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ORIGINAL RESEARCH

The Role of Lifestyle Behaviors in Shaping the Relationship Between Chronic Pain and Epigenetic Age Acceleration: Evidence From NHANES 1998 to 2002

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Background: Chronic pain is a prevalent and debilitating disorder. However, whether it accelerates aging remains contentious. Epigenetic clocks serve as robust instruments for evaluating biological aging. This study aims to examine the relationship between chronic pain and epigenetic clocks in a nationally representative US sample and discover potential shared systemic mechanisms.

Methods: This research comprised 2,532 individuals aged 50 and above from the 1999–2002 National Health and Nutrition Examination Survey (NHANES) database. Multivariable logistic regression combined with the propensity score matching (PSM) method was employed to investigate the relationship between chronic pain and six epigenetic clocks across three generations.

Results: There was a substantial correlation between chronic pain and epigenetic age acceleration before adjusting for confounders. This association slightly weakened upon adjustment for sociodemographic characteristics, drug uses, and comorbidities, and was utterly nullified after accounting for lifestyle behaviors. In the PSM model, which employed chronic pain as the dependent variable and included all covariates as predictors, no evidence of epigenetic age acceleration was detected. However, in the PSM model that excluded lifestyle behaviors as predictors, individuals experiencing chronic pain showed faster epigenetic age acceleration on GrimAge [$\beta = 0.94$, 95% confidence interval (CI): 0.05 to 1.83] and GrimAge2 [$\beta = 1.14$, 95% CI: 0.17 to 2.10].

Conclusion: Our findings highlight the role of lifestyle behaviors as confounders of the pain-aging relationship as potentially alterable risk factors for epigenetic age acceleration. It provides vital guidance for developing public health strategies to promote healthy aging among chronic pain patients.

Keywords: chronic pain, epigenetic clocks, propensity score matching, GrimAge2, NHANES

Introduction

Chronic pain, defined as pain persisting for three months or longer, is a prevalent and debilitating disorder affecting an estimated 11% to 40% of the global population,[1] resulting in significant disability and socio-economic challenges globally.[2] Advanced age is acknowledged as an independent risk factor for chronic pain,[3] yet the notion that chronic pain accelerates aging remains contentious. Particular academics have noted indicators of expedited aging in individuals with chronic pain, such as reduced life expectancy,[4] elevated mortality rates,[5] shortened telomere lengths,[6] and accelerated brain aging.[7] However, other scholars have not identified such a correlation.[8,9]

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Epigenetic clocks, derived from DNA methylation (DNAm) patterns, effectively quantify an individual's biological age and demonstrate strong correlations with the prevalence of age-related chronic diseases and the risk of age-associated mortality, offering a robust instrument for assessing the biological aging process.[10] The advancement of epigenetic clocks provides novel insights for a comprehensive examination of the correlation between pain and aging. The Yenisel Cruz-Almeida's team[11,12] and Edwin N. Aroke's team[13] identified connections between epigenetic age acceleration and pain-related metrics, such as pain status, intensity, disability, and pain-associated depressive symptoms. However, certain research has indicated that varying pain phenotypes do not result in expedited alterations in epigenetic age.[14] Nonetheless, these cohort studies exhibited limited sample numbers and insufficient population variety for comparative analysis.

Furthermore, numerous studies indicate that confounders linked to chronic pain, including sociodemographics,[15,16] analgesic consumption,[17] comorbidities,[18] and lifestyle behaviors,[19,20] also exert some influence on the epigenetic clock. A comprehensive examination of pertinent confounders in the relationship between chronic pain and epigenetic aging could facilitate the discovery of shared systemic mechanisms that may serve as targets for intervention or treatment. Additionally, researchers have recently developed an enhanced version of GrimAge, termed GrimAge2. GrimAge2 has demonstrated enhanced efficacy in forecasting mortality rates across multi-ethnic groups and age-related disorders relative to GrimAge.[21] To our knowledge, no research has yet employed GrimAge2, this potent epigenetic clock, to investigate the correlation between chronic pain and epigenetic age acceleration. Therefore, the objective of this study was to employ the National Health and Nutrition Examination Survey (NHANES) database, incorporating three generations of six epigenetic clocks, including GrimAge2, to thoroughly examine the intricate relationships between chronic pain and epigenetic clocks in a nationally representative US sample, with the intent of identifying potential shared systemic processes and determining the most suitable chronic pain-related epigenetic clocks.

