


Differential DNA methylation profiles of Alzheimer's disease-related genomic pathways in the blood of cognitively-intact individuals with and without high impact chronic pain

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Abstract

Background: Chronic pain and Alzheimer's disease (AD) are prevalent in older age and their etiologies remain to be understood and evidence supports potential associations between the two. Both high impact pain and AD have been previously associated with differences in the epigenome. Interactions with the epigenome may serve as a possible underlying mechanism linking high impact pain and AD.

Objective: To complete epigenetic canonical pathways analyses related to AD in individuals with and without high-impact knee pain.

Methods: This manuscript aimed to explore differences in DNA methylation patterns in genes and pathways associated with AD. Blood samples of cognitively intact, community-dwelling adults with high impact knee pain versus pain-free controls were compared on their DNA methylation levels of AD-related genes. Pathway enrichment analysis was performed on significantly different DNA Methylation probes by pain group.

Results: There were significant DNA methylation differences between the high impact versus the pain-free control groups in genes and pathways associated with AD ($p < 0.05$). We found a total of 17,563 differentially methylated CpG probes, including 13,411 hypermethylated CpG probes and 4152 hypomethylated CpG probes. Further, pathway analysis revealed these differences were significantly associated with AD-related pathways associated with AD progression.

Conclusions: This study sample showed AD-related DNA methylation differences and associated potential canonical pathways in those with and without high impact knee pain. These results highlight the need to study overlapping epigenetic modifications underlying high impact pain and AD pathologies. Further studies, including gene expression, are needed to further explore the relationship between epigenetics, chronic pain, and AD.

Keywords

Alzheimer's disease, chronic pain, epigenetics, high impact pain

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Introduction

Chronic pain is one of the most common health problems, and it is estimated that 1 in 5 individuals have a chronic pain condition at any given time in the United States. For many individuals, the pain they experience greatly impacts their well-being, and limits their daily functioning and productivity.¹ While the mechanisms underlying high-impact chronic pain are currently uncertain, risk factors such as age, race, and biological sex have been reported.^{2–8} Additionally, high-impact pain (HIP) is associated with a variety of other conditions, such as depression and anxiety, cancer, as well as Alzheimer's disease (AD) and related dementias.^{9–11} Thus, there is a need for research to explore the mechanisms that contribute to pain prevalence and maintenance, as well as pain's connections to other comorbid conditions.

Of the associated conditions, chronic pain is prevalent in AD. There were over 55 million adults globally living with some form of dementia in 2020, with the number expecting to increase in 139 million in 2050, implying 10 million new cases per year.¹² The most common form of dementia is AD, and is characterized with cognitive and behavioral impairments that can range from mild to severely life-limiting symptoms.¹³ While much of the disease remains a mystery, what is known about AD is common patterns of change in the brain, including abnormal accumulation of amyloid- β (A β) plaques and tau tangles, as well as the loss of connections between neurons in the brain and persistent neuroinflammation.^{14,15} Recently, the reported prevalence of chronic pain in AD patients was 45.8%, and may be grossly underestimated due to the inability to communicate the pain experience compared to cognitively healthy individuals.^{16,17} Additionally, evidence suggests that chronic pain may be a risk factor for AD, with chronic pain patients reporting significantly higher risk of AD and all-cause dementia.¹¹ Further, epidemiological evidence suggests that older individuals with persistent pain showed more rapid declines in cognitive function as they aged and were more likely to have dementia years later, although in other studies cognitive decline was dependent on various factors: (1) pain intensity (i.e., individuals with only moderate to severe or severe pain had greater cognitive decline); (2) number of pain sites (i.e., a greater pain sites were associated with significantly higher dementia risk, broader and faster cognitive impairment); and (3) pain interference or impact (i.e., greater pain interference was associated with a higher probability of developing dementia).^{18–22} Similarly, chronic inflammatory pain accelerated cognitive impairment in 5-month-old mice employing an AD/ADRD mouse model (APP/PS1), but not in wildtype controls. As APP/PS1 mice rarely develop overt cognitive deficits before 9–12 months of age, the development of memory impairment in this study suggests that chronic pain accelerates AD/ADRD pathogenesis and subsequent cognitive decline.²³ Given the large proportion of older adults who experience persistent

pain, research is urgently needed to understand the mechanisms underlying the relationship connecting AD and chronic pain.

