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Vitamin D Metabolism Genes Are Differentially Methylated in Individuals with Chronic Knee Pain

Larissa J. Strath[a, b] Lingsong Meng[c] Asha Rani[c] Zhiguang Huo[c]
Thomas C. Foster[d] Roger B. Fillingim[a, b] Yenisel Cruz-Almeida[a, b, d]

[a]Pain Research and Intervention Center of Excellence (PRICE) at the University of Florida, Gainesville, FL, USA; [b]Department of Community Dentistry and Behavioral Science, The University of Florida, Gainesville, FL, USA; [c]Department of Biostatistics, The University of Florida, Gainesville, FL, USA; [d]Department of Neuroscience, The University of Florida, Gainesville, FL, USA

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Epigenetics · Pain · Vitamin D · Vitamin D receptor

Abstract

Introduction: Recent evidence suggests that vitamin D may interact with the epigenome and play a role in the pain experience. In order for proper functioning to occur, there must be an adequate level of vitamin D present, made possible by enzymatic reactions that allow vitamin D to be biologically active. The purpose of this study was to explore the epigenetic landscape of genes involved in vitamin D metabolism in individuals with and without chronic knee pain. ***Methods:*** Community-dwelling individuals recruited as part of a larger study focused on knee pain provided demographic, clinical, and pain-related information, as well as an intravenous blood sample to determine DNA methylation levels at CpG sites. ***Results:*** There were differences in DNA methylation between those with and without pain in genes that code for enzymes related to vitamin D metabolism: CYP27B1 (1-α-hydroxylase). There was also hypermethylation on the gene that codes for the vitamin D receptor (VDR). ***Conclusions:*** The presence of chronic pain is associated with epigenetic modifications in genes responsible for the expression of enzymes involved in vitamin D metabolism and cellular function. These results lay groundwork in understanding the mechanism underlying the association between vitamin D and chronic pain.

Introduction

Chronic pain, defined as pain that persists for longer than 3 months, is a significant public health problem. More than 100 million individuals in the US report having chronic pain, a staggering statistic that is greater than the prevalence of cancer, heart disease, and diabetes combined. There is an urgent need for treatment options outside of traditional pharmaceutical practices that often include negative side effects that have their own independent impacts on the quality of living of individuals with chronic pain. Recent scientific endeavors have

Larissa J. Strath and Lingsong Meng share co-first authorship and have contributed equally to this work.

karger@karger.com
www.karger.com/lfg

Correspondence to:
Yenisel Cruz-Almeida, cryeni@ufl.edu

contributed to a growing body of research showing that nutrition status can have a considerable impact on many non-communicable diseases such as chronic pain. Study of nutritive factors associated with pain may offer unique insights into the prevention and treatment of many painful disorders with interventions free from burdensome side effects that characterize many current pharmacological treatment options. Many nutrients have been shown to interact directly with biological systems and processes related to pain (i.e., refined sugars, the immune system, and subsequent inflammation) [1], and we are still uncovering the influence of various macro- and micronutrients on these processes at a molecular level. Nutri-epigenomics, or the study of the effects of food and food nutrients on human health through epigenetic modifications, is revealing how nutrients can instigate, regulate, and mediate chronic pain states at the epigenome level [2].

One nutrient that has previously been linked to a variety of chronic pain states is 1,25-hydroxyvitaminD_3 (1,25(OH)D_3, calciferol, the active form of vitamin D). 1,25(OH)D_3 is a fat-soluble secosteroid that plays a key role in many systemic processes related to musculoskeletal, cardiac, immune, and nervous system function [3–5]. In order to reach the active form, a multistep process catalyzed by enzymes occurs in order for the inactive forms obtained by the diet (vitamin D_3) and synthesized in the skin via sun exposure from 7-dihydrocholesterol to be converted the active form of 1,25(OH)D_3 in order to be used by the body. Once active, 1,25(OH)D_3 can function as a hormone and communicate with cells through interactions with the vitamin D receptor (VDR) [6]. Thus, optimal levels of 1,25(OH)D_3, and effective metabolism from its inactive to active forms, are imperative for normal gene expression, and dysregulation of this metabolic pathway may play a role in a variety of health conditions and possibly the pain experience.