Methods

Study Population

NHANES is a significant public health survey administered by the National Centre for Health Statistics (NCHS) that provides essential data for analyzing the health and nutritional status of the US non-institutionalized population. NHANES utilizes a stratified multistage sampling technique to acquire a representative sample of US inhabitants. All participants submitted written informed permission at the time of recruitment. This study utilized de-identified NHANES data, and the Ethics Committee of the First Affiliated Hospital of Fujian Medical University exempted further Institutional Review Board review of these data, adhering to the guidelines of the US Department of Health and Human Services.[22] This study used data from two successive NHANES cycles (1999–2000, 2001–2002) to examine the relationship between chronic pain and epigenetic clocks. Participants were selected based on the availability of both blood DNAm data and completion of the Miscellaneous Pain Questionnaire (MPQ). The final cohort comprised 2,532 individuals aged 50 or older, with the recruitment process illustrated in Figure 1.

Assessment of Chronic Pain

Information regarding the duration and location of self-reported pain was gathered from respondents aged 20 and older through a series of home interview questions. Participants who had endured pain exceeding 24 hours in the preceding month were subsequently inquired about the duration of their discomfort. The 11th version of the International Classification of Diseases (ICD-11) defines chronic pain as pain persisting for over three months.[23] Consequently, participants enduring discomfort for over three months (MPQ100 = 1, MPQ110 = 3 or 4) were designated as the chronic pain group, and the remainder were classified as the non-chronic pain group.

Epigenetic Age and Epigenetic Age Acceleration

Samples from individuals aged 50 and above who had undergone whole-blood DNA purification, were obtained for DNAm analysis. The analysis was conducted at Duke University laboratory using Illumina EPIC microbead chip arrays. Raw methylation data in IDAT format was processed for preprocessing and normalization. Details of the DNAm

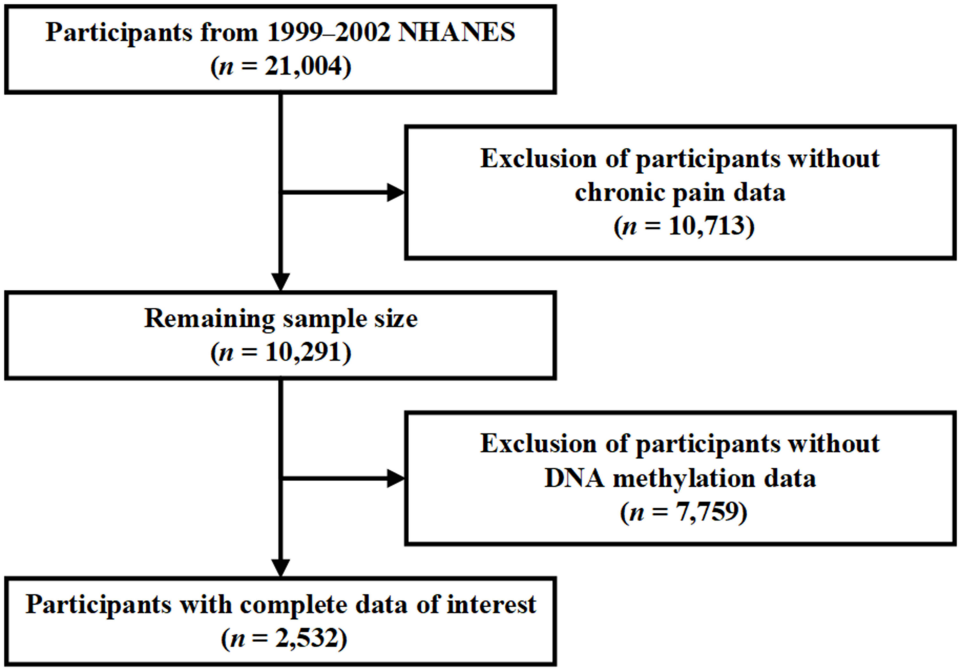

Figure 1 Flow chart of participants selection.
Abbreviation: NHANES, National Health and Nutrition Examination Survey.