While AD and chronic pain are related, the mechanisms underlying their relationship remains to be understood. It has been suggested that noradrenergic system dysfunction and persistent neuroinflammation may be potential links between chronic pain and AD.¹¹ A potential mechanism whereby one's life experience and environment can impact pathological manifestations of chronic pain and AD is epigenetic regulation of gene expression that may be an appropriate future therapeutic target for the treatment of each condition.^{24–26} Specifically, our previous study focused on differential DNA methylation by chronic pain status where we found an AD-related pathway to be one of the top 10 canonical pathways differentially enriched. Given the emerging evidence for both chronic pain and AD independently, the purpose of this study was to determine whether there were pain-related differences in DNA methylation profiles of AD-related genes, as well as enrichment of their functional pathways. We hypothesized that individuals with high impact knee pain would have a significant hyper- and/or hypomethylation across multiple AD-related genes and associated pathways.

Methods

Participants

All participants provided written informed consent and the study was IRB approved and conducted in accordance with the Declaration of Helsinki at the University of Florida (UF) and the University of Alabama at Birmingham (UAB). All participants provided written informed consent prior to study session commencement. Detailed inclusion/exclusion criteria have been previously reported.²⁷ The present study is a secondary investigation of a parent grant aimed at determining phenotypic differences in chronic pain populations, thus, only measures relevant to the current manuscript are included and presented below. While our group has examined various associations between DNA methylation with several lifestyle factors in this cohort, the present investigation is the first to examine the DNA methylation with a specific focus in AD-related genes and pathways. Specifically, we focused on studying a period of time before any overt cognitive deficit, and as such individuals with cognitive impairment were excluded from the present study.

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: clinical pain intensity and pain-related disability.²⁸ Details on how the GCPS pain grades are calculated have been reported elsewhere.^{27,29} GCPS pain

grades were used to derive knee pain impact groups accordingly: chronic pain-free controls = Grade 0; Low impact pain = Grades 1–2; and High impact pain = Grades 3–4.

Blood collection and processing. Blood samples were collected from the forearm or hand vein at the onset of the in-person study session in a 10 ml K² EDTA tube that was used for DNA methylation analyses. As these participants were alive at the time of study, we were unable to compare DNA methylation in the blood with DNA methylation in the brain. However, there is evidence that suggests that methylated CpGs in human blood and brain tissues are highly correlated.^{30,31}

DNA extraction and methylation analysis. Detailed methods for DNA extraction that was performed in-house has been previously reported.²⁷ Sodium bisulfite conversion and the Infinium MethylationEPIC 850 K BeadChip array was performed by Moffitt Cancer Center, Molecular Genomics Core located at 3011 Holly Dr, Tampa, FL, USA.

DNA methylation data preprocessing. DNA methylation preprocessing data has been previously reported.²⁷ After filtering out unusable data, 815,633 CpG probes remained in our final analysis.