1,25(OH)D_3 status has been implicated in a variety of painful conditions, with lower levels of 1,25(OH)D_3 being associated with greater pain severity and pain-related disability [7]. In particular, chronic pain that is not responsive to traditional treatment methodologies is often associated with chronically low 1,25(OH)D_3 status. It is hypothesized that 1,25(OH)D_3 may exert its effects through interactions with the immune system, as chronic immune system activation and dysregulation are well established in chronic pain. However, it is important to probe deeper and explore the metabolism of this nutrient, as dysfunction along 1,25(OH)D_3's metabolic pathway could be similarly detrimental to health and influence treatment options. The purpose of this analysis was to determine if there were differences in methylation status in the genes that code for the enzymes involved in vitamin D metabolism: cytochrome P450 family 2 subfamily R member 1 (*CYP2R1*), cytochrome P450 family 27 subfamily B member 1 (*CYP27B1*), cytochrome P450 family 24 subfamily A member 1 (*CYP24A1*), as well as the vitamin D receptor (*VDR*) in individuals with and without chronic knee pain. We also sought to examine differential DNA methylation differences by vitamin D clinical cut points (optimal, insufficient, and deficient), as well as examine the association between circulating vitamin D levels with degree of DNA methylation of the vitamin D metabolism genes.

Methods

Participants

Participants were adults between the ages of 45–85 with and without knee pain recruited from the communities surrounding the University of Florida (UF; Gainesville, FL, USA) and the University of Alabama at Birmingham (UAB; Birmingham, AL, USA). Individuals who self-identified as non-Hispanic black or non-Hispanic white and English speaking were eligible for inclusion. Detailed exclusionary criteria have been reported elsewhere [8]. All participants provided written informed consent, and the study was approved by the IRB at the University of Florida (approval #201400209). Participants were recruited as part of a larger prospective study designed to examine pain, physical psychosocial function, and brain structure and function in persons with or at risk for knee osteoarthritis. Thus, in the present study, only measures relevant to the proposed hypotheses are included and presented below.

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, blood draw, and physical exam were conducted during the HAS.

Study Measures

Graded Chronic Pain Scale

The GCPS is a robust, validated [9] self-reported questionnaire that measures two dimensions of chronic pain severity: pain intensity and pain-related disability. The questionnaire consists of seven items, with six scored on an 11-point Likert scale, asking participants to report their current, 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, which 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 difficulty performing daily, social, and work-related activities multiplied by 10, with each score ranging from 0 to 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?" Higher scores indicate greater pain and pain-related disability.

Pain Group Classification

Consistent with the Task Force for the Classification of Chronic Pain consensus for the 11th version of the International Classification of Diseases (ICD-11) of the World Health Organization (WHO) recommendations [10], incorporating both pain disability and its duration [11], individuals were categorized based on how limiting their pain in their daily lives using the *Graded Chronic Pain Scale* (*GCPS*) [12]. Scores from the *GCPS characteristic pain intensity scale* and disability points were then used to categorize participants according to a pain grade: grade 0 = no reported pain intensity; grade 1 = low disability (i.e., <3 disability points) and low pain intensity (i.e., <50); grade 2 = low disability-high intensity pain (i.e., ≥50); grade 3 = high disability-moderately limiting (i.e., 3–4 disability points), regardless of pain intensity; grade 4 = high disability-severely limiting (i.e., 5–6 disability points), regardless of pain intensity [12]. Pain groups were defined based on pain and disability grade as follows: grade 0 (i.e., no chronic pain), grades 1–2 (i.e., pain with low disability), and grades 3–4 (i.e., pain with high disability).

Blood Collection and Processing

Blood samples were collected by a trained phlebotomist from the forearm or hand vein at the onset of the session and included collection of a 10 mL K^2 EDTA tube and a 7 mL Corvac serum separator tube that were subsequently used for DNA methylation and 1,25(OH)D_3 analyses, respectively.

1,25(OH)D_3 Analysis

The Corvac tube is wrapped in foil to protect it from light. After 30 min, samples were centrifuged at 1,800 × *g* for 10 min and then transferred to a 0.5 mL serum aliquot into an amber cryovial and stored at −80°C until processed for assays. Vitamin D was measured on a TOSOH Bioscience AIA-900 (South San Francisco, CA) using immunofluorescence.