epigenetic clocks are documented in the DNA Methylation Array and Epigenetic Biomarkers Data Documentation. We calculated six epigenetic clocks across three generations, comprising the first generation (Horvath's epigenetic age,[24] Hannum's epigenetic age[25]), the second generation (PhenoAge,[26] GrimAge,[27] GrimAge2),[21] and the third generation [Dunedin Pace of Aging methylation (DunedinPoAm)[28]]. For the first and second generations, we determined age-adjusted epigenetic age acceleration, which is the residual from the linear regression of epigenetic age on chronological age. This algorithm encompassed Horvath's epigenetic age acceleration, Hannum's epigenetic age acceleration, PhenoAge acceleration (PhenoAgeAccel), GrimAge acceleration (GrimAgeAccel), and GrimAge2 acceleration (GrimAge2Accel), all quantified in units of years. Epigenetic age acceleration, rather than raw epigenetic age, is more strongly linked to the risk of age-related disorders and mortality. The difference between an individual's epigenetic age and chronological age may better predict health outcomes related to aging. Therefore, we focused on the correlation between chronic pain and epigenetic age acceleration.

Covariate Information

This study categorized the covariates that may affected the relationship between chronic pain and epigenetic clocks into sociodemographic characteristics, drug uses, comorbidities, and lifestyle behaviors. Sociodemographic characteristics included age (years); sex (male and female); ethnicity (non-Hispanic Black, non-Hispanic White, Mexican American, and other races); education level (less than high school, high school graduate, some college, and college graduate); poverty income ratio (PIR; PIR < 1, and PIR ≥ 1); and marital status (married and other status). Drug uses included analgesic use and antidepressant use. Analgesic use was defined as taking prescription or over-the-counter pain relievers almost daily for a month, and antidepressant use was defined as taking at least one prescribed antidepressant medication in the past 30 days. Comorbidities encompassed hypertension, diabetes, heart disease, cancer, asthma, and arthritis. Hypertension, diabetes, cancer, asthma, and arthritis were characterized as conditions diagnosed by a physician. Heart disease was characterized by a diagnosis of congestive heart failure, coronary heart disease, angina, or myocardial infarction, any of which qualified as a kind. Lifestyle behaviors encompassed smoking status (never: < 100 cigarettes/life; former: ≥ 100 cigarettes/life and ceased smoking; current: ≥ 100 cigarettes/life and still smoking); alcohol consumption (non-drinker: < 12 alcoholic beverages/year; drinker: ≥ 12 alcoholic beverages/year); body mass index

(BMI; underweight: < 18.5 kg/m², normal weight: 18.5–24.9 kg/m², overweight: 25.0–29.9 kg/m^2, and obese: ≥ 30.0 kg/m^2); and physical activity (PA). The PA level was assessed based on participants' involvement in moderate and vigorous physical activities during leisure time over the preceding 30 days. It was divided into no PA, insufficient PA for those under 150 minutes per week, and sufficient PA for those over 150 minutes per week.[29]

Statistical Analyses

Statistical analyses were performed using R (version 4.3.3). Due to the intricate multistage probability sampling design of NHANES, all analyses utilized weights, strata, and primary sampling units as stipulated by the NHANES Analytic Guidelines. A small percentage of participants in this study displayed missing data for variables. Examining the absent data types with the R's "mi" package (version 1.1) revealed a "Clustered by missingness" pattern, suggesting that the data deficiency was not wholly random. Therefore, we employed the Multiple Imputation by Chained Equations method to generate multiple imputations of the missing data across five separate datasets, with each interpolated dataset analyzed independently and consolidated the results.

Categorical variables were illustrated as weighted percentages, while continuous variables were presented as means with standard deviations. Categorical variables were compared using Chi-squared tests, while continuous variables were compared using the Complex Samples General Linear Model. Five weighted linear regression models were developed to evaluate the relationship between chronic pain and epigenetic clocks. Model 1 was the crude model. Model 2 incorporated adjustments for sociodemographic characteristics derived from Model 1. Model 3 further accounted for drug uses building upon Model 2. Model 4 enhanced Model 3 by incorporating adjustments for comorbidities. The final fully adjusted model (Model 5) enhanced Model 4 by incorporating lifestyle behavior variables.