Differentially methylated probes (DMPs) in AD-related genes associated with pain impact. We calculated the power for all putative methylation probes with $p < 0.05$ using R package pwr. By using a two-sided t-test, and assuming the 5% alpha level, and using the effect size obtained from our differential analysis, the power ranges 0.496–0.998. In the quality control step, X and Y sex chromosomes were excluded. To identify DMPs related to pain impact, we built a linear model followed by the empirical Bayes moderated t-statistics test.³² In these models, the methylation amount of a CpG probe was the outcome variable, the binary pain status was the predictor, while adjusting for white blood cell type (CD8T, CD4T, Natural Killer, B Cell, Monocytes and Granulocytes), age, sex, race, and study site as covariates. To explore the potential functional impact of pain-related DMPs, we annotated the DMPs to genomic features using the R package *GenomicFeatures*, including promoters, exons, introns, and intergenic regions.³³ The R package GenABEL was used to test for genomic inflation, with the factor sitting at 0.72 indicating no inflation issues. To account for the dependent methylation levels of nearby regions, we further performed differential methylated region (DMR) analysis. Methylation difference cutoff between pain groups was set to a probability level of 0.05. Multiple comparisons were accounted for using Benjamini-Hochberg FDR. Given the small sample size and exploratory nature of the study, only nominal p-values were reported.

Pathway enrichment analysis. Pathway enrichment analyses are used to help identify biological pathways that are—more

likely than by chance—enriched in a gene list.³⁴ To investigate the annotated pathways in relation to differential DNA methylation, we performed pathway enrichment analysis via Elsevier pathway database and Panther pathway database using EnrichR to identify canonical pathways. Annotated genes within ± 5 kb of the putative DMPs ($p < 0.005$) were used in the EnrichR pathway enrichment analysis.²⁷

Results

Demographics

The present study included 106 participants between 45 to 78 years old, the mean age was 57 (8.0), and 44 (41.5%) were male. These participants were categorized into no pain ($n = 31$) and high impact pain ($n = 75$) via their calculated GCPS pain grade scores. There was no significant difference in age, sex, and study site between no pain and high impact pain group ($p > 0.05$). Non-Hispanic black individuals were overrepresented in the severe pain groups. Table 1 contains full demographic information of the sample stratified by pain group.

AD-related DMPs associated with pain

There were significant DNA methylation differences between the groups ($p < 0.05$). We identified total 17,563 CpG probes, including 13,411 hypermethylated CpG probes (DNA methylation level is higher in the high impact pain group than the pain free group) and 4152 hypomethylated CpG probes (DNA methylation level is lower in the high impact pain group than the pain free group). The top DMPs are shown in Table 2 (full DMP list is shown in Supplemental Table 1). We also identified 488 DMRs under $p < 0.05$, seen in Supplemental Table 2. The remaining EPIC array data can be made available upon reasonable request. The range of % effect size is -0.156 – 0.177 , and can be seen in Supplemental Table 1.

Table 1. Characteristics of the study participants stratified by pain groups (pain grade).

	No pain ($n = 31$)	High Impact pain ($n = 75$)	p^*
Age, mean (SD), y	58.6 (9.2)	56.3 (7.3)	0.218
Sex, no. (%)			0.873
Male	12 (38.7)	32 (42.7)	
Female	19 (61.3)	43 (57.3)	
Race, no. (%)			0.041
Non-Hispanic black	12 (38.7)	47 (62.7)	
Non-Hispanic white	19 (61.3)	28 (37.3)	
Study site, no. (%)			1.000
University of Florida	18 (58.1)	42 (56.0)	
University of Alabama at Birmingham	13 (41.9)	33 (44.0)	

*p-values were calculated using student t test for continuous variables, and Chi-square test for binary outcomes. Bold values denote statistical significance.

Table 2. Top 20 differentially methylated probes (DMPs).

