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Characterizing *OPRM1* DNA methylation in prescription opioid users with chronic musculoskeletal pain

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Abstract

Introduction: Many patients with chronic pain use prescription opioids. Epigenetic modification of the μ-opioid receptor 1 (*OPRM1*) gene, which codes for the target protein of opioids, may influence vulnerability to opioid abuse and response to opioid pharmacotherapy, potentially affecting pain outcomes.

Objective: Our objective was to investigate associations of clinical and sociodemographic factors with *OPRM1* DNA methylation in patients with chronic musculoskeletal pain on long-term prescription opioids.

Methods: Sociodemographic variables, survey data (Rapid Estimate of Adult Health Literacy in Medicine-Short Form, Functional Comorbidity Index [FCI], PROMIS 43v2.1 Profile, Opioid Risk Tool, and PROMIS Prescription Pain Medication Misuse), and saliva samples were collected. The genomic DNA extracted from saliva samples were bisulfite converted, amplified by polymerase chain reaction, and processed for *OPRM1*-targeted DNA methylation analysis on a Pyrosequencing instrument (Qiagen Inc, Valencia, CA). General linear models were used to examine the relationships between the predictors and *OPRM1* DNA methylation.

Results: Data from 112 patients were analyzed. The best-fitted multivariable model indicated, compared with their counterparts, patients with > eighth grade reading level, degenerative disk disease, substance abuse comorbidity, and opioid use $\leq$ 1 year (compared with >5 years), had average methylation levels that were 7.7% (95% confidence interval [CI] 0.95%, 14.4%), 11.7% (95% CI 2.7%, 21.1%), 21.7% (95% CI 10.7%, 32.5%), and 16.1% (95% CI 3.3%, 28.8%) higher than the reference groups, respectively. Methylation levels were 2.2% (95% CI 0.64%, 3.7%) lower for every 1 unit increase in FCI and greater by 0.45% (95% CI 0.08%, 0.82%) for every fatigue T score unit increase.

Conclusions: *OPRM1* methylation levels varied by several patient factors. Further studies are warranted to replicate these findings and determine potential clinical utility.

Keywords: Epigenetics, Opioid abuse, Chronic musculoskeletal pain

1. Introduction

Pain is a complex and poorly understood condition affecting the lives of millions.[7,11,13] Many patients with chronic pain are consumers of long-term prescription opioids and are at risk for aberrant opioid use.[11,13] Numerous studies have identified demographic and psychosocial risk factors for aberrant prescription opioid use.[14,18,37] Several standardized instruments, incorporating these risk factors, are available for the clinical assessment of aberrant use.[20] Although patient risk factors are identified in the literature, a recent review demonstrated that some factors are inconsistently reported or are inconclusive, supporting the need for continued

efforts to definitively establish risk factors that can identify at-risk patients.[37]

Recent years have witnessed increased interest in genetic contributions to chronic pain, opioid misuse, and addiction. The μ-opioid receptor 1 (OPRM1) gene encodes the μ-opioid receptor and is an important endogenous target for prescription opioids; the cytochrome P450 2D6 (CYP2D6) gene metabolizes most opioid medications, and the catechol-O-methyl transferase (COMT) gene modulates pain response through breakdown and clearance of catecholamines. Polymorphisms in these genes are associated with differences in opioid consumption, metabolism, and efficacy.[36] Epigenetic modifications, another cause of genetic variability, are changes caused by environmental factors (medications, smoking, etc.), which do not alter the DNA sequence but, through DNA methylation or histone modification, influence gene expression producing phenotypic changes. Thus, less DNA methylation of the gene promoter is generally predicted to lead to increased gene transcription and more mRNA production. Epigenetic modifications to DNA structure, such as through methylation, may influence an individual's response to pharmacotherapy, including vulnerability to drug abuse.[21,22]