DNA Extraction and Methylation Analysis

The EDTA tube was centrifuged at 3,000 rpm for 10 min, and the buffy coat was carefully extracted and transferred to a cryovial for −80°C 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 6,000 rpm for 5 min at room temperature. The supernatant was discarded, and sodium EDTA solution was added to the pellet and vortexed gently to remove RBC clumps. Homogenate was incubated at 50–55°C with Proteinase K and SDS solution. Following incubation, an equal volume of phenol was added, mixed, and centrifuged at 10,000 rpm for 10 min. Supernatant was transferred to a fresh tube and equal volume of phenol-chloroform-isoamyl alcohol was added, mixed, and centrifuged at the same rpm. Supernatant was transferred to a fresh tube and equal volume of chloroform-isoamyl alcohol was added followed by centrifugation at the same conditions. The supernatant was transferred in a fresh tube and 1/10th volume of 3 M sodium acetate along with 2 volumes of absolute alcohol was added. The precipitated DNA was washed with 70% ethanol by centrifugation at 10,000 rpm for 5 min. The pellet was air dried and dissolved in Tris-EDTA buffer. The dissolved DNA was qubit quantified (Thermo Fisher Scientific, Waltham MA, USA) and visualized on agarose gel for quality assessment. Sodium bisulfite conversion and EPIC methylation array were performed by Moffitt Cancer Center, Molecular Genomics Core located at 3,011 Holly Dr., Tampa, FL 33612.

Preprocessing of DNA Methylation

To perform methylation data preprocessing and quality control, R package minfi [13] and Illumina Human Methylation EPIC annotation files hg19 were employed. To perform between-array normalization and regress out variability explained by the control probes, functional normalization was employed. Details on all CpG probes have been previously reported by our group [14].

Differentially Methylated Probes Associated with Pain-Disability Group

The aim of the present study was to investigate differences in DNA methylation on genes with roles in vitamin D metabolism and function: 1) *CYP2R1*, which codes for the enzyme 25-hydroxylase involved in the first step of metabolism, converting both 7-dihydrocholesterol (sun exposure) and vitamin D_3 (dietary intake) to 25-hydroxyvitaminD_3 (25(OH)D_3); 2) *CYP27B1*, which codes for the enzyme involved in the second step of vitamin D metabolism, 1-α-hydroxylase, that converts 25(OH)D_3 to active 1,25(OH)D_3; 3) *CYP24A1*, which codes for the enzyme involved in breaking down 1,25(OH)D_3 in order to maintain homeostasis, 24-hydroxylase; and 4) *VDR*, which codes for the vitamin D Receptor on cells and DNA that allows active 1,25(OH)D_3 to exert its effects. We only considered 144 CpG probes that fall within ±5 kb regions from these vitamin D metabolism and function genes. To identify differentially methylated probes (DMPs) related to GCPS grade (i.e., pain and disability status), we built a linear model followed by analysis of covariance (ANCOVA) tests, which are implemented in the limma package [15]. These methods have been described in detail elsewhere [8].

Differentially Methylated Probes Associated with 1,25(OH)D_3 Clinical Cut-Point Groups

Participants were grouped based on the current clinical cutoffs for serum vitamin D: deficient (<19.99 ng/mL), insufficient (20.00–29.99 mg/mL), and optimal (>30.00 ng/mL) [16]. Again, we only considered 144 CpG probes that fall within ±5 kb regions from these vitamin D metabolism and function genes. To identify DMPs related to 1,25(OH)D_3 groups, we built a linear model followed by ANOVAs which are implemented in the limma package [15]. These methods have been described in detail elsewhere [8].

Associations of Degree of Methylation of 1,25(OH)D_3 Genes and Circulating 1,25(OH)D_3

Partial correlation analyses were used to determine the relationships between circulating 1,25(OH)D_3 (as a continuous variable) and the degree of methylation with covariates of age, race, sex, and study site in *CYP2R1*, *CYP24A1*, *CYP27B1*, and *VDR* genes.

Table 1. Characteristics of the study participants

	GCPS grade 0 no pain (n = 31)	GCPS grades 1–2 pain-low disability (n = 107)	GCPS grades 3–4 pain-high disability (n = 75)	p*
Age, mean (SD), years	58.6 (9.2)	58.6 (7.7)	56.3 (7.3)	0.125
Sex, *n* (%)				
Male	12 (38.7)	40 (37.4)	32 (42.7)	0.770
Female	19 (61.3)	67 (62.6)	43 (57.3)	
Race, *n* (%)				
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, *n* (%)				
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)	
1,25(OH)D3 status, ng/mL	27.3 (±13.2)	28.3 (±13.0)	24.01 (±12.3)	0.046*

*p values were calculated using univariate analysis of variance (ANOVA).

