Original Research *Feature article*

HUMAN STUDY *COMT* and *DRD3* haplotype-associated pain intensity and acute care utilization in adult sickle cell disease

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Impact Statement

Pain intensity and acute care utilization for painful crises are major issues in management of sickle cell disease (SCD). Several catecholamine-related single-nucleotide polymorphisms (SNPs) in the catecholamine-O-methyltransferase (COMT) and dopamine receptor D3 (DRD3) genes have been associated with pain, analgesia, and disability indicators in various pain populations. A prior study indicated that a COMT SNP (rs4680) and DRD3 (rs6280) were associated with utilization for pain in adults with SCD as well as heterogeneity of other pain outcomes. In this study, we sought to extend the analysis to gene-sparing haplotypes of COMT SNPs and DRD3 SNPs, to investigate possible associations with pain-related acute care utilization in a cohort of individuals diagnosed with SCD. Of note, some of the SNPs included in this manuscript have never been reported on for pain and are significant. Improved understanding of the contributors to varying pain phenotypes in SCD could lead to improved pain management.

Abstract

A previous exploratory analysis of a COMT gene single-nucleotide polymorphism (SNP) and a DRD3 SNP by our group suggested possible contributions to pain-related acute care utilization in people with sickle cell disease (SCD). Our aim was to extend the analysis to gene-spanning haplotypes of COMT SNPs and DRD3 SNPs to investigate possible associations with pain intensity and pain-related acute care utilization in an SCD cohort. Genotyping was conducted, and clinical data were collected, including self-reported pain intensity using PAINReportIt® (average of current pain and least and worst in past 24 hours, average pain intensity [API]) and medical record-extracted, pain-related acute care utilization data of 130 adults with SCD. Haplotype blocks were identified based on linkage disequilibria (COMT = 7 haploblocks; DRD3 = 8 haploblocks). Regression analyses were tested for association between haplotypes and API and utilization, yielding several significant findings. For COMT block 1 (rs2075507, rs4646310, rs737865), the A-G-G haplotype was associated with higher API compared to the reference A-G-A ($p = 0.02$), whereas the A-A-A haplotype was associated with higher utilization ($p = 0.02$). For DRD3 block 2 (rs9817063, rs2134655, rs963468, and rs3773679), relative to reference T-C-G-C, the T-T-G-C haplotype was associated with higher utilization ($p = 0.01$). For DRD3 block 4 (rs167770, rs324029, and rs324023), the A-G-T haplotype was associated with higher API ($p = 0.04$) and utilization ($p < 0.001$) relative to reference G-A-T, whereas the A-A-T haplotype was associated with higher utilization ($p = 0.01$). We found COMT and DRD3 haplotypes associated with pain-related SCD features, suggesting that in future studies more emphasis be placed on cis effects of SNP alleles in evaluating genetic contributions to SCD pain and acute care utilization for pain.

Keywords: Polymorphism, linkage disequilibrium, haploblock, PainReportIt, healthcare

***Experimental Biology and Medicine* 2022; 247: 1601–1608. DOI: 10.1177/15353702221080716**

Introduction

Sickle cell disease (SCD), a hemoglobinopathy that affects approximately 100,000 individuals in the United States,[1] is marked by recurrent acute painful episodes from vaso-occlusion due to sickled red blood cells. Pain intensity and acute care utilization for painful crises are major issues in management of SCD. Several catecholamine-related SNPs in the catecholamine-O-methyltransferase (*COMT*) and dopamine receptor D3 (*DRD3*) genes have been associated with pain, analgesia, and disability indicators in various pain populations.[2,3] In particular, *COMT* SNPs (rs4680,[4] rs4633, and rs165599)[5] and a *DRD3* SNP (rs6280)[3] were associated with pain-related acute care utilization in SCD. Exploratory findings from studies examining *COMT* and *DRD3* variants have suggested a link to pain heterogeneity in patients who have SCD.[3,5,6] Thus, the purpose of our study was to test for association among haplotypes of 14 *COMT* and 16 *DRD3* SNPs with pain intensity and pain-related acute care utilization in an SCD cohort.

COMT and *DRD3* have functions relevant to pain. The COMT protein plays a role in the degradation of catecholamine neurotransmitters.[7] It is expressed in multiple tissues that include neurons. *COMT* gene variants have been associated with Alzheimer's, schizophrenia, major depressive disorder, and other neurological traits and conditions including pain sensation.[4] The dopamine receptor D3, encoded by *DRD3*, is a high-affinity receptor, mediating dopaminergic effects in the central nervous system.[2,3,8,9] This receptor is expressed in more primitive regions of the brain and is believed to be involved in emotional, endocrine, and cognitive function. The activity of this receptor is mediated by G proteins that inhibit adenylyl cyclase. This receptor mediates some effects of antipsychotic drugs and drugs used to treat Parkinson's disease like Levodopa[10] that has side effects of extrapyramidal reactions and tardive dyskinesia.

Apart from healthcare utilization, chronic pain is a common complication of SCD. In the Pain in Sickle Cell Epidemiology Study, Smith et al. found 29% of patients reported nearly daily pain and 54% reported pain on more than 50% of daily pain diary entries.[11] Similarly in a cross-sectional analysis, Wilkie et al. found nearly two-thirds of adult SCD patients reported pain at the time of a routine clinic visit and 80% described their pain as constant, continuous, or steady.[12]

The underlying causes of individual differences in SCD pain remain largely unknown. An improved understanding of the contributors to, and predictors of, varying pain phenotypes in SCD could lead to the design of personalized pain management plans to improve quality of life. Similar to other chronic pain syndromes, findings from several SCD studies suggest that genetic polymorphisms may account for at least some of the pain heterogeneity.[13] The mesolimbic monoamine system, which involves the COMT and DRD3 proteins among others, is of particular interest, given the availability of therapeutic agents to regulate dopamine expression and potentially pain-related phenotypes.[2,14,15]

A prior study by our group[3] indicated that a *COMT* SNP (rs4680) and *DRD3* (rs6280) were associated with utilization for pain in adults with SCD as well as heterogeneity of other pain outcomes. This finding led to the consideration that additional genetic variants in these genes may contribute to predicting SCD pain outcomes. Thus, the aim of this study is to evaluate *COMT* and *DRD3* multiple-SNP and haplotypes, to represent spans of the entire genes, for associations with SCD pain-relevant outcomes, pain intensity and utilization for pain, in the same cohort of adult outpatients with SCD.