Studying the relationship between chronic pain and the dynamic process of epigenetic remodeling may improve our understanding of the etiology and progression of chronic pain and the progression of chronic prescription opioid use to addiction. Evidence suggests methylation of the OPRM1 gene leads to reduced expression and has been linked to opioid-induced hyperalgesia, opioid tolerance, and addiction in former heroin users.[21,22] There is a dearth of studies examining OPRM1 methylation in long-time prescription opioid users for chronic noncancer pain. In one of few such studies, patients with chronic pain using prescription opioids for more than 1 year were shown to have higher total OPRM1 methylation levels compared with nonopioid-consuming patients with pain.[8] Moreover, a prospective study examining OPRM1 methylation in opioid naïve surgical patients who were prescribed opioids for postoperative pain management, demonstrated both higher opioid dose and duration of use predicted greater methylation levels at 39 days after discontinuation of opioids.[25] The limited available data suggest patients using prescription opioids for pain exhibit variable changes in OPRM1 methylation levels that may be influenced by factors such as duration of use, dose, or type of prescription opioid. These dynamic changes require further characterization to determine clinical implications gene modifications may have in pain management and the development of opioid use disorder. In addition, of the limited studies examining predictors of OPRM1 methylation in chronic noncancer pain prescription opioid users, few were conducted in a study population containing/reporting a substantial proportion of minority, socioeconomically disadvantaged participants.[4,8,25] Our objective was to investigate associations between clinical and sociodemographic factors with OPRM1 DNA methylation in patients with chronic pain treated with long-term prescription opioids, with a focus on underserved populations.

2. Methods

2.1. Study setting and enrollment

This was an Institutional Review Board approved observational study of patients with chronic musculoskeletal pain on chronic opioid therapy. Patients eligible for study enrollment included those aged 18 years or older with chronic musculoskeletal pain (pain present for 3 or more months) treated with prescription opioids on most days in the past 3 months. Patients who were non-English speaking, incarcerated, or unable to provide consent were excluded. Recruitment occurred from 4 enrollment sites: 2 pain management clinics, an emergency department, and an opioid maintenance pain management clinic. Two of the 4 recruitment sites are located on the campus of an urban safety-net hospital system caring for a predominantly socioeconomically disadvantaged population, with approximately 67% self-reporting as African American. The other sites, located in another city, also treat a diverse and vulnerable patient population (most of whom report receiving government assistance and 32% who identify as African American).

2.2. Variables collected

Demographic (such as age, sex, race, and highest education level attained), clinical (comorbidities, medication history including frequency of prescription opioid use, and indication for and duration of prescription opioid use), body mass index, height, and social (eg, smoking history) data were collected. Duration of prescription opioid use was categorized using a priori cutoff categories of <1 year, 1 year to 5 years, and >5 years, rather than discrete years to minimize patient recall bias. Health literacy was measured using the Rapid Estimate of Adult Health Literacy in Medicine-Short Form (REALM-SF).[2] Scores were categorized into grade ranges per the validated tool's instructions. These grade ranges were then regrouped into 2 categories (above eighth grade and eighth grade or less) based on whether the patient would be able to read most patient educational materials. Socioeconomic status was determined using the Area Deprivation Index.[27] In addition, the Patient-Reported Outcomes Measurement Information System (PROMIS) 43v2.1 Profile, Opioid Risk Tool (ORT), PROMIS Short Form v1.0-Prescription Pain Medication Misuse 7a, and Functional Comorbidity Index were completed by all patients.[10,23,38] To ensure adequate DNA sampling, 1 mL of saliva was obtained to determine OPRM1 DNA methylation levels and polymorphisms in CYP2D6, COMT (rs6269 A>G and rs4633 C>T), and OPRM1 (rs1799971 A>G) genes. These polymorphisms were included in our investigation because they have been reported to influence pain physiology and opioid medication effectiveness.[36]

2.3. Targeted DNA methylation assay design for OPRM1 gene

The PyroMark Assay Design software (Qiagen Inc, Valencia, CA) was used to design the assay. In designing the promoter CpG methylation assays, we used the Ensemble Genome Browser (https://useast.ensembl.org/index.html) to locate the gene sequence for the first exon and promoter region (1140 base pairs [bp] upstream of exon 1). We then copied the promoter sequence and used the UCSC (University of California, Santa Cruz) Genome Browser BLAT (BLAST-Like Alignment Tool) Search (http://genome.ucsc.edu/cgi-bin/hgBlat?command=start) to align the sequence with the UCSC genome sequence to confirm sequence match. Based on the ENSG00000112038 OPRM1 sequence, there were 8 CpG sites within the 1140 bp sequence fragment (Chr6: 154008740-154009880). We selected 2 CpG sites located at sequence coordinates upstream of the first exon (CpG site 1: 154009292 and CpG site 2: 154009356), which had not been previously described in association with opioid response.