Results

Participant Demographics

Our study included 216 participants between 45 and 78 years old (mean age = 57.7 ± 7.9) and mostly female (n = 132, 61.1%). Based on self-reported pain and disability, these 213 participants were further categorized into those with no chronic pain (n = 31), pain with low disability (n = 107), and pain with high disability (n = 75). Table 1 shows demographic characteristics, and $1,25(OH)D_3$ means stratified by pain and disability status (no chronic pain, pain-low disability, and pain-high disability). No significant differences in age, sex, study site were observed across groups ($p > 0.05$). Non-Hispanic black individuals were overrepresented in the pain with high disability group ($p = 0.003$).

DMPs Associated with Pain Status (GCPS-Derived Pain Impact Groups)

In terms of GCPS-derived impact groups, at $p < 0.05$ cutoff, we identified total 3 CpG probes, including 1 hypermethylated CpG probes (DNA methylation level is higher in the GCPS grades 3–4, followed by grades 1–2, and grade 0/no pain), and 2 hypomethylated CpG probes (DNA methylation level is lower in the GCPS grades 3–4, followed by grades 1–2, and grade 0/no pain). The 3 DMPs are shown in Table 2.

DMPs Associated with $1,25(OH)D_3$ Clinical Cut-Point Groups

In terms of $1,25(OH)D_3$ clinical cut-point groups, at $p < 0.05$ cutoff, we identified a total of 3 hypermethylated CpG probes where DNA methylation level is higher in the deficient group, followed by insufficient and optimal groups. The 3 DMPs are shown in Table 3.

Associations of Degree of Methylation of 1,25(OH)D_3 Genes and Circulating 1,25(OH)D_3

The correlations between the degree of methylation in CYP2R1, CYP24A1, CYP27B1, VDR, and circulating vitamin D levels are $r = 0.20$ ($p = 0.008$), $r = 0.18$ ($p = 0.020$), $r = -0.18$ ($p = 0.022$), $r = -0.15$ ($p = 0.050$), respectively.

Discussion

Chronic pain is a significant and debilitating problem in Western society that has many biopsychosocial and economic costs. Pain is a complex phenomenon, with many factors contributing to its experience. Over recent years, the effects of nutrition status on pain outcomes have gained significant traction, since nutritional interventions can be tailored to individual lifestyles and cultures and are free from the adverse effects common to many current pharmaceutical solutions [17]. Additionally, various macro- and micronutrients can interact with the epigenome, influencing the way genes are expressed [18]. Concurrently, our increased understanding of the relationship between epigenetic modifications and the pain experience invites examination of the relationship between nutrient status and pain, employing an epigenetic lens. A growing body of evidence suggests that $1,25(OH)D_3$ has the ability to participate in gene

Table 2. Differentially methylated probes (DMPs) associated with pain status (high impact pain vs. low impact pain vs. no pain)

CpG probe	Estimate	95% CI	Chromosome	Start	End	Feature	Direction*	*p* value	Genes[a]
cg13865595	−0.0020	(−0.0039, −0.0001)	12	48,298,924	48,298,924	promoters	−	0.0377	*VDR*
cg24110768	−0.0031	(−0.0061, −0.0001)	12	58,159,478	58,159,478	promoters	−	0.0411	*CYP27B1*
cg13301841	0.0038	(0, 0.0076)	12	48,237,092	48,237,092	exons	+	0.0479	*VDR*

*+ indicates hypermethylation (higher methylation level in the pain group as compared to the no-pain group); and − indicates hypomethylation (lower methylation level in the pain group as compared to the no-pain group). [a]Annotated genes within ±5 kb of the CpG probe.