CpG probe	Estimate	Chr	Start	End	Feature	Direction*	p	Genes†
cgl6830944	0.017123465	14	62200995	62200995	exons	+	4.79E-06	HIF1A
cgl2173150	0.086115361	6	170338591	170338591	intergenic	+	7.98E-06	
cgl3477812	0.040691119	4	186435506	186435506	introns	+	8.80E-06	PDLIM3
cg05226506	-0.013953581	2	118845797	118845797	promoters	-	1.74E-05	INSIG2
cgl4931071	0.017495921	6	12717776	12717776	promoters	+	1.93E-05	PHACTRI
cgl9733255	0.03238186	9	74918970	74918970	intergenic	+	3.14E-05	
cg09749703	-0.012095051	2	153192460	153192460	promoters	-	4.30E-05	FMNL2
cg21523574	0.035811882	14	23595597	23595597	exons	+	5.48E-05	SLC7A8
cg00057476	0.030546165	22	29708246	29708246	exons	+	6.10E-05	GAS2L1; RASL10A
cg26991453	0.016475112	2	28117271	28117271	introns	+	6.51E-05	RBKS; BRE-ASI; BABAM2
cgl1146114	0.022733903	12	4671731	4671731	promoters	+	7.13E-05	RAD51API; DYRK4
cg08765940	0.035434289	11	133789708	133789708	exons	+	7.29E-05	IGSF9B
cg00119127	0.038617455	7	1422999	1422999	intergenic	+	7.34E-05	
cg07307994	0.07135518	2	3828216	3828216	intergenic	+	7.88E-05	
cg23781022	-0.030334011	12	96589925	96589925	introns	-	8.04E-05	ELK3
cg26485159	0.045642026	5	4511755	4511755	intergenic	+	8.90E-05	
cgl3668657	-0.034681529	2	242042464	242042464	promoters	-	9.76E-05	MTERF4; PASK
cgl5060115	0.038396571	11	45672248	45672248	promoters	+	9.81E-05	CHST1
cg04745703	0.020943617	9	130859347	130859347	introns	+	1.02E-04	SLC25A25
cgl0886173	0.017123465	14	97104878	97104878	intergenic	+	1.06E-04	

*+indicates hypermethylation (higher methylation level in the severe-pain group as compared to the no-pain group); and - indicates hypomethylation (lower methylation level in the severe-pain group as compared to the no-pain group).

†Annotated genes within ± 5 kb of the CpG probe.

Functional annotation by genomic features

To examine the potential functional impact of identified DMPs, we annotated the putative DMPs ($p < 0.05$) to predetermined genomic features (Figure 1). Compared to the null distribution of CpG probes included in the Illumina EPIC array, hypermethylated probes were enriched in introns (37.5% versus 32.9%), intergenic probes (33.7% versus 27.3%), and exons (9.2% versus 8%) but depleted in promoters (19.6% versus 31.8%). Hypomethylated probes were most enriched in promoters (58.9% versus 31.8%), but depleted in exons (3.8% versus 8%), intergenic probes (19.6% versus 27.3%), and introns (17.7% versus 32.9%). All contrasts are statistically significant at $p < 0.05$.

Pathway enrichment analysis

In terms of canonical Elsevier pathway database, Amyloid beta Clearance in Alzheimer Disease ($p = 0.0014$) and Amyloid beta Traffic and Degradation in Extracellular Matrix in Alzheimer's Disease ($p = 0.0368$) were significant. In terms of canonical Panther pathway database, Alzheimer disease-presenilin pathway Homo sapiens P00004 was significant ($p = 0.0024$).

Discussion

Chronic pain and AD are significant problems affecting an ever-aging population that can significantly interfere with daily functioning.^{16,35} Historically, chronic pain and AD

have been studied as separate entities. A bidirectional association is present between chronic pain and AD in previously reported literature, though a clear mechanistic link remains to be understood. Approximately 48.5% of AD patients report having chronic pain, and pain intensity is positively correlated with dementia severity.¹⁶ Recent reports have proposed that chronic widespread pain may predict cognitive diseases, as chronic pain, peaking in prevalence ~ 20 –30 years prior to a diagnosis, was associated with a 43% increase in all-cause dementia, and a 47% increase in AD and related dementia risk.^{11,18–23} It is imperative that these disease states be studied in tandem if we are to promote positive health outcomes in an aging population. Moreover, studying these disease states before any impairment is incredibly important to understand early disease symptoms and pathologies to target early interventions. As we had previously noted significant differences in an AD-related enrichment pathway by pain status, this exploratory study sought to observe DNA methylation profile associations of AD-related genes in individuals with high-impact chronic pain compared to pain-free controls, and employed computational analysis to identify differences in enrichment of common target pathways and genes with differentially methylated CpG sites. Presently, the discussion will focus on the functions and potential downstream effects of the differentially methylated genes. Further, we will discuss the three enriched pathways identified that are reflective of AD disease progression that these genes may likely influence: (1) the Amyloid beta Clearance in Alzheimer Disease pathway; (2) the Amyloid beta Traffic