The DNA sequence was first bisulfite converted by the software, and then, the designated and depicted CpG sites were

targeted to design polymerase chain reaction (PCR) and sequencing primers for PCR assay. The Pyrosequencing PCR and sequencing primers and CpG sites information for *OPRM1* DNA methylation assay are summarized in **Table 1**.

2.4. DNA methylation analysis

Genomic DNA from collected samples was extracted using a Qiagen DNA isolation kit (Qiagen Inc). The isolated DNA samples were quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and normalized to 20 ng/ul. A total of 400 ng of genomic DNA was then bisulfite converted using EpiTect Bisulfite Kit (Qiagen Inc) and quantified. The bisulfate-converted DNA was amplified by PCR and processed for DNA methylation analysis on a PSQ HS 96 Pyrosequencing instrument (Qiagen Inc).[15,26]

2.5. Cytochrome P450 2D6 genotyping and phenotype assignment

Samples were genotyped using a TaqMan genotyping assay for *CYP2D6 *2*, *3*, *4*, *6*, *8*, *10*, *17*,* and *41* (Applied Biosystems, Thermo Fisher Scientific). The TaqMan genotyping assays were performed per manufacturer's recommendations (Applied Biosystems, Thermo Fisher Scientific). The *CYP2D6* gene copy number variation (CNV) analysis for all DNA samples was determined by TaqMan Copy Number Assay (Applied Biosystems, Thermo Fisher Scientific). The RNase P assay was used as the internal control for copy number analysis (Applied Biosystems, Thermo Fisher Scientific). All samples for CNV analysis were run in quadruplicates along with Corriel DNA samples with known copy number (1, 2, and 3 *CYP2D6* gene copies) used as positive controls.[15,24] The TaqMan CNV assay was performed according to the manufacturer's recommendations and published protocol.[15] Relative quantification of *CYP2D6* gene copy number was performed by using CopyCaller software (Applied Biosystems, Thermo Fisher Scientific).

Alleles were assigned an activity score following Clinical Pharmacogenetics Implementation Consortium (CPIC) recommendations.[6] Normal activity alleles (ie, *1* and *2*) were assigned

a score of 1. Alleles with decreased activity (ie, *10*, *17*, and *41*) received a score of 0.5. Alleles with no activity (ie, *3*–*8*) received a 0 score. The sum of the activity scores for each allele of the diplotype (or gene score) was used to assign phenotype: 0, poor metabolizer (PM); 0.25 to 0.75, intermediate metabolizer (IM); 1 to 2, normal metabolizer (NM); and >2, ultra-rapid metabolizer (UM). Using methodology previously described and according to CPIC guidelines, phenotypes for patients who were prescribed a strong or moderate CYP2D6 inhibitor during enrollment were recategorized to reflect alterations in CYP2D6 enzyme activity resulting from medication interactions (phenoconversion).[28] Activity scores for these patients were multiplied by an inhibitor factor, 0 for strong (eg, bupropion, fluoxetine, paroxetine, quinidine, and terbinafine) and 0.5 for moderate (eg, abiraterone, cinacalcet, duloxetine, lorcaserin, mirabegron) inhibitors.[9]

The genotyping assay detected allele duplication but not which allele was duplicated or the number of allele copies, so ranged phenotypes were also a possibility in some patients (eg, NM to UM). Patients with ranged CYP2D6 phenotypes of IM to NM or NM to UM were excluded from analysis because phenotype could not be definitively assigned. Poor metabolizer and intermediate metabolizer phenotypes were combined as 1 variable.