Table 3. Differentially methylated probes (DMPs) associated with 1,25(OH)D_3 clinical cut-point groups (deficient vs. insufficient vs. optimal)

CpG probe	Estimate	95% CI	Chromosome	Start	End	Feature	Direction*	*p* value	Genes[a]
cg02470587	0.0069	(0.0005, 0.0134)	12	48,302,191	48,302,191	Intergenic	+	0.0348	*VDR*
cg01886921	0.0204	(0.0003, 0.0404)	12	48,280,406	48,280,406	Introns	+	0.0458	*VDR*
cg20372759	0.0058	(0, 0.0115)	12	58,162,287	58,162,287	Intergenic	+	0.0491	*CYP27B1*

*+ indicates hypermethylation (higher methylation level in the pain group as compared to the no-pain group); and − indicates hypomethylation (lower methylation level in the pain group as compared to the no-pain group). [a]Annotated genes within ±5 kb of the CpG probe.

transcription, gene expression, and epigenetic modifications [5] on genes potentially linked to chronic pain and epigenetic aging [8]. However, because vitamin D undergoes many chemical reactions in the body to become its active form, it is also important to examine the epigenetic integrity of the biochemical machinery that allows these processes to occur. Thus, the current investigation aimed to explore the epigenetic landscape of genes that encode the enzymes that catalyze the reactions to make vitamin D biologically active as 1,25(OH)D_3: *CYP2R1* (25-hydroxylase), *CYP27B1* (1-α-hydroxylase), and *CYP24A1* (24-hydroxylase). A schematic detailing this biological process can be seen in Figure 1. Additionally, we sought to explore the epigenetic landscape of the *VDR* gene, which allows 1,25(OH)D_3 to exert its biological effects in various tissues and systems. We also examined the differences in methylation of these genes in the context of clinical cut points of 1,25(OH)D_3 (deficient, insufficient, and optimal). Finally, we sought to examine associations of the amount of circulating 1,25(OH)D_3 and the degree of methylation of these genes. The present study found that circulating levels of 1,25(OH)D_3 were lowest in our high-impact pain group, followed by the low-impact pain and no pain groups as expected based on current literature [19, 20]. We also noted differences in the amount of DNA methylation of the *VDR* and *CYP27B1* genes between individuals with varying pain impacts, as well as differences in these same genes when examined by clinical cut point. Additionally, there were significant positive correlations of CYP2R1 and CYP24A1 (methylation increases as 1,25(OH)D_3 level increases), as well as significant negative correlations of CYP27B1 and VDR with circulating levels of 1,25(OH)D_3 (methylation decreases as 1,25(OH)D_3 level increases). These results may lay the groundwork for understanding mechanisms underlying the association of vitamin D and the pain experience.

Epigenetics is being studied in a variety of health outcomes [21]. Epigenetic control of gene expression is normal and natural, but some epigenetic changes can lead to detrimental behavioral and health outcomes to the organism. One of the ways that cells mitigate gene expression is the epigenetic modification of DNA methylation [22]. The end result of hypermethylation of a gene is generally a downregulation in expression of that gene, as well as its subsequent proteins. On the contrary, hypomethylation of a gene in the promoter region generally allows for increased efficiency of transcription, allowing an upregulation of gene expression and increased quantities of the encoded protein [23]. Presently,

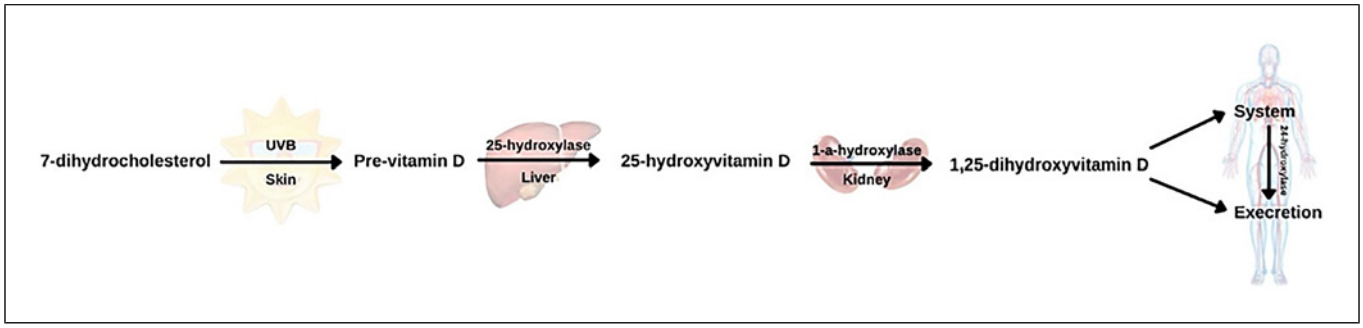

Fig. 1. Vitamin D metabolism, enzymes catalyzing the reactions and the locations the reactions occur.

we found that individuals with high impact pain had significantly different methylation statuses on a CpG site related to *CYP27B1* gene, as well as significantly different methylation on CpG sites associated with the *VDR* when comparing no pain and low impact pain. This would – in theory – lead to differential gene expression and the proteins that they code for.

