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Epigenetic age predictors in community-dwelling adults with high impact knee pain

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Abstract

Gerontological research reveals considerable interindividual variability in aging phenotypes, and emerging evidence suggests that high impact chronic pain may be associated with various accelerated biological aging processes. In particular, epigenetic aging is a robust predictor of health-span and disability compared to chronological age alone. The current study aimed to determine whether several epigenetic aging biomarkers were associated with high impact chronic pain in middle to older age adults (44–78 years old). Participants (n = 213) underwent a blood draw, demographic, psychosocial, pain and functional assessments. We estimated five epigenetic clocks and calculated the difference between epigenetic age and chronological age, which has been previously reported to predict overall mortality risk, as well as included additional derived variables of epigenetic age previously associated with pain. There were significant differences across Pain Impact groups in three out of the five epigenetic clocks examined (DNAmAge, DNAmPhenoAge and DNAmGrimAge), indicating that pain-related disability during the past 6 months was associated with markers of epigenetic aging. Only DNAm-PhenoAge and DNAmGrimAge were associated with higher knee pain intensity during the past 48 h. Finally, pain catastrophizing, depressive symptomatology and more neuropathic pain symptoms were significantly associated with an older epigenome in only one of the five epigenetic clocks (i.e. DNAmGrimAge) after correcting for multiple comparisons (corrected p's < 0.05). Given the scant literature in relation to epigenetic aging and the complex experience of pain, additional research is needed to understand whether epigenetic aging may help identify people with chronic pain at greater risk of functional decline and poorer health outcomes.

Keywords

Biological aging, epigenetic aging, chronic pain, pain biomarker, aging biomarker

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Introduction

Osteoarthritis (OA) is a common and disabling condition among older adults, with the knee being the most commonly affected joint.[1] Lifetime risk of developing symptomatic knee OA exceeds 40%, and OA prevalence in the U.S. population is rising.[1,2] With the number of older adults over 65 expected to double from 40 to 88 million by 2050, the health concerns related to OA for middle-aged and older individuals and society at-large will significantly increase in the coming decades.

Emerging evidence suggests that high impact chronic pain may be associated with various accelerated biological aging processes.[3–5] Chronological aging is easily determined by birth date; however, there is substantial inter-individual variability in functional and physiological integrity during aging, whereby individuals with the same *chronological* age may express a different *biological* age. Biological aging processes using previously described candidate hallmarks of aging appear to be better predictors of functional decline, health-span and ultimately mortality than chronological age alone.[6] Specifically, epigenetic alterations with age have been shown to be powerful predictors of an individuals' biological age and can be easily measured in humans. Epigenetic clocks derived from high-throughput, high-resolution DNA methylation data, have enabled the construction of extremely accurate age estimators, termed "Epigenetic clocks" or "DNA methylation clocks". Specifically, our own preliminary work supported associations between chronic pain and accelerated epigenetic aging in community-dwelling older individuals.[5] However, the study sample size was relatively small and lacked diversity in several important demographic factors. However, a follow-up study by Kwiatkowska and colleagues,[7] failed to find any associations between epigenetic aging and pain variables across various pain cohorts. Instead, they reported that pain variables were associated with other measures derived from the methylation analyses (i.e. CD8+ T cell counts, naive CD4+ T cell counts, natural killer cell counts, GDF15, leptin, and DNA methylation-derived telomere length). In our previous study, we examined only the initial epigenetic clock derived by Horvath,[8] and no other newly studied epigenetic clocks nor individual measures, which may be more relevant to pain and aging. Therefore, the present investigation was designed to test differences across epigenetic aging clocks and other measures previously studied in relation to chronic pain within a larger, more racially diverse sample of middle- and older-aged community-dwelling individuals with varying levels of knee pain impact (i.e. knee pain with/without pain interference), and controls without chronic pain. Our primary hypothesis was that individuals with high impact knee pain will have accelerated epigenetic aging using five epigenetic clocks and derived variables, compared to those with low impact pain and pain-free controls. In addition, we tested associations between the epigenetic variables with clinical pain characteristics, as well as psychological, somatosensory and physical and cognitive function consistent with the biopsychosocial conceptualization of chronic pain.[9]

Methods

Participants

Participants were adults between the ages of 45–85 with and without knee pain recruited from the University of Florida (UF; Gainesville, Florida, USA) and the University of Alabama at Birmingham (UAB; Birmingham, Alabama, USA). Individuals who self-identified as (1) non-Hispanic, and (2) "African American/Black" or "White/Caucasian/European", and (3) English speaking, were eligible for inclusion. Individuals were excluded if they reported: (1) significant surgery to the index (i.e. most painful knee (e.g. total knee replacement surgery); (2) cardiovascular disease or history of acute myocardial infarction; (3) uncontrolled hypertension (blood pressure >150/95 mmHg); (4) systemic rheumatic diseases (e.g. rheumatoid arthritis, systemic lupus erythematosus, and fibromyalgia); (5) neuropathy; (6) chronic opioid use; (7) serious psychiatric illness; (8) neurological disease (e.g. Parkinson's, multiple sclerosis, stroke with loss of sensory or motor function, or uncontrolled seizures); (9) pregnant; (10) significantly greater pain in a body site other than the knee. All participants provided written informed consent and the study was IRB approved (IRB201400209) and conducted with accordance with the Declaration of Helsinki.

Procedures

Demographic information including age, ethnicity/race, and sex were self-reported during initial phone screening. Eligible individuals were scheduled for a Health Assessment Session (HAS), at which informed consent was obtained prior to study procedures. A health history and pain history, and physical exam were conducted during the HAS including an X-ray to obtain a Kellgren-Lawrence scores in both knees. Approximately 1 week later, participants attended a quantitative sensory testing (QST) session. Blood draws occurred prior to QST testing. Clinical pain measures were collected within 24 h preceding the QST session.