2.6. Catechol-O-methyl transferase and OPRM1 polymorphisms

Genotyping for *COMT* (rs6269 A>G and rs4633 C>T) and *OPRM1* (rs1799971 A>G) was performed by TaqMan allelic discrimination using the fluorescence-based TaqMan Quant Studio Real-Time PCR System (Life Technologies/Fisher Scientific, Foster City, CA). The genotyping probes for TaqMan assays were purchased from Applied Biosystems/Fisher Scientific (Life Technologies/Fisher Scientific). The genotyping for single nucleotide polymorphism assays was performed and analyzed according to the manufacturer's recommendations (Life Technologies/Fisher Scientific).

2.7. Data analysis

Summary statistics are counts and percentages for categorical data and medians (quartiles) for continuous data. Bivariate and multivariable analyses were performed to investigate the association between variables and *OPRM1* DNA methylation (**Tables 2 and 3**). The average methylation of the CpG sites both together and individually were used to determine associations. In the bivariate analyses, associations of the *OPRM1* DNA methylation with categorical variables were investigated using the non-parametric Wilcoxon rank sum test. Correlations between continuous variables were assessed using the nonparametric Spearman ρ correlation coefficient. The Shapiro-Wilk test was used to test whether continuous data were normally distributed. In the multivariable analyses, general linear models were used to examine the relationships between the predictors and *OPRM1* DNA methylation. Any variable having a significant bivariate test at 0.15 level of significance was selected as a candidate for the multivariable analyses and entered the full multivariable model. This was performed to allow inclusion of other variables that might be important but did not make the cut at a $P < 0.05$ in the bivariate analyses. Then, we used an F test to investigate whether the reduced multivariable model including only the significant predictors at 0.05 level of significance would perform as well as the full model. The level of significance was set at 5%. That is, a P value was significant if it was less than 0.05. In addition, a

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Table 1

Pyrosequencing polymerase chain reaction and sequencing primers and CpG sites information for *OPRM1* DNA methylation assay.

Gene	*OPRM1* Chr:6 ENSG00000112038, NCBI Build 38
Location	Chromosome 6: 154008740-154009880, forward strand
Amplicon size	222 bp (154009211-154009433)
Bisulfite converted PCR primers	Forward-AGGAAGTAGAGATTGAATGAGTGATAA Reverse Biotinylated-AAAATCTATATCACAAACCATTATTTCTTA
Bisulfite converted Pyrosequencing primer	ATGAGTTTGAAGGAAATATATG
Sequence to analyze (CpG sites are highlighted, and their sequence coordinates are shown)	AAGTGGTAAA TAGAAGGAGA TA**Y** (154009292 CpG site 1) GGGATAAA AGGAGGTAAT TTATAAATAT AGATGGTTTT TTAATGTGTT TATATATATT TTTTTA**Y** (154009356 CpG site 2) GTT TTTTATTAAA ATAAATTTTG AATAAAATAT TTTTT

OPRM1, µ-opioid receptor 1; PCR, polymerase chain reaction.

Table 2

Findings from bivariate analyses (continuous variables).

Variable	N	Median (1st quartile, 3rd quartile)	Spearman ρ correlation with *OPRM1* % DNA methylation	*P* for correlation with *OPRM1* % DNA methylation
Age, y	112	56.5 (48.5, 63)	−0.141	0.140
Area Deprivation Index national percentile	111	78 (56, 90)	−0.096	0.315
Body mass index	111	32 (26.6, 39)	−0.044	0.648
Functional Comorbidity Index	112	4 (2, 5)	−0.149	0.118
Fatigue T score	112	58.8 (52.4, 65)	0.186	0.050
Depression T score	112	53.4 (48.3, 60.5)	−0.005	0.955
Anxiety T score	112	59.4 (49.85, 64.6)	0.069	0.470
Physical function T score	112	35 (31.1, 38.5)	0.053	0.578
Sleep disturbance T score	112	61 (52.3, 66.5)	0.022	0.820
Social roles T score	112	42.9 (36.2, 46.9)	−0.114	0.233
Pain interference T score	112	66.7 (63, 71.8)	0.076	0.425
Pain intensity score	112	8 (7, 9.5)	0.018	0.852
Opioid risk tool total score	112	1 (1, 6)	−0.038	0.690
Prescription pain medication T score	112	36.3 (36.3, 45.5)	0.047	0.623
OPRM1 % DNA methylation:	112	46 (30.5, 59.5)	—	—