CYP27B1 is a gene that encodes for an enzyme that is involved in vitamin D metabolism. *CYP27B1* codes for the enzyme 1-α-hydroxylase, an important catalyst involved in 25(OH)D_3 anabolism and the conversion to 1,25(OH)D_3 – its active, useable formation [24]. The inactive form of vitamin D can be obtained in three ways: either through consumption of vitamin D_3 rich foods (fish, fortified dairy, eggs, etc.), oral supplementation with vitamin D_3 or by exposure to sunlight that catalyzes the conversion of 7-dihydrocholesterol into vitamin D_3 in the epidermis. From there, the inactive vitamin D circulates to the liver and kidneys, where it undergoes chemical reactions to become its biologically active form 1,25(OH)D_3. 1-α-hydroxylase catalyzes the second step of this reaction and is an extremely important enzyme to have present if the body wants to have adequate levels of biologically active 1,25(OH)D_3. Interestingly, when examined by both pain group and 1,25(OH)D_3 clinical cut-point groups, those who fell into the high impact pain group or deficient group in our sample had significantly differential hypomethylation on CpG regions associated with the gene *CYP27B1*, that codes for this critical enzyme. These changes, along with a theoretical differential expression of 1-α-hydroxylase, may either be 1) a reflection of the body's attempt to have the biochemical machinery available to process as much of the small amount of vitamin D_3 present (inadequate sunlight and vitamin D rich foods) to catalyze; or 2) it could also be an epigenetic error, whereby modifiers have methylated these regions inappropriately in response to other environmental factors.

Another difference in methylation status in this sample when examined by both pain group and 1,25(OH)D_3 clinical cut-point groups was the differential of CpG sites associated with the *VDR* gene in the chronic knee pain group compared to the no pain group. The *VDR* gene subsequently codes for the VDR [25]. Interactions with the VDR are what allow 1,25(OH)D_3 to be a powerful regulator or gene expression and cellular functions. The VDR is present in every type of cell in the human body, as well as present in many promoter regions as a factor involved in the transcription of genes [26]. The 1,25(OH)D_3/VDR complex further goes on to influence vitamin D response elements (VDREs) that are involved in gene expression. VDREs are dependent on the liganded vitamin D/VDR complex in order for successful transcription. Thus, if there are inadequate levels of 1,25(OH)D_3, or no VDR present, there is typically a down-regulation of VDRE expression. Because so many genes, spanning across systems, have been discovered to be VDREs, it appears as though 1,25(OH)D_3 is an incredibly important compound for the homeostasis and function of the organism. Many VDREs are those that are involved in healthy immune system function and inflammation [27, 28]. Chronic inflammation and immune system activation have been well-established to be a part of the development and maintenance of chronic pain [29]. Thus, it is hypothesized that differential methylation of the *VDR* gene and subsequent differences in gene expression could lead to immune system dysregulation. This may be one of the mechanisms that gives rise to the associations seen to 1,25(OH)D_3 status and levels of pain severity and disability. The VDR gene could be epigenetically modified by a lack of circulating 1,25(OH)D_3 or other environmental

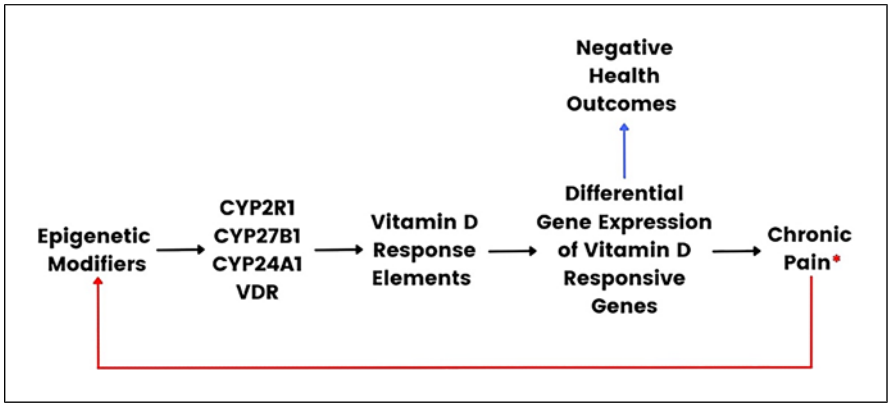

Fig. 2. Schematic of the potential pathways by which epigenetics, vitamin D, and chronic pain could be involved.

factors, rendering adequate levels of circulating, active 1,25(OH)D_3 irrelevant.