Study measures

Clinical pain

Graded chronic pain scale (GCPS). The GCPS is a robust, validated, self-reported questionnaire that measures two dimensions of chronic pain severity: pain intensity and pain-related disability.[10] The questionnaire consists of seven items, with six scored on an 11-point Likert scale asking participants to report their current, and average and worst pain over the last 6 months (i.e. 0 = "no pain" to 10 = "pain as bad as it can

be"), and how much pain has interfered with daily activities, recreation/social/family activities, and ability to work (i.e. 0 = "no interference" to 10 = "unable to carry out activities"). Scores are then calculated for the two subscales: characteristic pain intensity is calculated as the mean intensity ratings for the current, worst and average pain multiplied by 10; and the pain-related disability score, which is calculated as the mean rating for difficult performing daily, social and work-related activities multiplied by 10, with each score ranging from 0–100. One open-ended question asks participants to report "how many days in the last 6 months have you been kept from your usual activities because of pain" (i.e. disability days). Higher scores indicate greater pain and pain-related disability. To operationalize knee pain impact groups, we employed the GCPS pain grades as previously reported,[10] where pain free controls had a GCPS Grade 0; participants with GCPS Grades 1–2 were classified as low impact pain; and those with Grades >3 were assigned to the high impact pain category.

WOMAC-pain. The Western Ontario and McMaster Universities Osteoarthritis Index pain subscale (WOMAC-Pain) assesses lower extremity pain experienced in the past 48 h in relation to five common activities (e.g. going down stairs, walking on a flat surface), using a 5-point Likert type scale ranging from 0 "none" to 4 "extreme".[11] Higher scores indicate greater pain severity during these activities.

Pain-DETECT. The pain-DETECT assesses the quality, pattern, and radiation of pain and was developed for the purpose of identifying neuropathic-type pain.[12] The pain-Detect consists of nine items with possible scores ranging from −1 to 38, with higher scores indicating possible neuropathic-like pain. While originally developed for individuals with low back pain, it has been successfully used in OA populations.[13,14]

# of painful sites. Participants were asked to report if they experienced pain across 14 body sites, including hands, arms, neck, shoulders, head/face/jaw, chest, stomach, pelvis, upper back, lower back, knees, legs, feet, and/or one 'other' body region (i.e. open response). Participants were able to report pain unilaterally or bilaterally, with possible scores ranging from 0 to 28.

Experimental pain

Consistent with our prior work,[15] experimental pain was assessed using a standardized multimodal quantitative sensory testing (QST) battery. QST procedures occurred in one 2.5 h session. Participants were seated in a comfortable chair with an ambient room temperature and given standardized instructions prior to each test. Full QST procedures have been previously reported.[13]

Heat pain. Heat pain threshold (HPTh) and heat pain tolerance (HPTol) were assessed at the medial joint line of the index (i.e. most painful) knee and on the ipsilateral ventral forearm using a 16 × 16 mm thermode (Medoc Pathway, Ramat Yishai, Israel), that increased at a rate of 0.5°C/sec from a baseline temperature of 32°C. The thermode position was moved between trials to avoid sensitization and/or habituation of cutaneous receptors. HPTh was indicated by participants pressing button when they first experienced pain (HPTh), and preceded HPTol trials. HPTol was indicated by participants pressing a button when they could no longer tolerate the pain. The mean temperature of each of three trials within 3°C for each parameter at each testing site were calculated and Z-transformed. Z-scores from these trials were combined with the Z-transformed average of all heat pain ratings obtained during suprathreshold heat testing (described below) to compute an overall Heat Pain Index, with higher values indicating greater thermal sensitivity.

Heat pain temporal summation. Heat pain temporal summation (TS) was assessed at the same anatomical sites using a contact heat-evoked potential stimulator thermode. Participants were asked to verbally rate their pain at the peak of each suprathreshold heat pulse on a 0 "no pain" to 100 "most intense pain imaginable" numerical rating scale (NRS). Each trial started at a baseline temperature of 35°C and increased at a rate of 20°C/sec to the target temperature (i.e. 44°C, 46°C, or 48°C) in a train of five repetitions for each temperature. The trial was terminated if the participant rated the pain at 100. Participants were also asked to rate their pain 15 and 30-s after the last heat pulse as a measure of heat after-sensations. Heat pain temporal summation (TS) was calculated as the difference between the maximum pain rating and the first stimulus pain rating. Heat TS scores were standardized (i.e. Z-transformed) and combined to form a composite Heat Pain TS Index, with higher values indicating greater temporal summation of heat pain.

Punctate pain. Sensitivity to punctate mechanical stimuli was assessed using a nylon monofilament (Touchtest Sensory Evaluator 6.65) calibrated to bend at 300 g of pressure. Testing was completed at the patella of the index knee and the back of the ipsilateral hand in a randomized order. Participants were asked to verbally rate pain after a single contact, and then after a series of 10 contacts delivered at a rate of 1 contact/second. Pain ratings were made on a 0 "no pain" to 100 "most intense pain imaginable" NRS. The procedure was repeated and ratings over the two single contact trials were averaged separately, Z-transformed, and combined to produce a Punctate Pain Single Index, with higher values indicating greater sensitivity to mechanical stimuli.

Punctate pain temporal summation. To determine temporal summation of punctate mechanical pain, the pain rating for the single contact was subtracted from the pain rating for the series of contacts, and averaged over the two trials separately by site. Averages were then Z-transformed and combined to

form a Punctate Pain TS Index that included both trials at each testing site, with higher values indicating greater mechanical temporal summation.

Pressure pain. Pressure pain threshold was assessed using a digital, handheld pressure algometer (Algomed, Medoc, Ramat Yishai, Israel). Pressure was applied to the medial and lateral joint lines of the index knee, ipsilateral quadriceps, and trapezius muscle, in a randomized order, at a constant rate of 30 kPa/s. Participants were asked to press a button when they first felt pain from the pressure, and the trial terminated. A maximum pressure level of 600 kPa for knee sites, and 1000 kPa for other sites, was set to maintain participant safety. The average of three trials (at each site) within 40 kPa were Z-transformed and combined to calculate a Pressure Pain Index that included all testing sites. Higher values indicated greater pressure pain sensitivity.