To enhance the validity of the results, we employed the propensity score matching (PSM) method for sensitivity analysis. We fitted two PSM models, each utilizing chronic pain as the dependent variable but encompassing distinct predictor variables. PSM Model 1 encompassed all covariates, including sociodemographic characteristics, drug uses, comorbidities, and lifestyle behaviors as predictors, whereas PSM Model 2 excluded lifestyle behaviors as predictors. We calculated each participant's propensity score using multivariable logistic regression and matched chronic and non-chronic pain participants at a 1:1 ratio using nearest neighbor matching with a caliper width of 0.2 standard deviations. Balance was assessed by the absolute standardized mean difference (ASMD), with an ASMD value less than 0.1 signifying adequate covariate balance.[30] Simultaneously, we produced kernel density estimation to visualize the distribution of propensity scores before and after matching. Considering NHANES's complex sample design, we created a new sampling weight by dividing each participant's weight by the study's average weight and incorporated it into the analysis. These PSM procedures were performed using R's "MatchIt" package (version 4.5.5).

Results

Characteristics of the Study Population

The study encompassed 2,532 individuals, with 396 assigned to the chronic pain cohort and 2,136 to the non-pain cohort (Table 1). Notable differences in baseline characteristics between the two groups were evident, as indicated by their ASMD exceeding 0.1. The most pronounced discrepancies were in the prevalence of arthritis (ASMD = 0.629), followed by obesity (ASMD = 0.355), and the use of analgesics (ASMD = 0.269). Chronic pain sufferers tended to be younger, have a lower income ratio, exhibit higher rates of analgesic antidepressant usage, exhibit obesity, engage in minimal PA, smoke currently, and suffer from comorbidities associated with hypertension, cardiovascular disease, and arthritis (all $P < 0.05$).

Association Between Chronic Pain and Epigenetic Age Acceleration

Table 2 presents a series of weighted linear regression models that explore the link between chronic pain and six epigenetic clocks across three generations. No significant differences were identified between chronic pain and the first-generation clocks (Horvath and Hannum) and the second-generation PhenoAge clock in any of the models. By contrast, individuals with chronic pain were observed to have significantly faster epigenetic age acceleration on GrimAgeAccel (β

Table 1 Baseline Characteristics of the Study Population by Chronic Pain Status (n = 2532)

Variables	With Chronic Pain (*n* = 396)	Without Chronic Pain (*n* = 2196)	Standardized Mean Differences	*P*
Sociodemographic				
Chronological age (years)	62.02 ± 9.50	63.84 ± 10.33	0.192	0.014
Sex (%)				0.170
Female	56.9	52.5	0.089	
Male	43.1	47.5	−0.089	
Race (%)				0.265
Mexican American	5.2	5.9	−0.032	
Non-Hispanic Black	12.2	12.2	0	
Non-Hispanic White	71.4	66.5	0.108	
Other	11.2	15.4	−0.133	
Education level (%)				0.297
College graduate	17.4	22.3	−0.129	
High school graduate	24.9	25.7	−0.018	
Less than high school	32.1	30.2	0.040	
Some college	25.6	21.8	0.087	
Marital status (%)				0.585
Married	63.8	65.4	−0.033	
Other	36.2	34.6	0.033	
PIR (%)				0.008
< 1	18.0	12.8	0.134	
≥ 1	82.0	87.2	−0.134	
Drug uses				
Analgesics (%)	45.9	32.9	0.269	< 0.001
Antidepressants (%)	18.0	9.0	0.265	< 0.001
Comorbidities				
Hypertension (%)	50.6	43.4	0.144	0.049
Diabetes (%)	16.5	13.1	0.091	0.154
Heart disease (%)	22.7	14.5	0.195	0.021
Cancer (%)	14.9	15.3	−0.011	0.889
Asthma (%)	14.5	11.2	0.093	0.081
Arthritis (%)	64.3	34.2	0.629	< 0.001
Lifestyle behaviors				
BMI (%)				< 0.001
Normal	21.1	29.8	−0.213	
Obese	47.7	30.0	0.355	
Overweight	29.4	39.4	−0.218	
Underweight	1.8	0.8	0.070	
Physical activity (%)				0.004
No	56.7	46.4	0.208	
Insufficient	7.1	5.3	0.072	
Sufficient	36.2	48.3	−0.252	
Smoking status (%)				0.006
Never	36.3	46.3	−0.207	
Current smoker	24.8	15.2	0.222	
Former smoker	38.9	38.5	0.008	
Alcohol consumption (%)				0.385
Drinker	62.0	65.0	−0.062	
Non-drinker	38.0	35.0	0.062	

Abbreviations: PIR, the ratio of family income to poverty; BMI, body mass index.