OPRM1, μ-opioid receptor 1.

sensitivity analysis was performed to examine significant associations with DNA methylation at each site independently and compared with the results from the analysis of the 2 sites combined. All analyses were performed in SAS for Windows version 9.4 or later.

3. Results

Data from 112 patients were analyzed. The median age was 56.4 years, 67% were women, and 56% self-reported as non-White (62 African American, 49 White) (**Tables 2 and 3**). The sensitivity analyses revealed that DNA methylation levels for the 2 sites were highly correlated (Spearman correlation coefficient of 0.997, $P < 0.0001$). The bivariate analyses revealed that the same independent predictors were associated with DNA methylation levels when each site was considered separately and when combined, as the average DNA methylation between both sites. In the multivariable analyses, the magnitude of effects for each individual site were within 10% of the magnitude of effects found when the average DNA methylation was analyzed; therefore, we decided to report the average methylation levels for sites 1 and 2 together.

Based on their association with the *OPRM1* DNA methylation in the bivariate analyses, the candidate predictors for inclusion in multivariable analyses were age (Spearman $\rho = -0.14$, $P = 0.140$), Functional Comorbidity Index (FCI) score (Spearman $\rho = -0.15$, $P = 0.118$), fatigue T score (Spearman $\rho = 0.19$, $P = 0.050$), sex ($P = 0.102$), REALM-SF score ($P = 0.072$), degenerative disk disease ($P = 0.017$), substance abuse comorbidity ($P = 0.040$), prescription opioid use for chronic head or neck pain ($P = 0.133$), prescription opioid use for knee, hip, or shoulder ($P = 0.082$), and opioid use duration ($P = 0.061$) (**Table 3**). The full model had an $R^2 = 0.306$, F(11,98) = 3.93, $P < 0.0001$ (**Table 4**). The reduced model including FCI score, fatigue T score, REALM-SF score, degenerative disk disease, substance abuse comorbidity, and opioid use duration had an $R^2 = 0.290$, F(7, 102) = 5.95, $P = < 0.0001$, with all the predictors having a significant contribution to the model. This reduced model performed as well as the full model, R^2-change = 0.014, F(3,

99) = 0.700, $P = 0.554$. Thus, the best-fitted multivariable model indicated, compared with their counterparts, in patients with chronic musculoskeletal pain, reading level >eighth grade, degenerative disk disease, substance abuse comorbidity, and opioid use ≤1 year (compared with >5 years) were associated with average methylation levels that were 7.7% (95% CI 0.95%, 14.4%), 11.7% (95% CI 2.7%, 21.1%), 21.7% (95% CI 10.7%, 32.5%), and 16.1% (95% CI 3.3%, 28.8%) higher than the reference group, respectively. Each 1 unit increase in FCI was associated a 2.2% (95% CI 0.64%, 3.7%) lower methylation level, while each fatigue T score unit increase was associated with 0.45% (95% CI 0.08%, 0.82%) higher methylation level.

4. Discussion

μ-opioid receptor 1 methylation levels varied by several factors in our study population of patients with chronic musculoskeletal pain using prescription opioids. Patients with a history of degenerative disk disease, substance abuse comorbidity, reading level over eighth grade, and prescription opioid use of 1 year or less were associated with greater methylation levels compared with their counterparts. This increase in methylation levels suggests these patients may have reduced *OPRM1* gene transcription and, subsequently, less μ-opioid receptor production, potentially diminishing the effectiveness of consumed opioid medications. A history of heroin addiction has previously been linked with increased *OPRM1* methylation, and our findings associating substance abuse with higher *OPRM1* methylation reaffirm these findings.[22] However, we also identified novel associations, in a mostly minority disadvantaged population, which highlight the complexity of the relationship between epigenetics, pain, and the use of prescription opioid medications.