Cold pain. Cold pain was rated on a 0–100 NRS during the Conditioned Pain Modulation procedure (see below) at 30 s of hand immersion and immediately upon removing hand from the cold water bath. Pain ratings were averaged, Z-transformed and combined to form a composite Cold Pain Ratings Index, with higher values indicating greater cold pain sensitivity.

Conditioned pain modulation. Conditioned pain modulation (CPM) was determined by assessing the ability of a conditioning stimuli (i.e. cold water bath) to reduce perceived pain of a test stimulus (i.e. algometer delivered pressure pain). First, pressure was applied to the left trapezius (as previously described), and participants indicated when they first felt pressure pain. Next, participants were instructed to place their right hand (opened flat, palm down) into a cold water bath maintained at 12°C using a refrigeration unit (Neslab, Portsmouth, NH, USA). Pressure pain threshold (PPT) was again assessed after 30 s of cold water immersion. After 60 s of immersion, participants were instructed to remove their hand from the water and pressure pain threshold was assessed again. The participant's hand was then covered with a warm pack for 1 min, and the procedure was repeated following a 10-min rest period. CPM-During was calculated as the difference between pre-immersion PPT and PPT at 30 s of cold water immersion; CPM-Post was calculate as the difference between pre-immersion PPT and PPT taken immediately after hand was removed from the cold water. Mean PPT differences were Z-transformed and used for analysis.

Psychosocial function

In vivo coping (IVC). Participants were asked to indicate the degree to which they employed 10 coping strategies during the QST session (i.e. In Vivo Coping).[16] Items were rated on a 5-point Likert type scale ranging from 1 "not at all" to 5 "very much" and consisted of passive (e.g. "I felt that if the pain got any worse I wouldn't be able to tolerate it"), and active (e.g. "I thought of other things to get my mind off of the pain") strategies/cognitive responses to pain. Items for each domain were averaged, with higher scores indicating greater use of those coping strategies.

Somatization. The Patient Health Questionnaire 15-item somatic symptom severity scale (PHQ-15) was used to assess somatic symptom severity.[17] The PHQ-15 was derived from the Patient Health Questionnaire (PHQ) and has shown good reliability and validity. The PHQ-15 includes 15 physical symptoms (e.g. stomach pain, shortness of breath) that are rated on a 0 "not bothered at all" to 2 "bothered a lot" scale, with scores ranging from 0–30, and higher scores indicating greater somatization.

Coping strategies questionnaire-revised. The Coping Strategies Questionnaire-Revised (CSQ-R) consists of 27 items to assess the use of pain coping strategies within six domains: Distraction, Catastrophizing, Ignoring pain sensations, Reinterpreting, Coping self-statements, and Praying.[18,19] Participants were asked to rate the frequency of using specific strategies within each domain on a 0 "never do that" to 6 "always do that" Likert-type scale. Responses within each domain are averaged to produce separate subscales. Active coping was defined as the mean across the Distraction, Ignoring pain sensations, Reinterpreting, and Coping self-statements subscales, and Passive coping was defined as the mean of the Catastrophizing and Praying subscales. Higher scores indicated greater use of that type of coping.

Positive affect negative affect scale. The Positive Affect Negative Affect Scale (PANAS) is a 20-item measure that assesses positive (10 items) and negative (10 items) valence.[20] Participants were asked to rate "to what extent do you generally feel this way" on a 5-point Likert-type scale anchored at 1 "very slightly or not at all" and 5 "extremely". Items are summed to calculate trait positive (PA) and negative affect (NA) subscale scores ranging from 10–50, with higher scores indicating greater PA or NA.

Perceived stress scale. The Perceived Stress Scale (PSS) is a 10-item measure used to assess individuals' perception of stress.[21] Participants were asked to rate statements about their feelings and thoughts during the past month of a 5-point Likert-type scale ranging from 0 "never" to 4 "very often". Higher scores indicated greater perception of stress.

PROMIS measures. Anxiety. The PROMIS Anxiety (7a)[22] consists of seven items that ask participants to rate the frequency with which they experienced emotions such as fear, stress, and anxiety in the past 7 days ("never" to "always"), with higher scores indicating greater anxiety.

Depression. The PROMIS Depression (8b)[22] consists of eight items that ask participants to rate the frequency that they

have experienced feelings of worthlessness, hopelessness, and sadness over the past 7 days on a ("never" to "always") scale, with higher scores indicating greater depression.

Sleep. The PROMIS Sleep-Related Impairment (8b)[22,23] consists of eight items that assess signs of sleep impairment over the prior 7 days (e.g. alertness, tiredness, sleepiness, and functional impairments associated with sleep problems). Items are measured on a 5-point Likert scale ("not at all" to "very much"), with higher scores indicating greater sleep impairment.

Physical and cognitive function

Short physical performance battery. The Short Physical Performance Battery (SPPB) was used as an objective measure of physical function.[24] The SPPB asks participants to complete three tasks to assess function of the lower extremities, including a balance task, a chair stand task, and a gait speed task. Items are scored from 0 (unable to complete) to 4 (highest level of performance), and summed for an overall score ranging from 0–12, with higher scores indicating greater functional ability. In addition, participants were asked to rate the pain experienced during each task (i.e. balance, chair stand, and walking) on a 0 "no pain" to 100 "worst pain imaginable" NRS, for a measure of movement-evoked pain.[25,26] *Cognitive Function*

Montreal cognitive assessment. The Montreal Cognitive Assessment (MoCA) is a brief screening tool used to detect mild cognitive impairment in community-dwelling adults.[27] The MoCA is a 30 question assessment which takes approximately 10 min to administer and tests short-term memory, visuospatial abilities, executive function, language, and orientation. Higher scores indicate better cognitive function.