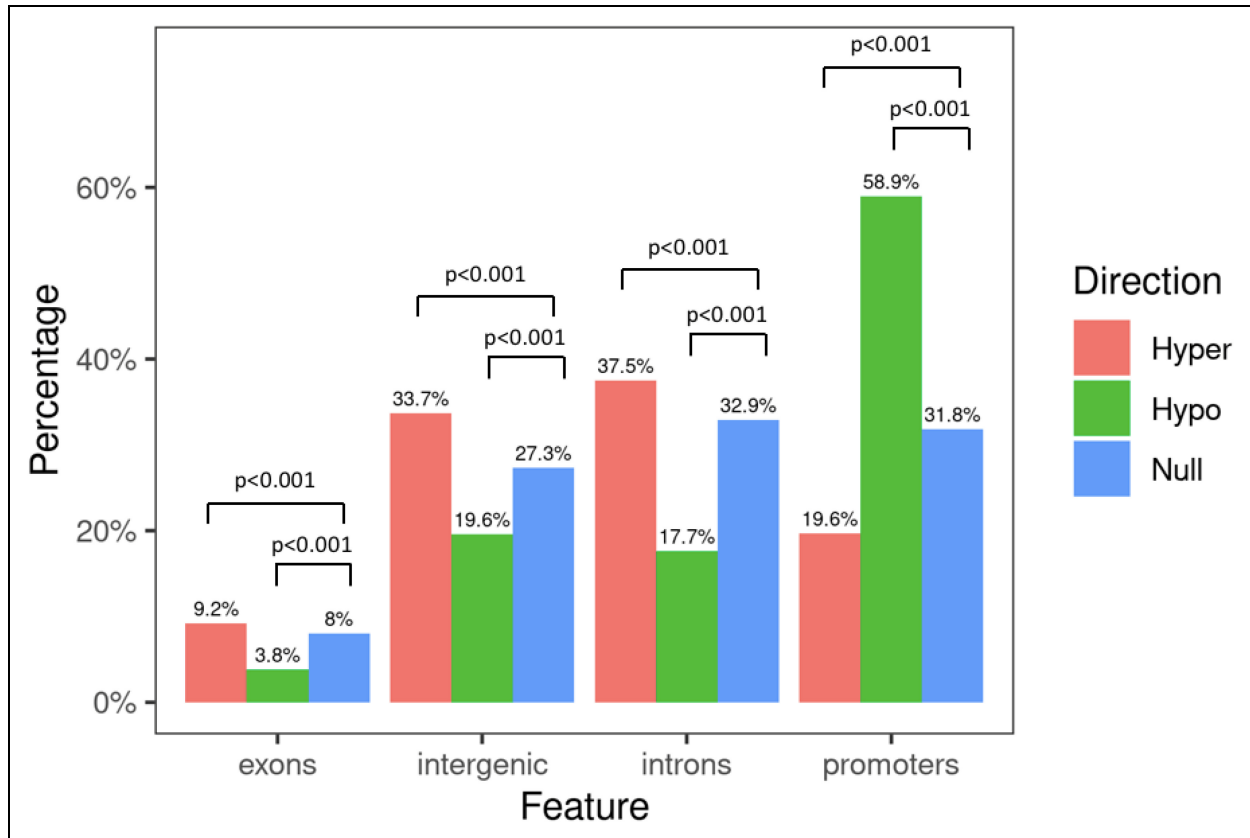


Figure 1. Genomic feature distributions of all putative DMPs (raw $p < 0.05$). p -values were calculated using Chi-square test.

and Degradation in Extracellular Matrix in Alzheimer's Disease pathway; and (3) the Alzheimer disease-presenilin pathway in Homo sapiens.