Patients with degenerative disk disease in our study population had higher *OPRM1* methylation levels compared with those with other painful conditions, such as fibromyalgia, rheumatoid arthritis, osteoarthritis, lupus, and diabetes. Certain pain conditions have previously been associated with variation in DNA methylation levels.[26,29] For example, osteoarthritis has been linked to hypomethylation of inflammatory-associated genes,

Table 3

Findings from bivariate analyses (categorical variables).

Variable	Category	N (%)	Median *OPRM1* DNA methylation	*P**
Gender	Female	67 (60)	41 (28, 58)	0.102
	Male	45 (40)	50 (36, 60)	
Race	White	49 (44)	46 (29, 60)	0.610
	Non-White	63 (56)	46 (31, 59)	
Education	Less than high school	23 (21)	41 (24, 59)	0.752
	High school	46 (41)	42.5 (33, 61)	
	Some college, vocational, or technical school, or 2-year college degree	34 (30)	50 (32, 60)	
	Four-year college or advanced degree, postgraduate degree	9 (8)	53 (31, 56)	
Smoking status	Smoker	69 (62)	46 (29, 61)	0.988
	Nonsmoker	43 (38)	46 (34, 56)	
Health literacy†	Reading level < eighth grade	52 (46)	38 (29.5, 56.5)	0.072
	Reading level $\geq$ eighth grade	59 (54)	51 (33, 62)	
Medications, *CYP2D6* phenotype, polymorphisms				
Opioid duration of action	Short + long acting	2 (2)	42.5 (24, 61)	0.595
	Long acting only	2 (2)	57 (48, 66)	
	Short acting only	108 (96)	46 (30.5, 59)	
Opioid treatment duration (y)	<1	9 (8)	59 (46, 67)	0.061
	1–5	59 (53)	51 (32, 61)	
	>5	43 (39)	39 (28,53)	
CYP2D6 phenotype	Normal metabolizer (AS 1–2)	73 (65)	46 (31, 58)	0.410
	Poor metabolizer (AS 0)+ intermediate metabolizer (AS 0.25–0.75)	30 (35)	40 (27, 58)	
OPRM1 rs1799971 A>G	AA	94 (85)	46.5 (31,60)	0.809
	AG	16 (15)	50.5 (25.5, 60.5)	
COMT rs6269 A>G	AA	41 (37)	51 (32, 61)	0.820
	AG	57 (52)	46 (32, 59)	
	GG	11 (11)	47 (31, 55)	
COMT rs4633 C>T	CC	39 (36)	42 (31, 57)	0.631
	CT	52 (47)	51 (31.5, 62)	
	TT	19 (17)	46 (28, 57)	
Comorbidities				
Substance abuse	Yes	13 (12)	57 (36, 69)	0.040
	No	99 (88)	46 (28, 59)	
Hypertension	Yes	85 (76)	46 (30, 59)	0.380
	No	27 (24)	51 (32, 60)	
Obesity	Yes	58 (52)	50.5 (32, 61)	0.348
	No	54 (48)	42 (29, 58)	
Diabetes	Yes	37 (33)	48 (33, 59)	0.542
	No	75 (67)	46 (28, 60)	
Osteoarthritis (not including spine)	Yes	60 (54)	45 (31.5, 59.5)	0.868
	No	52 (46)	48.5 (29, 59.5)	
Degenerative disk disease	Yes	17 (15)	59 (51, 64)	0.017
	No	95 (85)	42 (29, 58)	
Other chronic musculoskeletal pain (nonimmunologic)	Yes	101 (90)	47 (32, 59)	0.434
	No	11 (10)	39 (21, 62)	
Rheumatoid arthritis	Yes	8 (7)	55 (35.5, 59)	0.307
	No	104 (93)	46 (29.5, 59)	
Fibromyalgia	Yes	9 (8)	52 (24, 56)	0.690
	No	103 (92)	46 (31, 60)	
Lupus	Yes	4 (4)	42 (21.5, 59)	0.695
	No	108 (96)	46 (30.5, 59.5)	
Indication for chronic opioid use				
Surgery	Yes	5 (4)	41 (28, 51)	0.751
	No	107 (96)	46 (31, 60)	
Head or neck pain	Yes	31 (28)	36 (28, 61)	0.133
	No	81 (72)		
Back pain	Yes	97 (87)	46 (30, 60)	0.786
	No	15 (13)	46 (31, 56)	
Arthritis pain (more than 1 area/joint)	Yes	40 (36)	43.5 (30.5, 62)	0.990
	No	72 (64)	48.5 (30.5, 59)	
Fibromyalgia	Yes	8 (7)	50 (32, 55.5)	0.933
	No	104 (93)	46 (30.5, 60)	