Blood collection and processing

Blood samples were collected from the forearm or hand vein at the onset of the quantitative sensory testing session and included collection of a 10 mL K^2 EDTA tube that was subsequently was centrifuged at 3000 r/min for 10 min. After separation, the buffy coat was carefully extracted and transferred to a cryovial for −80-degree storage. To isolate genomic DNA, the frozen buffy coat samples were thawed at 37°C to dissolve homogeneously. ∼200 µL (or 150–200 µL) of sample was lysed in R.B.C lysis buffer and centrifuged at 6000 r/min for 5 min at room temperature. The supernatant was discarded and sodium EDTA solution was added to the pellet and vortex gently to remove RBC clumps. Homogenate was incubated at 50–55°C with Proteinase K and SDS solution. Following incubation, equal volume of phenol was added, mixed, and centrifuged at 10,000 r/min for 10 min. Supernatant was transferred in a fresh tube and equal volume of phenol-chloroform-isoamyl alcohol was added, mixed and centrifuged at the same rpm. Again, supernatant was transferred in a fresh tube and equal volume of chloroform-isoamyl alcohol was added followed by centrifugation at same rpm conditions. Supernatant was transferred in a fresh tube and 1/10th volume of 3M sodium acetate along with two volumes of absolute alcohol was added. The precipitated DNA was washed with 70% ethanol by centrifugation at 10,000 r/min for 5 min. The pellet was air dried and dissolved in Tris-EDTA buffer. The dissolved DNA was qubit quantified and visualized on agarose gel for quality assessment. Sodium Bisulfite conversion and EPIC methylation array was performed by Moffitt Cancer Center, Molecular Genomics Core located at 3011 Holly Dr, Tampa, FL 33,612.

Methylation age calculation. Raw methylation idat files of the EPIC arrays were processed using R package *minfi*. The resulting methylation beta values (percentage of methylation for each CpG site) were uploaded to the Horvath epigenetic age calculator Web site, which is publicly accessible at http://dnamage.genetics.ucla.edu. The Normalize Data and the Advanced Analysis options were employed as recommended by the calculator tutorial. Consistent with previous work,[5] we estimated epigenetic-predicted age differences (i.e. epigenetic-predicted age differences = epigenetic-predicted age – chronological age). Five distinct types of predicted epigenetic age were obtained by the epigenetic age calculator:

1. **DNAmAge** originally derived by Horvath, is a pan-tissue epigenetic clock estimated based on 353 CpG sites using samples in virtually all human cell types and tissues, which was used in our previous investigation in older adults;[8]
2. **DNAmAgeHannum** derived by Hannum,[28] estimates methylation based on 71 CpG sites from the whole blood of 656 human individuals aged 19 to 101;
3. **DNAmAgeSkinBloodClock** estimates biological age based on the methylation of 391 CpG sites for human fibroblasts, keratinocytes, buccal cells, endothelial cells, lymphoblastoid cells, skin, blood, and saliva samples also developed by Horvath[8] and showed better prediction of chronological ages than the DNAmAge for the pre-mentioned skin and blood related tissues;
4. **DNAmPhenoAge** is an age estimate of phenotypic age derived from the methylation of 513 CpG sites and is capable of capturing risks of many aging outcomes including all-cause mortality;[29]
5. **DNAmGrimAge** uses an age model built on eight different DNAm-based measures consisting of 1030 unique CpGs for smoking pack-years as well as a selection of plasma proteins (i.e. DNAmADM, DNAmB2M, DNAmCystatinC, DNAmGDF15, DNAmLeptin, DNAmPACKYRS, DNAmPAI1, DNAmTIMP1) that were associated with mortality or morbidity. The term "Grim" represented the better predictive power for time-to-death or time-to-disease event (i.e. grim news) compared to other methylation age clocks.[30]

Finally, additional DNA methylation-based predictions previously reported to be associated with pain in one study[7] were also included in the analysis: CD8+ T cell counts, naive CD4+ T cell counts, natural killer cell counts, GDF15, leptin, and DNA methylation-derived telomere length.

Statistical analysis

All data and statistical analyses were performed using R software. Data were examined for important underlying assumptions for each test (Figure S1). Given the multidimensional level of pain, and the potential complex associations with epigenetic aging, we performed analyses using both nominal (i.e. pain groups) and continuous (i.e. WOMAC-Pain levels) variables.

Epigenetic Age Biomarkers Across Pain Impact Groups. The predicted epigenetic age difference was calculated as the difference between the epigenetic age and the chronological age. This was performed for each of the five epigenetic ages (DNAmAge, DNAmAgeHannum, DNAmAgeSkinBloodClock, DNAmPhenoAge, and DNAmGrimAge), where a larger difference represents an older epigenome. To evaluate whether the epigenetic age biomarkers were associated with pain impact, one-way analysis of covariance (ANCOVA) was conducted, where the epigenetic age biomarker was the outcome variable, the 3-level pain impact (i.e. pain-free controls, low impact pain, and high impact pain) was the predictor, with chronological age, sex, race and study site entered as covariates. To further narrow down which pairs of pain impact groups yielded the most significant contrast in the epigenetic age difference, post-hoc ANCOVAs were further performed by using the Tukey's HSD (honestly significant difference) test.

Epigenetic Age Biomarkers and WOMAC-Pain Levels. To examine the association between each predicted epigenetic age and WOMAC-Pain levels, linear regression models were deployed, where the one of the predicted epigenetic age differences was the outcome variable, the WOMAC-Pain was the predictor, adjusting for chronological age, sex, race and study site.

Epigenetic Age Biomarkers and Other Clinical Variables. To examine the association of epigenetic age with clinical variables reflecting multiple domains relevant to the study of pain using a biopsychosocial framework (i.e. clinical pain, experimental pain, cognitive function, physical function, and psychosocial function), partial correlation analyses were utilized, adjusting for chronological age, sex, race and study site as covariates. To reduce multiple testing burden, only the epigenetic age biomarkers that were significantly different by pain groups were examined. Further, we employed a Benjamini-Hochberg correction[31] to adjust for multiple comparisons in the partial correlation analysis (i.e. the clinical variables across the five domains), and the false discovery rate (i.e. q-value) was reported.