In the current study, we found that there were significant differences in DNA methylation in individuals with high impact pain compared to pain-free controls on CpG sites found within genes themselves associated with or in intergenic regions in close proximity to genes associated with: (1) cell structure, development and proliferation (PDZ and LIM domain 3 (*PDLIM3*), phosphatase and actin regulator 1 (*PHACTR1*), growth arrest specific 2 like 1 (*GAS2L1*), dual specificity tyrosine phosphorylation regulated kinase 1 (*DYRK4*) and formin 2 (*FMN2*)); (2) DNA transcription and repair (hypoxia inducible factor 1 subunit alpha (*HIF1A*), BRISC and BRCA1 A complex member 2 (*BABAM2*), RAD51 associated protein 1 (*RAD51AP1*), ETS transcription factor (*ELK3*) and mitochondrial transcription termination factor 4 (*MTERF4*); (3) macronutrient metabolism (solute carrier family 7 member 8 (*SLC7A8*), RAS like family 10 member A (*RASL10A*), carbohydrate sulfonotransferase 1 (*CHST1*), solute carrier family 25 member 25 (*SLC25A25*), insulin induced gene 2 (*INSIG2*), and PAS domain containing serine/threonine kinase (*PASK*)); and (4) neurotransmission (immunoglobulin superfamily member 9B (*IGSF9B*)). Specifically, of the

genes related to cell structure, *PDLIM3*, *PHACTR1*, *GAS2L1*, and *DYRK4* were hypermethylated in the HIP group compared to the pain-free controls. Because we do not presently have gene expression data, we can only hypothesize that this hypermethylation may lead to decreased gene expression of their protein constituents in the HIP group. These aforementioned genes are involved in cytoskeletal assembly, tubule formation, cell survival, proliferation and differentiation, in which disruption of these processes have been implicated in AD. In AD, the histopathological hallmarks are not only A β plaques, but also neurofibrillary tangles (NFTs) composed primarily of tau.³⁶ Basic and clinical science experiments have led to many hypothesizing that abhorrent cell structure in the brain may prompt an environment that allows for the upregulation of AD-related genes and formation of NFTs in AD.^{36,37} Dysregulation of genes related to cytoskeleton and microtubule formation may lead to this type of environment in individuals with HIP. In addition to this, NFTs contain tau, a microtubule-associated protein that works with the FMN2 protein, coded for by the *FMN2* gene that was contrastingly hypomethylated in the HIP group. In the neurons of the brain, microtubule-actin cross-talk is primarily controlled by tau, which simultaneously acts as a stabilizer and promotor of growth along actin bundles. FMN2 works alongside tau, guiding along

actin and promoting the capture of microtubules and aiding in the formation of NFTs.^{37–40} Thus, the epigenetic modifications seen in our HIP sample may in theory contribute to an environment suitable for structural abnormalities that promote the development of NFTs and increased AD development risk.