Table 3 (*continued*)

Findings from bivariate analyses (categorical variables).

Variable	Category	N (%)	Median *OPRM1* DNA methylation	*P**
Hip, knee, or shoulder pain	Yes	64 (57)	39.5 (28, 57.5)	0.082
	No	48 (43)	51.5 (33.5, 61.5)	
Trauma	Yes	7 (6)	50 (29, 61)	0.904
	No	105 (94)	46 (31, 59)	

Data are medians (first and third quartiles).
* Wilcoxon rank sum test.
† REALM-SF scores 0 to 6 = reading level ≤ eighth grade, scores 7+ = reading level > eighth grade.
OPRM1, μ-opioid receptor 1.

resulting in increased gene expression.[29] However, increased methylation levels in *OPRM1* have not previously been associated with specific pain conditions. Our finding may be the result of a global increase in methylation levels that is not specific to the *OPRM1* gene, but rather due to increased methylation in several yet to be identified genes, as was discovered in patients with osteoarthritis.[28] Still, it is unclear why patients in our study population with degenerative disk disease would have higher methylation levels than patients with other chronic painful conditions, when controlling for other reported methylation-increasing factors such as history of substance abuse and duration of opioid use. It is also unclear why lower *OPRM1* methylation was associated with higher FCI scores. These findings require further investigation to determine whether other unmeasured factors (such as *COMT* methylation levels) may account for these associations.

Ethnicity, age, smoking, socioeconomic status, and obesity have all previously been associated with variability in DNA methylation.[21,33,35] African-American heroin users, for example, were shown to have lower *OPRM1* methylation levels compared with non-African American heroin users.[21] In our diverse chronic musculoskeletal pain population, age, ethnicity, and health literacy were significantly associated with *OPRM1* methylation in univariate testing; however, only health literacy remained significant in the final model. Low socioeconomic status is associated with increased

morbidity and mortality,[1] and both health literacy and DNA methylation have been suggested as mediators of the association between socioeconomic status and health-related outcome disparities.[5,12,16,30–32] Previous studies have identified several life course socioeconomic status indicators associated with variable methylation levels depending on the indicator and specific period measured during the life course (eg, childhood vs adulthood).[3,19] Socioeconomic status indicators associated with global hypomethylation included living in an area of deprivation as an adult, occupations requiring manual labor, and, conversely, higher adult educational attainment.[17,34] However, contrary to the findings of the latter study, we found patients with higher health literacy (or higher socioeconomic status) had increased methylation levels compared with those with lower health literacy levels. This discrepancy may indicate that health literacy, although a socioeconomic status indicator, may be associated with biological processes differently than other described indicators, such as manual labor occupations or education level.

Previous studies have linked chronic opioid exposure with increased DNA methylation in both patients with opioid addiction and those with pain.[8] However, our results suggest *OPRM1* epigenetic changes may be more nuanced, with degree of methylation varying depending on the duration of chronic opioid use (16.1% higher in opioid users of ≤1 year than those >1 year). This contrasts somewhat with findings from a recent study in

Table 4

Regression coefficients (β) and 95% confidence interval from general linear models.