Results

Demographics

Our study included 213 participants between 44 and 78 years old, with a mean age of 57.7 (±7.9) years, and 84 (39.4%) were male. According to the self-reported pain impact as described in the methods, these 213 participants could be further categorized into no pain ($n = 31$), low impact pain ($n = 107$), and high impact pain ($n = 75$). Table 1 shows the detailed epigenetic ages and demographic characteristics stratified by pain impact groups. There was no significant difference in chronological age, epigenetic ages, sex, or study site among pain groups. Individuals self-identifying as Non-Hispanic black were over represented in the high impact pain group ($p = 0.003$).

Epigenetic age and pain impact

Figure 1 depicts the differences between the five methylation clocks and chronological age. DNAmAge ($p = 0.041$), DNAmPhenoAge ($p = 0.003$), and DNAmGrimAge ($p < 0.001$) were significantly different by pain impact groups while DNAmAgeHannum ($p = 0.574$) and DNAmAgeSkinBloodClock ($p = 0.367$) were not. Post-hoc comparisons revealed there was a significant difference in DNAmPhenoAge between the no pain group (−11.60 ± 0.34) and the high impact pain group (−7.21 ± 0.53, $p = 0.039$, Figure 1d); and a significant difference in DNAmGrimAge between the no pain group (1.12 ± 0.30) and the high impact pain group (5.34 ± 0.41, $p = 0.007$) as well as between the low impact pain group (1.71 ± 0.38) and the high impact pain group (5.34 ± 0.41, $p = 0.004$, Figure 1e). Differences in DNAmAge did not survive post-hoc multiple comparison corrections. Additional DNA methylation derived measures were not significantly different by pain impact: $CD8^+$ T cell counts ($p = 0.882$), naive $CD4^+$ T cell counts ($p = 0.826$), natural killer cell counts ($p = 0.123$), GDF15 ($p = 0.091$), leptin ($p = 0.115$), and DNA methylation-derived telomere length ($p = 0.079$).

Epigenetic age and WOMAC pain

As shown in Figure 2, WOMAC pain was positively associated with DNAmPhenoAge (Figure 2b, $\beta = 0.225$ years/unit increase of WOMAC pain, 95% CI = [0.025, 0.425], $p = 0.028$) and DNAmGrimAge (Figure 2d, $\beta = 0.198$ years/unit increase of WOMAC pain, 95% CI = [0.067, 0.330], $p = 0.003$). WOMAC pain was not significantly associated with the DNAmAge ($p = 0.762$), the DNAmAgeSkinBloodClock ($p = 0.828$), or the DNAmAgeHannum ($p = 0.765$). Additional DNA methylation derived measures were not significantly associated with WOMAC pain: CD8+ T cell counts ($p = 0.802$), naive CD4+ T cell counts ($p = 0.714$), natural killer cell counts ($p = 0.082$), GDF15 ($p = 0.343$),

Table 1. Epigenetic ages and demographics stratified by pain groups.

	Mean (SD) or no. (%)			
	No pain	Low impact pain	High impact pain	p[a]
N	31	107	75	
Chronological age	58.6 (9.2)	58.6 (7.7)	56.3 (7.3)	0.125
DNAmAge	59.9 (8.2)	60.2 (7.8)	59.5 (8.3)	0.876
DNAmAgeHannum	48.4 (8.7)	48.2 (9.1)	46.8 (8.7)	0.533
DNAmAgeSkinBloodClock	57.6 (8.2)	57.3 (7.6)	55.8 (7.9)	0.359
DNAmPhenoAge	47.0 (9.4)	48.6 (9.2)	49.1 (9.4)	0.581
DNAmGrimAge	59.8 (7.0)	60.3 (7.4)	61.7 (7.9)	0.369
Sex				
Male	12 (38.7)	40 (37.4)	32 (42.7)	0.770
Female	19 (61.3)	67 (62.6)	43 (57.3)	
Race				
Non-hispanic black	12 (38.7)	41 (38.3)	47 (62.7)	**0.003**
Non-hispanic white	19 (61.3)	66 (61.7)	28 (37.3)	
Study site				
University of Florida	18 (58.1)	73 (68.2)	42 (56.0)	0.212
University of Alabama at birmingham	13 (41.9)	34 (31.8)	33 (44.0)	

[a]The p-values were obtained by ANOVA for continuous variable, or χ^2 -test for categorical variables.

leptin (p = 0.163), and DNA methylation-derived telomere length (p = 0.928).

Epigenetic age and other functional domains

As shown in Figure 3, partial correlation analysis suggested that older DNAmAge was associated with higher IVC-Active Coping (r = 0.152, p = 0.027, corrected p = 0.390) and higher CSQ-Reinterpreting (r = 0.213, p = 0.002, corrected p = 0.052). Moreover, older DNAmPhenoAge was associated with higher painDETECT (r = 0.200, p = 0.003, corrected p = 0.100), higher CSQ-Catastrophizing (r = 0.183, p = 0.007, corrected p = 0.109), higher CSQ-Reinterpreting (r = 0.149, p = 0.030, corrected p = 0.293), and lower PSS (r = −0.140, p = 0.048, corrected p = 0.347). Additionally, older DNAmGrimAge was associated with higher CSQ-Catastrophizing (r = 0.273, p < 0.001, corrected p = 0.002), lower SPPB (r = −0.257, p < 0.001, corrected p = 0.002), higher painDETECT (r = 0.192, p < 0.005, corrected p = 0.047), higher PROMIS-Depression (r = 0.187, p < 0.007, corrected p = 0.047), higher CSQ-Passive Coping (r = 0.175, p = 0.010, corrected p = 0.061), lower MoCA (r = −0.143, p = 0.038, corrected p = 0.172), and higher PROMIS-Anxiety (r = 0.141, p = 0.042, corrected p = 0.172). There were no significant associations with any of the QST measures (p's > 0.05).