Accumulation of DNA damage throughout the body, and especially in the brain, is a well-known aging factor.⁴¹ As such, a substantial amount of evidence points to DNA damage as a critical player in the pathogenesis AD. In clinical studies, DNA damage has been shown to have accumulated in the brains of AD patients, and currently, abnormalities in DNA damage repair can be used as a diagnostic biomarker for AD. In the context of Mendelian genetics, noted disruptions in DNA damage repair that have resulted from point mutations in the *BRAC1* gene, among other DNA damage repair genes have been linked to AD pathogenesis.⁴¹ We also hypothesize that epigenetic modifications to DNA repair genes may possibly facilitate AD pathology. In our sample, *HIF1A*, *BABAM*, *RAD51AP*, and *ELK3* were hypermethylated in the HIP group compared to the pain-free group, which has the possibility to lead to decreased DNA repair and dysfunction in gene transcription in the HIP group. In contrast, *MTERF4* was hypomethylated, the gene used to transcribe and translate the Mitochondrial Transcription Termination Factor 4 (mTERF) protein and influencing mitochondrial function.^{42,43} Interestingly, basic science experiments have shown that mTERF is also upregulated in animal models of AD. mTERF over expression has also been demonstrated to promote amyloidogenic processing by suppressing a disintegrin and metalloproteinase 10 (ADAM10), resulting in a significant increase in amyloid precursor protein (APP).⁴² Without gene expression being quantified, we can only hypothesize that individuals with high impact pain may be exhibiting changes in expression of ADAM10 and APP, possibly due to the hypomethylation of *MTERF4* compared to pain-free individuals. Future studies are urgently needed to confirm this hypothesis.

In addition to genes related to cellular function and DNA repair, we also noted differential DNA methylation in genes related to macronutrient metabolism, primarily carbohydrate metabolism, in HIP versus pain-free individuals: *SLC7A8*, *RBKS*, *RASL10A*, *CSHT1*, *SCL25A25*, *INSIG2*, and *PASK*. Though the brain only contributes a small percentage of an individual's overall mass, it is one of the most energy demanding sources of the organism. Ultimately, all macronutrients are converted to glucose to be used by cells throughout the body and in the brain. It is essential for neuronal activity and is used to undergo cellular respiration by glucose transporters expressed in the brain and peripheral endothelium, astrocytes and neurons.⁴⁴ *RBKS*, *RASL10A*, *CSHT1*, and *SCL25A25* were all hypermethylated in individuals with high impact pain and are all genes encoding for proteins and enzymes

critical for carbohydrate metabolism. Evidence suggests that subsequent down-regulation of expression of these genes would lead to aberrant carbohydrate metabolism. Interestingly, patients at risk for or with AD show decreased glucose metabolism in the brain, and one study in a fly model of AD demonstrated that enhancing glucose metabolism showed neuroprotective effects.⁴⁴ In addition, there is evidence to suggest changes in glucose metabolism in various regions of the brain in individuals with chronic pain.⁴⁵ *INSIG2* and *PASK* were hypomethylated in our sample of high impact pain individuals. *INSIG2* and *PASK* are both influenced in their expression by the presence or absence of insulin, corroborating the theoretical shift in carbohydrate metabolism associated with differential methylation of the above genes, as insulin concentrations fluctuate with the amount of carbohydrate present in the bloodstream.⁴⁶ Ultimately, it is well-demonstrated that errors in carbohydrate metabolism can lead to increases in reactive oxygen species (ROS) and oxidative stress. We have previously shown significant associations between ROS/oxidative stress and chronic pain, and previous literature has linked ROS/oxidative stress with AD.^{4,47,48} Thus, it is hypothesized that these phenomena may be a possible underlying link between the two disease states.

Finally, in our sample, *IGSF9B* was hypermethylated in the HIP group compared to controls. This gene codes for an immunoglobulin family protein that is a brain-specific adhesion molecule that is found in GABAergic interneurons. The resulting IGSF9B participates in inhibitory synaptic organization by participating in synaptic adhesion.⁴⁹ Decreased expression of the protein may be associated with GABA-related dysfunction in neurons.⁵⁰ This potential dysfunction would be consistent with the literature that human chronic pain patients have lower concentrations of GABA in the brain, and animal models of chronic pain showing GABAergic dysfunction.^{51–54} Additionally, there are noted GABAergic inhibitory interneuron defects in AD.⁵⁵ More research is needed to determine whether the epigenetic differences described in our sample may be important mechanisms by which the two disease conditions potentially interact.