Variable	Full model β (95% CI)	Reduced Model β (95% CI)
Intercept	55.34 (21.30, 89.38)	34.35 (10.71, 57.99)
Functional Comorbidity Index	−1.89 (−3.60, −0.18)	−2.16 (−3.69, −0.64)
Fatigue T score	0.43 (0.06, 0.81)	0.45 (0.08, 0.82)
REALM_SF, score 7 or higher REALM_SF, score 0–6	8.10 (14.94, 1.26) Ref	7.69 (0.95, 14.44) Ref
Degenerative disk disease, yes	11.62 (2.11, 21.12)	11.87 (2.66, 21.07)
Degenerative disk disease, no	Ref	Ref
Substance abuse, yes Substance abuse, no	21.45 (10.39, 32.50) Ref	21.68 (10.86, 32.49) Ref
Opioid use duration, <1 y Opioid use duration, 1-5 y Opioid use duration, >5 y	14.89 (1.84, 27.94) 5.68 (−1.79, 13.17) Ref	16.07 (3.33, 28.81) 6.91 (−0.16, 13.98) Ref
Age, y	−0.02 (−0.34, 0.31)	—
Gender, female Gender, male	−2.38 (−9.72, 4.96) Ref	— —
Prescription opioid use for head/neck, yes Prescription opioid use for head/neck, no	−1.88 (−9.99, 6.22) Ref	— —
Prescription opioid use for hip/knee/shoulder, yes Prescription opioid use for hip/knee/shoulder, no	4.44 (2.42, 11.30) Ref	— —

CI, confidence interval; REALM-SF, rapid estimate of adult health literacy in medicine-short form.

opioid-naïve patients prescribed opioids after dental surgery, where higher opioid dose and duration of use predicted increased *OPRM1* methylation within weeks of opioid discontinuation.[25] Of course, these previous findings were in the context of acute pain and brief opioid use, which may explain the discrepant results. Nonetheless, the clinical consequences of variability in *OPRM1* methylation have yet to be fully elucidated, as evidenced by conflicting findings reported in the literature. DNA methylation at *OPRM1* was associated with increased pain intensity in surgical nonchronic opioid users; however, global methylation and not *OPRM1* methylation was associated with increased pain in patients with chronic pain using prescription opioids for more than 1 year.[4,8] We also did not find *OPRM1* methylation levels to be associated with pain intensity in our study population of patients with chronic musculoskeletal pain on long-term prescription opioids. We did, however, find PROMIS fatigue T scores to be positively associated with methylation levels. Thus, it may be that the effect of *OPRM1* methylation on the pain experience may depend on the history of opioid use and duration of opioid use, with nonchronic opioid users and those using opioids for 1 year or less having higher levels of methylation and pain.

Our findings should be interpreted in light of the limitations of the study. First, our sample size was relatively small and quite heterogeneous, which may have reduced our power to detect some significant associations, particularly those pertaining to *CYP2D6*, *OPRM1*, and *COMT* polymorphisms. Secondly, we examined only 2 CpG sites of the 8 sites present in the 1140 bp sequence upstream of exon 1, limiting our ability to detect other important associations. However, this limitation does not diminish our reported findings. Thirdly, the cross-sectional design precludes conclusions regarding the temporal direction of the observed associations or whether a cause-and-effect relationship exists. Future prospective studies are needed to address this issue. In addition, we did not collect information regarding the duration of chronic pain; therefore, how long the patients have been with pain might affect the association observed between duration of opioid use and DNA methylation level. Lastly, we examined only methylation of 1 gene, and additional research is needed to determine whether epigenetic changes extend to other genes. However, given our limited understanding of the interindividual differences in pain and response to opioid medications, the novel associations identified in our mostly minority and disadvantaged study population should be further explored and characterized in a larger population and to determine clinical utility. Characterizing epigenetic changes along the continuum of opioid use, misuse/abuse, and addiction may provide a comprehensive picture of the complex interplay between environmental and genetic influences leading to opioid addiction. This may offer an opportunity to improve the management of chronic pain and possibly steer a patient's trajectory away from opioid addiction.

Disclosures

The authors have no conflict of interest to declare.

Acknowledgments

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