Discussion

In our prior work, we conducted the first investigation of how chronic musculoskeletal (MSK) pain relates to epigenetic aging in healthy community-dwelling older adults,[5] using Horvath's "first generation" DNA methylation aging clock (i.e. DNAmAge).[8] The current investigation extends our previous work to a larger, more demographically diverse sample of individuals with chronic knee pain using the five primary epigenetic clocks. Several important findings emerged. First, there were significant differences across Pain Impact groups in three out of the five epigenetic clocks examined, indicating that chronic pain with pain-related disability during the past 6 months may be associated with markers of epigenetic aging. Second, higher knee pain intensity during the past 48 hours (i.e. WOMAC-Pain) was associated with an older epigenome, but only in two of the examined clocks (i.e. DNAmPhenoAge and DNAmGrimAge). Finally, pain catastrophizing, depressive symptomatology and more neuropathic pain symptoms were significantly associated with an older epigenome in only one of the five epigenetic clocks (i.e. DNAmGrimAge) even after correcting for multiple comparisons.

To the best of our knowledge, only two previous studies have investigated epigenetic age acceleration in chronic pain conditions.[5,7] In our initial study, we found a younger epigenome in nine age-matched control (i.e. pain-free) individuals compared to 20 individuals reporting chronic MSK pain.[5] A subsequent study found no differences in epigenetic age estimates among three cohorts of individuals with various pain profiles: (1) a healthy, but heat pain sensitive (HPS) cohort including 20 monozygotic twin pairs discordant for heat pain temperature threshold; (2) a fibromyalgia (FM) cohort including 24 cases and 20 controls; and (3) a headache cohort including 22 chronic migraine and medication overuse headache patients, 18 episodic migraine patients, and 13 healthy controls. Interestingly, our current findings partially align with both of these previous investigations. First, we

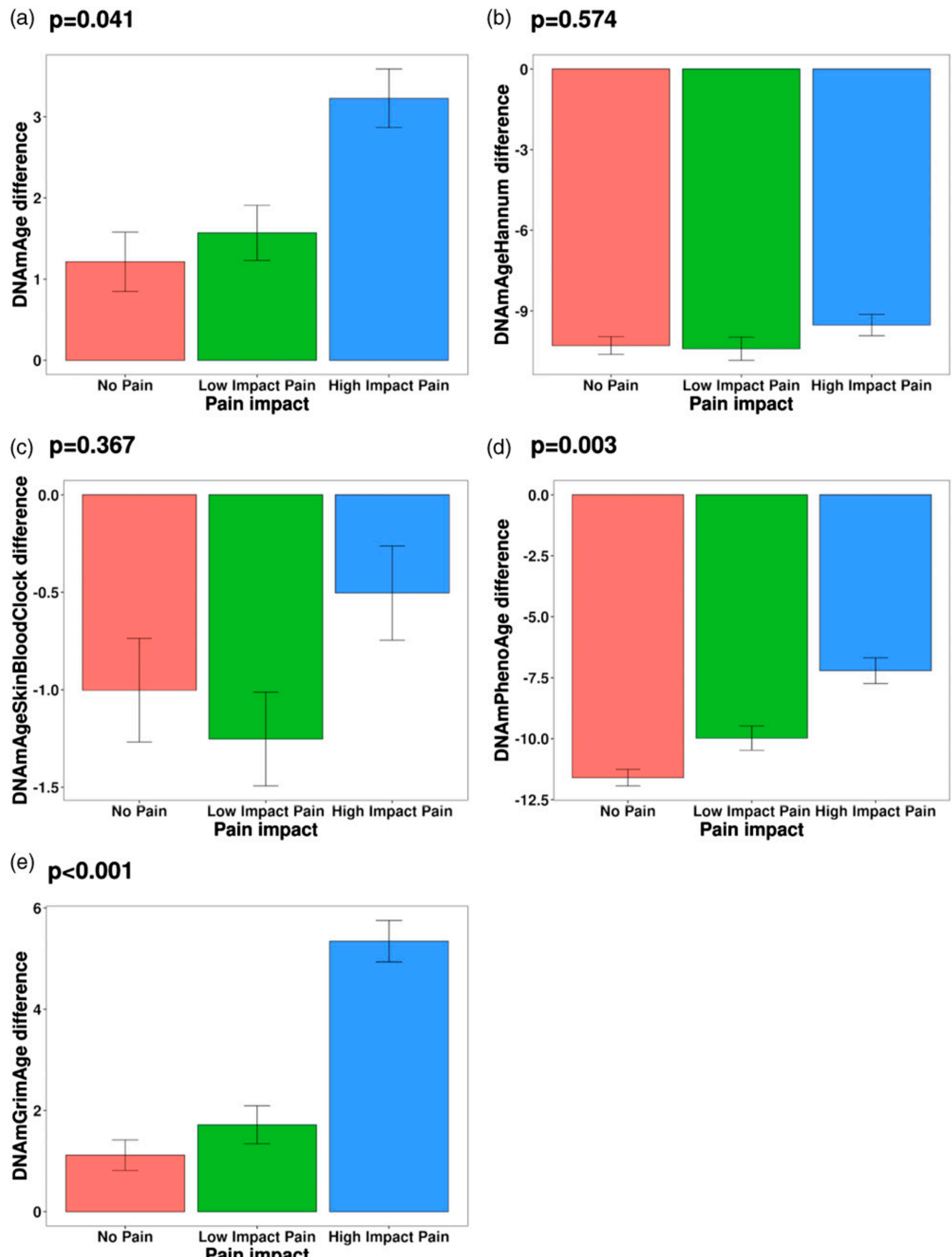

Figure 1. Bar plot of predicted epigenetic age difference (predicted epigenetic age—chronological age) with respect to the pain impact (a) DNAmAge; (b) DNAmAgeHannum; (c) DNAmAgeSkinBloodClock; (d) DNAmPhenoAge, and (e) DNAmGrimAge. *p*-values were obtained by ANCOVAs adjusting for age, sex, study site, and chronological age as covariates. Standard errors were marked on the bar plot.