Table 2 Weighted Linear Regression Analysis of the Association Between Chronic Pain and Epigenetic Age Acceleration (n = 2532)

Epigenetic Clocks	Model 1	Model 2	Model 3	Model 4	Model 5
	β (95% CI)				
ΔHorvath	0.10 (−0.71, 0.91)	0.12 (−0.70, 0.95)	0.05 (−0.74, 0.84)	0 (−0.88, 0.88)	−0.07 (−1.79, 1.65)
ΔHannum	0.06 (−1.02, 1.13)	0.12 (−0.91, 1.16)	0.05 (−0.94, 1.04)	0.05 (−1.08, 1.18)	−0.06 (−2.22, 2.10)
ΔPhenoAge	1.00 (−0.19, 2.19)	0.93 (−0.24, 2.10)	0.79 (−0.28, 1.86)	0.64 (−0.63, 1.91)	0.33 (−2.12, 2.78)
ΔGrimAge	1.46 (0.65, 2.27)[a]	1.38 (0.58, 2.18)[b]	1.30 (0.53, 2.08)[b]	1.15 (0.27, 2.03)[c]	0.44 (−0.70, 1.59)
ΔGrimAge2	1.67 (0.81, 2.52)[a]	1.56 (0.73, 2.40)[a]	1.46 (0.65, 2.27)[b]	1.24 (0.30, 2.19)[c]	0.44 (−0.89, 1.77)
DunedinPoAm	0.03 (0.01, 0.04)[b]	0.02 (0.01, 0.04)[c]	0.02 (0, 0.04)[c]	0 (0, 0.04)[c]	0.01 (−0.02, 0.03)

Notes: Model 1: unadjusted model; Model 2: adjusted for sociodemographic characteristics, including gender, chronological age, race, education level, marital status, and the ratio of family income to poverty; Model 3: adjusted for sociodemographic characteristics and drug uses, including gender, chronological age, race, education level, marital status, the ratio of family income to poverty, analgesic use, and antidepressant use; Model 4: adjusted for sociodemographic characteristics, drug uses, and comorbidities, including gender, chronological age, race, education level, marital status, the ratio of family income to poverty, analgesic use, antidepressant use, hypertension, diabetes, heart disease, cancer, asthma, and arthritis; Model 5: adjusted for sociodemographic characteristics, drug uses, comorbidities, and lifestyle behaviors; including gender, chronological age, race, education level, marital status, the ratio of family income to poverty, analgesic use, antidepressant use, hypertension, diabetes, heart disease, cancer, asthma, arthritis, body mass index, physical activity, smoking status, and alcohol consumption. ΔHorvath: Horvath's epigenetic age acceleration; ΔHannum: Hannum's epigenetic age acceleration; ΔPhenoAge: PhenoAge acceleration; ΔGrimAge: GrimAge acceleration; ΔGrimAge2: GrimAge2 acceleration. [a]$P < 0.001$; [b]$P < 0.01$; [c]$P < 0.05$.

Abbreviations: CI, confidence interval; DunedinPoAm, Dunedin Pace of Aging methylation.

= 1.46, 95% CI: 0.65 to 2.27, $P < 0.001$), GrimAge2Accel (β = 1.67, 95% CI: 0.81 to 2.50, $P < 0.001$), and DunedinPoAm (β = 0.03, 95% CI: 0.01 to 0.04, $P < 0.01$) in the crude model compared to those without chronic pain. However, when incremental adjustments were made for sociodemographic characteristics, drug uses, and comorbidities, the strength of these associations weakened. In Model 4, the association was reduced to 1.15 years for GrimAgeAccel (95% CI: 0.27 to 2.03, $P < 0.05$), 1.24 years for GrimAge2Accel (95% CI: 0.30 to 2.19, $P < 0.05$), and the acceleration for DunedinPoAm became nearly negligible (95% CI: 0 to 0.04, $P < 0.05$). Finally, further adjustments for lifestyle behavior variables in Model 5 completely attenuated the observed associations across all epigenetic clocks.