Finally, the canonical pathways enriched in our sample were associated with the presenilin pathway in AD, A β clearance in AD and A β traffic and degradation in extracellular matrix in AD. A β is currently one of the most well-known molecules associated with AD progression and mortality through the accumulation and deposition of this molecule in the brain. A β is both neurotoxic and prone to self-aggregation. The 'A β clearance in AD' and the 'A β traffic and degradation in AD' pathways, are a series of enzymatic and non-enzymatic reactions focused on reducing the amount of A β deposited in the brain. The 'Presenilin' pathway was also associated with differential DNA methylation of genes in high impact pain patients in our sample. The presenilins (presenilin-1 and

presenilin-2) are transmembrane proteins responsible for the regulation of cleavage of other proteins in their domain. Ultimately, dysregulation of this pathway by these proteins increases the production of A β .⁵⁶ Dysfunction of these pathways are crucial to the pathogenesis of AD. It should be emphasized that in our sample, individuals with HIP that were deemed “cognitively normal” at the time of study, already show differences in DNA methylation of genes associated with these pathways. These DNA methylation differences associated with HIP may predispose individuals with chronic pain to AD or it may accelerate existing ongoing processes.

We acknowledge that this study presented its own unique limitations. First, its cross-sectional nature does not indicate causality. Second, there are other epigenetic modifications (histone acetylation and microRNA expression) that work together with DNA methylation to regulate gene expression that we did not assess. Additionally, bisulfite conversion generates a greater amount of DNA fragmentation and lower yields than enzyme conversion; however, quality control steps were employed in order to minimize the DNA damage. It should be noted that the 850k EPIC array was developed for cancer research and only includes a small proportion of the 28 million CpG candidates found in the human genome. As such, there were additional CpGs not included in the analyses. Third, we did not measure gene expression, thus, it is not clear whether these epigenetic differences impacted function. Finally, peripheral blood is heterogenous, and we did not account for the different cell types in the analyses, however, in our previous study cell type proportion did not impact our findings. We also acknowledge that peripheral amyloid-beta clearance as measured by peripheral blood samples may be key to understanding these mechanisms and may provide a useful biomarker and validation of epigenetic changes. Future research should aim to be longitudinal, compare specific tissue types, include SNPs and other genetic variants, and measure both epigenetic modifications, differences in gene expression and A β in the periphery to truly capture the essence of the relationship between AD and chronic pain.


Both chronic pain and AD are significant, debilitating disease affecting aging populations. As chronic pain and AD are more prevalent in older adults and the number of adults over the age of 65 is expected to increase, the burden of these diseases for both older individuals and society at large are also expected to increase in the near future.⁵⁷ Here, we showed that individuals with chronic HIP had differentially methylated genes associated with various AD-related pathways. Our findings provide preliminary evidence to support the chronic pain-AD link corroborating other literature, but more research is needed to confirm the directionality of this relationship.^{11,18–23} Because of the epigenome’s readiness to respond to changes in its environment, targeting interventions that

enhance the epigenome’s ability to effectively regulate shared genes and pathways may help to prevent and/or treat subsequent AD and chronic pain.

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Statements and declarations

Author contributions

Larissa Strath (Conceptualization; Formal analysis; Methodology; Validation; Visualization; Writing – original draft; Writing – review & editing); Lingsong Meng (Data curation; Formal analysis; Writing – review & editing); Yutao Zhang (Formal analysis; Writing – reviewing & editing); Asha Rani (Methodology; Writing – review & editing); Zhiguang Huo (Supervision; Writing – review & editing); Thomas C Foster (Supervision; Writing – review & editing); Roger B Fillingim (Supervision; Writing – review & editing); Yenisel Cruz-Almeida (Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Resources; Supervision; Validation; Writing – review & editing).

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Declaration of conflicting interests

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

Data availability

Data can be made available upon reasonable request.

Supplemental material

Supplemental material for this article is available online.

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