found differences in epigenetic aging in only three of the five epigenetic clocks when considering pain's impact during the past 6 months, and only two of those clocks (i.e. DNAm-PhenoAge and DNAmGrimAge), were directly associated with knee pain during the past 48 hours. This is similar to our prior work using Horvath's clock. Second, in the current study there were no associations between the epigenetic clocks and any experimental pain measure, which is consistent with Kwiatkowska and colleagues'[7] findings. Ultimately, only DNAmGrimAge epigenetic clock was related to emotional aspects of the pain experience and its neuropathic characteristics after correcting for multiple comparisons. These findings suggest that DNAmGrimAge clocks may be particularly sensitive to pain-related biological alterations. This is not surprising because DNAmGrimAge was built using a composite biomarker based on the DNA methylation surrogates of seven plasma proteins and of smoking pack-years[30] and has previously outperformed the

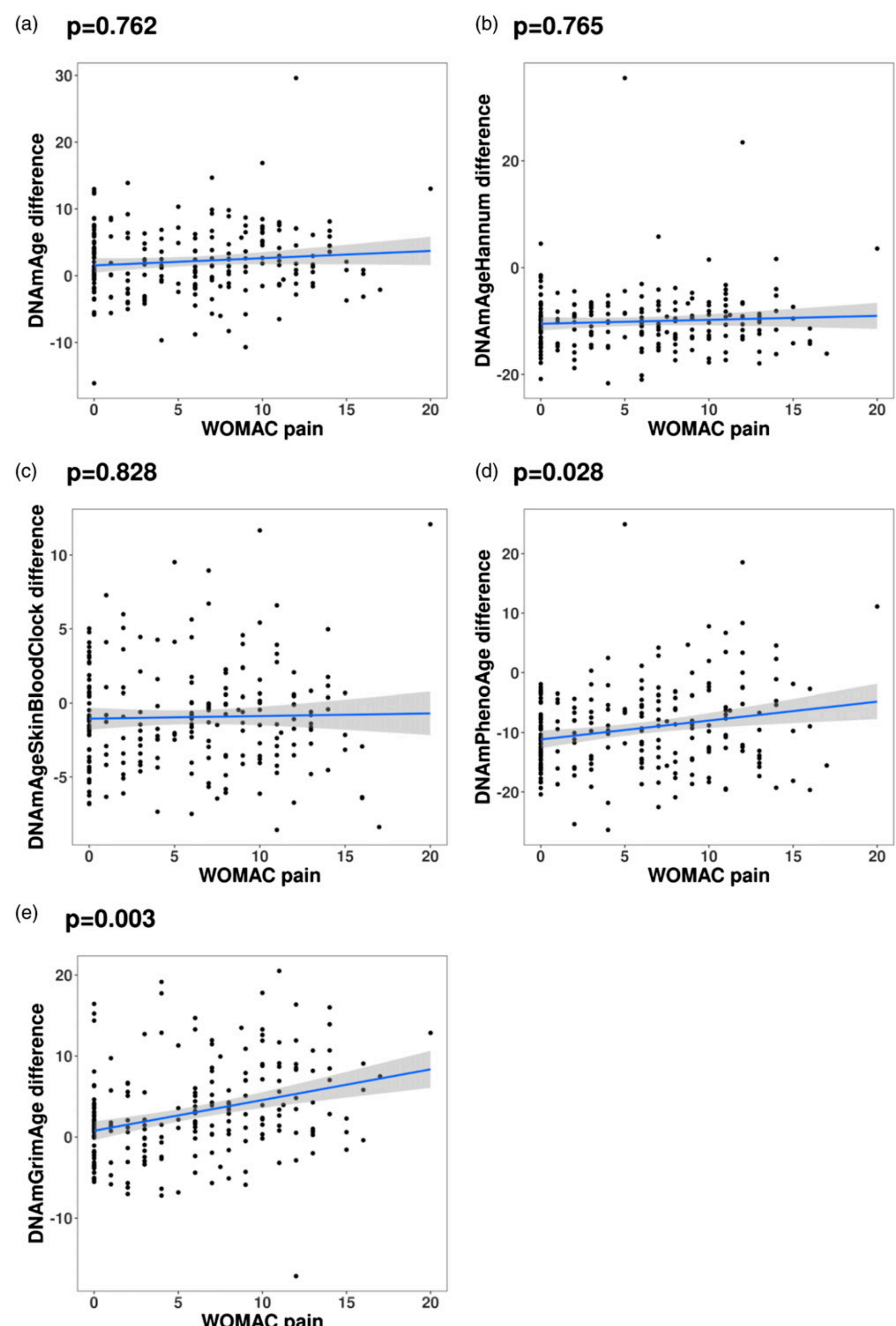

Figure 2. Scatter plot of predicted epigenetic age difference (predicted epigenetic age—chronological age) with respect to WOMAC pain. (a) DNAmAge; (b) DNAmAgeHannum; (c) DNAmAgeSkinBloodClock; (d) DNAmPhenoAge, and (e) DNAmGrimAge. *p*-values were obtained by linear regression model adjusting for age, sex, study site, and chronological age as covariate.

other epigenetic clocks in their associations with age-related conditions and mortality.[32] DNAmGrimAge has also been shown to be greater among individuals with major depressive disorder compared to healthy controls.[33] Thus, our findings highlight the nuanced differences between the various epigenetic aging clocks. For example, DNAmGrimAge may be better suited to understand epigenetic aging in complex, multidimensional conditions such as chronic pain because it covers the largest number of DNA methylation sites (i.e. > 1000 CpGs) compared to the other clocks (i.e. 71–513 CpGs)