Sensitivity Analyses

The PSM Model 1 matched 389 pairs (totaling 778 individuals) between those with chronic pain and those without, while PSM Model 2 matched 395 pairs (totaling 790 individuals). Both PSM models achieved a balanced distribution across all covariates, as demonstrated by the kernel density plots in eFigure 1, which delineates the distribution of propensity scores before and after matching, alongside the sample characteristics and standardized mean differences detailed in eTable 1 and eTable 2.

Figure 2 presents the correlations between chronic pain and six epigenetic clocks across three generations after matching. In PSM Model 1, there was no significant correlation between chronic pain and any of the epigenetic clocks. However, in PSM Model 2, individuals experiencing chronic pain showed faster epigenetic age acceleration on GrimAgeAccel (β = 0.94, 95% CI: 0.05 to 1.83) and GrimAge2Accel (β = 1.14, 95% CI: 0.17 to 2.10). Notably, the association between chronic pain and DunedinPoAm did not reach statistical significance.

Discussion

This study employed multivariable logistic regression combined with the PSM method to investigate the presence of epigenetic age acceleration in middle-aged and older Americans suffering from chronic pain between 1999 and 2002. Our findings yielded two significant insights. Firstly, no correlation was observed between chronic pain and epigenetic age acceleration after controlling for sociodemographic characteristics, drug uses, comorbidities, and lifestyle behaviors, with lifestyle behaviors identified as potentially modifiable risk factors. Secondly, among the various epigenetic clocks developed, the GrimAge2 clock demonstrated the highest sensitivity to epigenetic alterations linked to chronic pain.