We acknowledge potential limitations to our study. First, the study sample was relatively small, including only non-Hispanic black and white participants, and future studies are needed including other ethnic and racial groups. Second, vitamin D may be more important in combination with other nutrients also known to interact with the epigenome; thus, future studies should include a variety of nutrients in order to truly understand to what extent 1,25(OH)D_3 alone or in combination impact gene expression and subsequent pain outcomes [30, 31]. Also, the present study only measured methylation level and did not include other epigenetic modifiers or levels of gene expression. Presently, as this was a targeted, exploratory endeavor, there were no corrections for multiple comparisons as there would be in larger epigenetic-based studies. Future large cohorts are warranted to replicate the findings in this manuscript. Finally, this was a cross-sectional study; thus, no causal inference can be established. Future longitudinal studies including placebo-controlled supplementation studies are needed to establish causality.

Conclusions

Research has demonstrated that individuals with chronic pain are often 1,25(OH)D_3 deficient. However, in our sample, the genes that code for enzymes influencing vitamin D use by the body are differentially methylated based on pain experience. We are unsure if this is a response to low levels of inactive vitamin D_3 from sunlight exposure or dietary quality, or if this differential methylation is potentially leading to a lack of the enzyme to create the 1,25(OH)D_3 used in the blood measurements. Therefore, future research should aim to elucidate whether the hypermethylation of these sites is related to the conservation of energy or epigenetic errors in metabolism as this could drastically change the way we approach research and treatment related to the relationship 1,25(OH)D_3 and chronic pain. Moreover, future research should explore the directionality of these relationships (Fig. 2). In addition to other environmental factors, it is also possible that pain then goes on to act as an epigenetic modifier itself, further involving itself in the methylation of these genes. In any case, it is important to better understand the complex influences of 1,25(OH)D_3 on the pain experience, as this could reveal valuable intervention targets that could greatly improve the lives of those who are suffering from chronic pain.

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UPLOAD2 study participants and study team.

