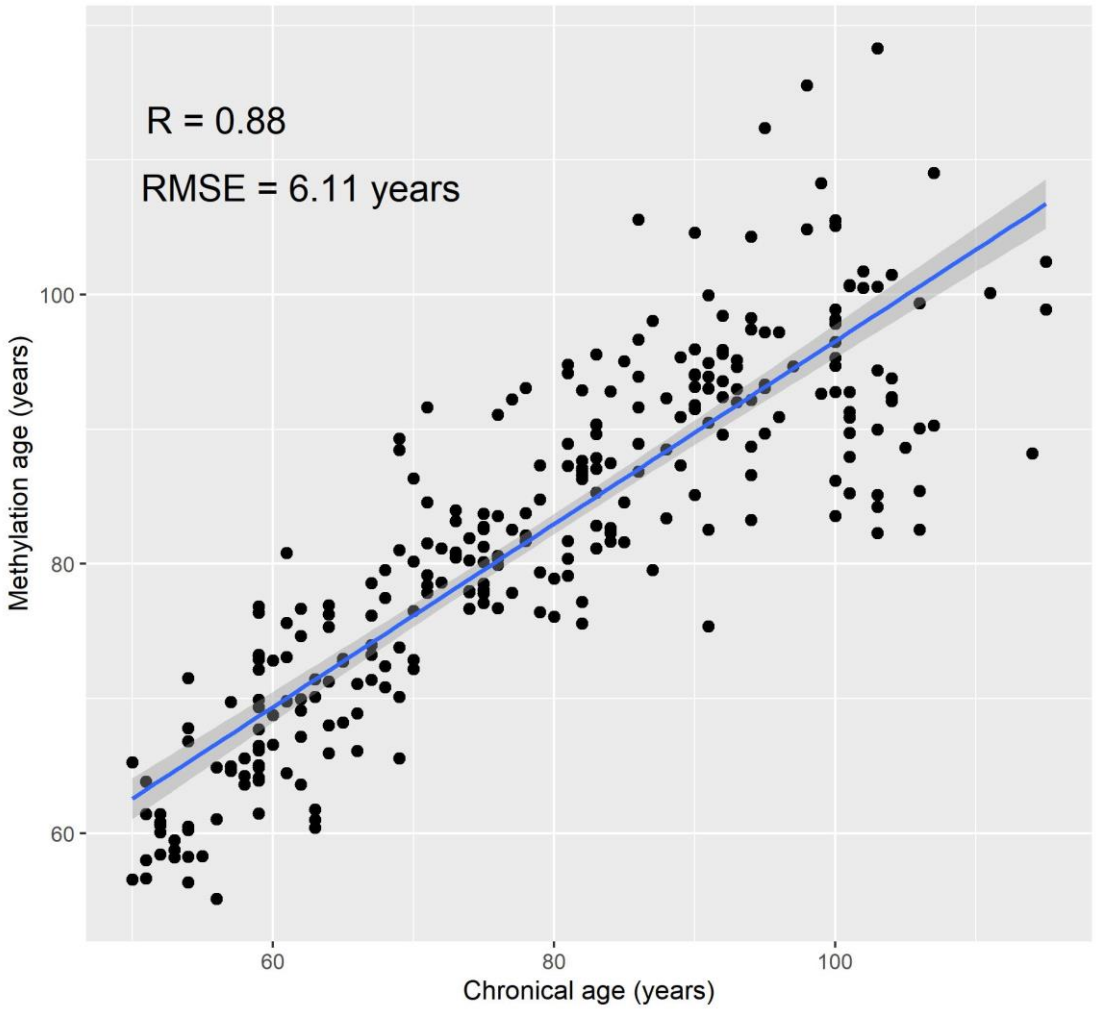

Figure S1. Scatter plot for the correlation between chronological age and Horvath's DNAm age in the elder study population (N=280). R denotes the Pearson's correlation coefficients. RMSE denotes the root-mean-square error.

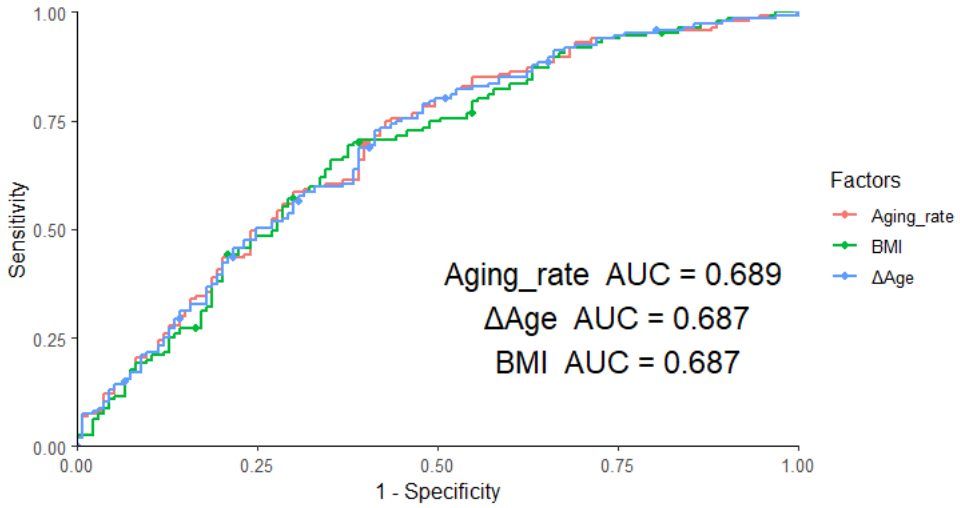

Figure S2. Receiver operating characteristic (ROC) curve analysis for epigenetic age acceleration (ΔAge and aging rate) and BMI to distinguish subjects with hypertension (N=280).