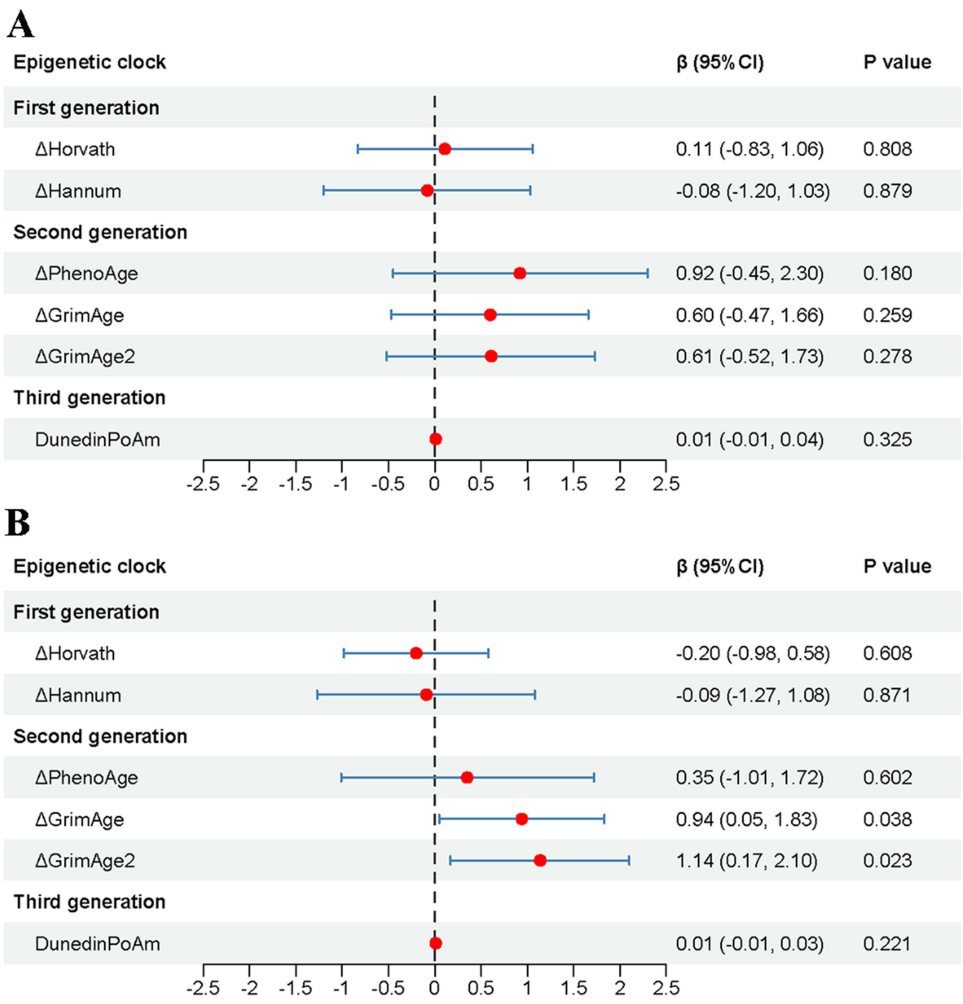

Figure 2 Forest plots of the association between chronic pain and epigenetic age acceleration after matching in different models.
Notes: (**A**) PSM Model 1 (*n* = 778): employed chronic pain as the dependent variable and encompassed sociodemographic characteristics, drug uses, comorbidities, and lifestyle behaviors as predictors; (**B**) PSM Model 2 (*n* = 790): employed chronic pain as the dependent variable and excluded lifestyle behaviors as predictors. ΔHorvath: Horvath's epigenetic age acceleration; ΔHannum: Hannum's epigenetic age acceleration; ΔPhenoAge: PhenoAge acceleration; ΔGrimAge: GrimAge acceleration; ΔGrimAge2: GrimAge2 acceleration.
Abbreviations: PSM, propensity score matching; CI, confidence interval; DunedinPoAm, Dunedin Pace of Aging methylation.

The current study demonstrated notable differences between the chronic pain and non-chronic pain cohorts for various baseline variables. Patients with chronic pain were typically younger, possessed a lower socioeconomic status, were more likely to take analgesics and antidepressants, engaged in less healthy lifestyle behaviors, and were accompanied by related comorbidities. These findings were primarily congruent with prior epidemiological research on chronic pain, aside from the age covariate.[31–34] Chronic pain is more prevalent in the elderly compared to younger individuals; however, this study focused on middle-aged and older participants aged 50 and above, rendering the age difference clinically insignificant. Epigenetic aging, like chronic pain, is a multifaceted process shaped by various internal and external factors. Research on chronic pain and epigenetic aging may uncover shared systemic mechanisms that could be targeted for intervention or treatment.

Consequently, the selection of covariates in the current study was undertaken more remarkably than in prior research, including the study by Edwin N. Aroke's team,[13] which adjusted solely for gender, race, and BMI, and the recent study by Yenisel Cruz-Almeida's team,[35] which overlooked comorbidities in the elderly population. Furthermore, in contrast to other research, the current study employed the PSM method combined with multivariable regression correction to mitigate selection bias from confounding factors. In observational studies, the PSM method is superior to traditional regression adjustment as it mitigates confounding by employing propensity scores independent of the study outcome.

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This distinction allows for a more precise separation between study design and analysis, thereby more accurately simulating a randomized controlled trial. At the same time, traditional regression adjustment may involve subjective decisions regarding the variable selection and model specification.[36] In our study, sensitivity analysis enhanced the reliability of the findings, the attenuation of the association underscores the significance of lifestyle behaviors in the link between chronic pain and epigenetic age acceleration as potentially alterable risk factors.

Prior research has demonstrated a biological connection between chronic pain and epigenetic aging. Epigenetic alterations, especially DNAm, may influence injury and pain perception by modulating the expression of pro-injurious and anti-injurious genes within the injury pathway;[37] simultaneously, accelerated DNAm reprogramming may contribute to the chronicity of pain.[38] Additionally, persistent stress and neuroinflammation linked to chronic pain may promote DNA damage and cellular senescence accumulation, expediting epigenetic aging.[39] Our research indicated that, alongside direct biological connections, confounding variables, particularly lifestyle behaviors, significantly influence the relationship between chronic pain and epigenetic aging. This discovery aligned with recent research indicating that epigenetic aging is a multifaceted biological phenomenon intricately linked to the subjective experience of pain and influenced by an amalgamation of biological, psychological, social, and environmental elements.[40] Lifestyle behaviors offer a more accessible target for change among chronic pain patients than other confounders, particularly regarding unhealthy behaviors such as smoking, alcohol abuse, physical inactivity, and obesity.[41] Consequently, this study provides vital guidance for developing personalized public health interventions to promote healthy aging among patients with chronic pain.