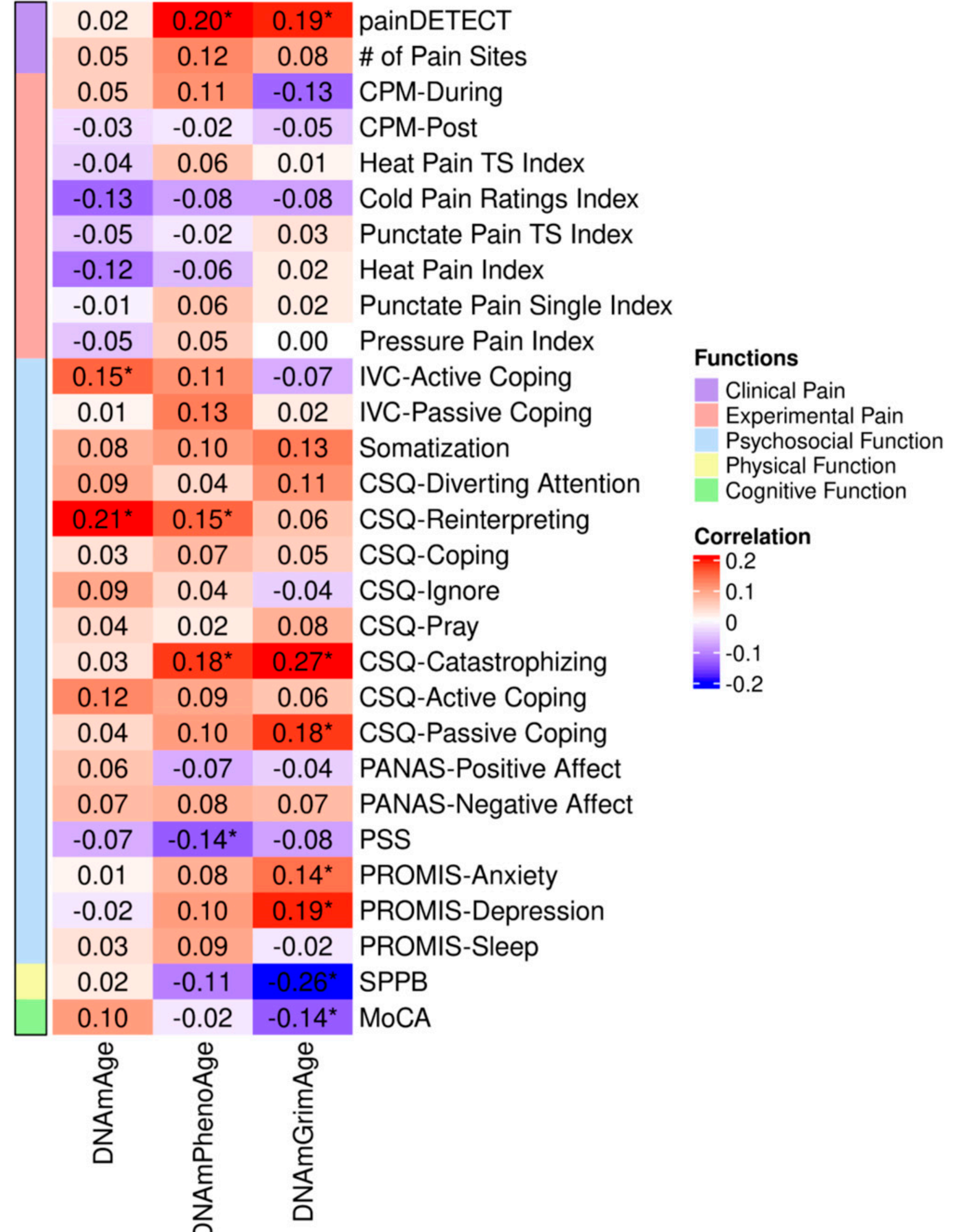

Figure 3. Heatmap of the partial correlation coefficients between predicted epigenetic age and variables across five domains, including clinical pain, cognitive function, experimental pain, physical function, and psychosocial function. Each row is a clinical variable and each column is a type of the predicted epigenetic age difference (predicted epigenetic age - chronological age), including DNAmAge, DNAmPhenoAge, and DNAmGrimAge. Chronological age, sex, race and study site were adjusted as covariates. The partial correlation coefficient was indicated by a red-blue color scale, where red indicated positive correlation, blue indicated negative correlation. Significant partial correlations (i.e. $p \leq 0.05$) were indicated by *.

and considers health and lifestyle-related factors that are not part of the other epigenetic clocks.

The current study advances the work by Kwiatkowska and colleagues'[7] by considering pain's impact (i.e. pain-related disability), not just pain presence, on epigenetic aging. Epigenetic aging in the three clocks (i.e. DNAmAge, DNAmPhenoAge, and DNAmGrimAge) was most evident in those reporting high impact knee pain, while those reporting low impact knee pain were more similar to the chronic knee pain-free controls. Prior studies examining epigenetic aging and chronic pain did not consider the distinction between pain impact and pain

presence, and it is plausible that epigenetic aging is only accelerated by pain that is significantly limiting. High-impact chronic pain is a relatively new chronic pain classification that was conceptualized by the U.S. National Pain Strategy to overcome limitations in previous chronic pain definitions that relied solely on pain duration.[9] Population-based studies indicate high-impact chronic pain places individuals at increased odds of severe pain, physical disability, and cognitive impairment.[9,34–36] While the effects of high-impact chronic pain are just beginning to be examined, the relationship with epigenetic aging is similar to that seen with brain aging[3,4,37] as well as in other debilitating chronic diseases.[38–42]

Both differences and similarities between the current and previous study findings in persons with chronic pain are likely due to disparities in the sample sizes, chronological age inclusion and exclusion criterion, racial diversity, age distribution, as well as pain characteristics of the included participants. Overall, our findings suggest an older epigenome in persons with severely limiting knee pain, although including younger individuals less than 45 years of age would have enabled us to directly compare epigenetic aging across the lifespan. However, by including individuals with high- and low-impact chronic knee pain, compared to pain-free age-matched controls, we were able to examine epigenetic aging in accordance with the biopsychosocial model of pain in persons with the highest risk of functional decline. Given this is an emerging area of research, and the large number of associations tested in our study, the associations between epigenetic aging and pain as well as the lack of associations with other pain-related measures (e.g. experimental pain) needs to be further explored in future studies and in other pain conditions. Finally, our study was cross-sectional, thus, causality cannot be determined. It is likely that the relationship between epigenetic aging and pain is bidirectional. Future longitudinal studies aimed at understanding the clinical significance of measuring epigenetic aging, and its ability to predict treatment outcomes in pain are warranted.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Data availability statement

The data that support the findings of this study are available from the corresponding author, [Y.C.-A.], upon reasonable request.

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Supplemental Material

Supplemental material for this article is available online.

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