Statement of Ethics

Participants completed written informed consent prior to the commencement of study procedures. The study protocols were approved by the IRB at the University of Florida (approval #201400209).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Larissa J. Strath: formal analysis, visualization, and writing – original draft: Lingsong Meng: software, formal analysis, data curation, and writing – review and editing Asha Rani: methodology, investigation, data curation, and writing – review and editing: Zhiguang Huo: software, formal analysis, data curation, supervision, and writing – review and editing: Thomas C. Foster: methodology, investigation, resources, data curation, supervision, and writing – review and editing Roger B. Fillingim: methodology, investigation, resources, and writing – review and editing: Yenisel Cruz-Almeida: conceptualization, methodology, resources, writing – review and editing, supervision, project administration, and funding acquisition.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Strath LJ, Sorge RE. Racial differences in pain, nutrition, and oxidative stress. Pain Ther. 2022;11(1):37–56.
2 Remely M, Stefanska B, Lovrecic L, Magnet U, Haslberger AG. Nutriepigenomics: the role of nutrition in epigenetic control of human diseases. Curr Opin Clin Nutr Metab Care. 2015;18(4), 328–33,
3 Kongsbak M, Levring TB, Geisler C, von Essen MR. The vitamin D receptor and T cell function. Front Immunol. 2013;4:148.
4 Dursun E, Gezen-Ak D. Vitamin D basis of Alzheimer's disease: from genetics to biomarkers. Hormones. 2019;18(1):7–15.
5 Fetahu IS, Hobaus J, Kallay E. Vitamin D and the epigenome. Front Physiol. 2014;5:164.
6 Carlberg C, Seuter S. A genomic perspective on vitamin D signaling. Anticancer Res. 2009; 29(9):3485–93.
7 Glover TL, Horgas AL, Fillingim RB, Goodin BR. Vitamin D status and pain sensitization in knee osteoarthritis: a critical review of the literature. Pain Manag. 2015;5(6):447–53.
8 Strath LJ, Meng L, Rani A, Sinha P, Johnson AJ, Huo Z, et al. Accelerated epigenetic aging mediates the association between vitamin D levels and knee pain in community-dwelling individuals. The journal of nutrition health and aging. 2022;26. 318–23.
9 Elliott AM, Smith BH, Smith CW, Chambers AW. Changes in chronic pain severity over time: the chronic pain grade as a valid measure. Pain. 2000;88(3):303–8.
10 Nugraha B, Gutenbrunner C, Barke A, Karst M, Schiller J, Schafer P, et al. The IASP classification of chronic pain for ICD-11: functioning properties of chronic pain. Pain. 2019;160(1):88–94.
11 Pitcher MH, Von Korff M, Bushnell MC, Porter L. Prevalence and profile of high-impact chronic pain in the United States. J Pain. 2019;20(2):146–60.
12 Von Korff M, Ormel J, Keefe FJ, Dworkin SF. Grading the severity of chronic pain. Pain. 1992;50(2):133–49.
13 Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, et al. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics. 2014;30(10):1363–9.
14 Montesino-Goicolea, S, Sinha P, Huo Z, Rani A, Foster TC, Cruz-Almeida Y. Enrichment of genomic pathways based on differential DNA methylation profiles associated with chronic musculoskeletal pain in older adults: an exploratory study. Mol Pain. 2020. 16: p. 1744806920966902,
15 Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015; 43(7):e47.
16 National Acadamies Press. Calcium, Io.MCt. RDRIf.VDa. Washington (DC): National Acadamies Press; 2011.
17 Shipton EA, Shipton EE, Shipton AJ. A review of the opioid epidemic: what do we do about it? Pain Ther. 2018;7(1):23–36.
18 Sales NMR, Pelegrini PB, Goersch MC. Nutrigenomics: definitions and advances of this new science. J Nutr Metab. 2014;2014: 202759.
19 Overstreet DS, Strath LJ, Hasan FN, Sorge RE, Penn T, Rumble DD, et al. Racial differences in 25-hydroxy vitamin D and self-reported pain severity in a sample of individuals living with non-specific chronic low back pain. J Pain Res. 2022;15:3859–67.
20 Strath LJ, Meng L, Rani A, Sinha P, Johnson AJ, Huo Z, et al. Accelerated epigenetic aging mediates the association between vitamin D levels and knee pain in community-dwelling individuals. J Nutr Health Aging. 2022;26(4): 318–23.
21 Weinhold B. Epigenetics: the science of change. Environ Health Perspect. 2006; 114(3):A160–7.
22 Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. Nat Rev Genet. 2016;17(8):487–500.
23 Greenberg MVC, Bourc'his D. The diverse roles of DNA methylation in mammalian development and disease. Nat Rev Mol Cell Biol. 2019;20(10):590–607.
24 NCBI. CYP27B1 cytochrome P450 family 27 subfamily B member 1 [Homo sapiens (human)]. Gene. 2022 Feb 27 [cited 2022; Available from: https://www.ncbi.nlm.nih.gov/gene/1594.
25 NCBI. VDR vitamin D receptor [Homo sapiens (human)]. Gene. 2022 March 20 [cited 2022]; Available from: https://www.ncbi.nlm.nih.gov/gene/7421.
26 Andraos C, Koorsen G, Knight JC, Bornman L. Vitamin D receptor gene methylation is associated with ethnicity, tuberculosis, and TaqI polymorphism. Hum Immunol. 2011; 72(3):262–8.
27 Wang M, Kong W, He B, Li Z, Song H, Shi P, et al. Vitamin D and the promoter methylation of its metabolic pathway genes in association with the risk and prognosis of tuberculosis. Clin Epigenetics. 2018;10(1):118.
28 Ong LTC, Schibeci SD, Fewings NL, Booth DR, Parnell GP. Age-dependent VDR peak DNA methylation as a mechanism for latitude-dependent multiple sclerosis risk. Epigenetics Chromatin. 2021;14(1):9.
29 Totsch, SK, RE Sorge. Immune system involvement in specific pain conditions. Mol Pain. 2017. 13: p. 1744806917724559,
30 Remely M, Ferk F, Sterneder S, Setayesh T, Kepcija T, Roth S, et al. Vitamin E modifies high-fat diet-induced increase of DNA strand breaks, and changes in expression and DNA methylation of Dnmt1 and MLH1 in C57bl/6J male mice. Nutrients. 2017;9(6):607.
31 Bar-El Dadon S, Reifen R. Vitamin A and the epigenome. Crit Rev Food Sci Nutr. 2017; 57(11):2404–11.