This study assessed the link between chronic pain and three generations of epigenetic clocks, our findings revealed that the second-generation clock, GrimAge, and its derivative, GrimAge2, exhibited heightened sensitivity to pain-related epigenetic alterations, supporting the findings of prior researchers.[11,35] Developed using 1,030 distinct CpG sites near genes in various gene sets, including MHC class II genes, cytokine-mediated signaling pathways, and genes from the GO, KEGG, and PANTHER databases, the GrimAge-associated clock covers more CpG sites than other epigenetic clocks. The genes adjacent to these sites involve a wider range of biological processes and functions. Additionally, the GrimAge-associated clock also shows a stronger connection with mental mood fluctuations, such as autism[42] and depression,[43] suggesting its potential effectiveness in comprehending the epigenetic aging associated with chronic pain, which is influenced by a complex interplay of biological, psychological, social, and environmental factors. More importantly, our study demonstrated for the first time that GrimAge2 surpasses the original GrimAge in evaluating epigenetic alterations in chronic pain. GrimAge2 employs the identical 1030 CpG sites and, in addition to the original GrimAge's incorporation of chronological age, female sex indicator, and eight DNAm biomarkers, add two novel biomarkers: high-sensitivity C-reactive protein and hemoglobin A_1C. These additional biomarkers enhance the assessment of an individual's biological age and health status, potentially leading to a more robust correlation with age-related phenotypes. Further research is needed to fully evaluate GrimAge2's role in epigenetic changes associated with chronic pain.

Our work possesses several features. Firstly, in the study design, we meticulously examined the intricate relationship between chronic pain and epigenetic clocks, and the selection of covariates was rigorously evaluated, rendering it more complete than prior studies. For the first time, this work incorporated six epigenetic clocks spanning three generations, including the novel GrimAge2 clock, thereby enhancing the data available for elucidating the connection between chronic pain and epigenetic aging. We employed multivariable logistic regression combined with the PSM method, thereby augmenting the robustness and trustworthiness of our findings. Indeed, our study has several limitations. Like other cross-sectional studies, this research can only establish associations, not causations. The connection between chronic pain and epigenetic age acceleration is probably bidirectional, necessitating future longitudinal investigations. Additionally, despite the study accounting for biological, social, and environmental confounders, unrecognized or uncontrolled confounders influencing the outcomes may still exist, such as mental health issues. The 1999–2002 NHANES exclusively evaluated the mental health condition of adults aged 20 to 39, and the lack of mental health data may influence our assessment of the relationship between chronic pain and epigenetic clocks in the middle-aged and older demographic. In addition, the NHANES database's limitations precluded the evaluation of more granular data concerning pain duration, pain intensity, and functional interference, thus constraining our capacity to investigate the

dose-response relationship between chronic pain and accelerated epigenetic aging. Future investigations on the dose-response relationship are essential. Furthermore, for the third-generation epigenetic clock, the existing NHANES database provides data only on DunedinPoAm, limiting our ability to examine it with the more advanced Dunedin Pace of Aging Calculated from the Epigenome (DunedinPACE) clock. Subsequent research on chronic pain and DunedinPACE is essential.

Conclusions

In middle-aged and older persons in the United States suffering from chronic pain, lifestyle behaviors serve as confounders in the pain-aging association and represent modifiable risk factors for epigenetic age acceleration. This offers essential direction for formulating public health policies to enhance healthy aging in individuals with chronic pain. Moreover, among the various epigenetic clocks developed, the GrimAge2 clock has the most sensitivity to epigenetic alterations linked to chronic pain, positioning it as a promising biomarker in the epigenetic investigation of pain.

Data Sharing Statement

The datasets generated and/or analyzed for this study can be found in the NHANES website (https://www.cdc.gov/nchs/nhanes/).

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Disclosure

The authors report no conflicts of interest in this work.

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