Materials and methods

Design

This cross-sectional study was approved by the Institutional Review Board (IRB) at the University of Illinois at Chicago (UIC). The IRB at the University of Florida approved the study as exempt for use of de-identified data obtained via a data use agreement.

Participants

Participants were recruited from the Sickle Cell Clinic at the University of Illinois Hospital and Health System (UI). One hundred-thirty individuals met eligibility criteria and completed the study. Eligibility criteria included: age $\geqslant$ 18 years of age, able to read and speak English, diagnosed with SCD, scheduled for continuing care at the UI Sickle Cell Clinic, in addition to routine laboratories, the participant was able to withstand the removal of an additional 8.5 mL of blood. Exclusion criteria included: participants on a chronic transfusion program, legally blind, and/or incapable of physically completing the study questionnaire. Participants provided written informed consent prior to the start of all procedures. This study was conducted in accordance with the Declaration of Helsinki.

Procedures

Participants were approached at the UI sickle cell clinic, during scheduled clinic visits, if they met eligibility criteria. A research assistant explained the study procedure to the patients and obtained written informed consent if they agreed to participate. During the visit, participants completed their initial data collection, prior to hospital discharge, or at home. There was a 24-month period in which participants were followed, they received phone calls at 2 week intervals to capture acute care visit data outside of the UI. Buccal or blood samples were obtained for the purpose of DNA extraction.

Measures

DNA and genotyping. SNPs were chosen across the two genes based on having an African American minor allele frequency of at least 0.025, including tag SNPs, to represent haploblocks spanning the length of each gene. As previously reported, DNA was extracted from blood using the QuickGene-mini80 isolation device and QuickGene DNA whole blood extraction method (Autogen, Holliston, Massachusetts) or from buccal samples using a modified phenol/chloroform procedure adopted from Vandenbergh et al.[3,16] DNA samples were aliquoted and stored at –80°C.

COMT and *DRD3* polymorphisms were genotyped using the MassARRAY iPLEX Platform (Sequenom, CA, USA) according to previously published methods, and Hardy–Weinberg equilibrium were examined for each.[2,17]

Pain intensity. Participants utilized PAIN*Report*It,[12,18] a multidimensional computerized pain assessment tool, to record pain intensity. PAIN*Report*It is an electronic touch screen format of the 1970 version of the McGill Pain Questionnaire,[19] it requires little to no previous computer experience, and may be self-administered by the patient. We computed average pain intensity (API, as the average of current, least and worst pain in the past 24 hours) rated on a 0 to 10 pain intensity number scale (PINS), which is part of PAIN*Report*It.[20] The PINS is a valid and reliable measure of pain intensity and is predictive of future acute care utilization for SCD.[18] Participants were not in a sickle crisis at the time of sample collection or pain reporting.

Utilization. Utilization was defined as visits to the SCD acute care center to treat SCD pain, or to the emergency department or hospital admissions. At the UI site, acute care utilization events were mined from the electronic health record with excellent inter-rater reliability as reported elsewhere.[21] Documentation of acute care utilization at other facilities was obtained from logs of phone calls to participants every 2 weeks. [21] The number of pain-related utilization events over 12 months served as a surrogate marker for acute pain utilization in SCD.

Haplotype and statistical analysis. There is well-established precedence for using haplotypes instead of individual SNPs in genetic association studies, as haplotypes can confer a stronger function than individual SNPs, such as in the *COMT* gene.[22] We examined the pairwise linkage disequilibrium (LD) between SNPs using the scaled metric D.' The LD heat maps were generated using the *R* package LDHeatmap with the magnitude of D' represented by gray scale and positions of SNPs marked on the diagonal line.[23] SNPs were then partitioned into haplotype blocks, within which there was little evidence of historical recombination in the cohort, based on the values of D',[24] using the *R* package trio (Bioconductor.org).

Estimates of the haplotype probabilities for each haplotype block were then obtained using maximum likelihood methods accounting for linkage phase ambiguities as well as missing genotype values, followed by regression analysis of association between haplotypes and the pain outcomes.[25] In our regression analysis of each haplotype block, the most frequent haplotype for that block was always used as the reference. Population reference haplotype frequency data for Southwestern US African Americans were available from the LDhap tool (https://ldlink.nci.nih.gov/?tab=ldhap), based on the 1000 Genomes project, and are shown in Tables 3 and 4 (LDhap freq) along with cohort haplotype frequencies. As this was an exploratory study of haplotypes, multiple testing correction was not utilized.

Table 1. Summary of sample demographics (N = 130).

Variable	Category	Frequency (%)
Sex	Female	86 (66%)
	Male	44 (34%)
Race	Black	127 (98%)
	White	3 (2%)
Ethnicity	Hispanic	2 (2%)
	Non-Hispanic	128 (98%)
Sickle cell type	SS	99 (76%)
	SC	15 (12%)
	Other	16 (12%)
	Statistic	**Value**
Age (years)	Mean (SD)	35.0 (11.4)
	Range	19 – 70
Utilization	Mean (SD)	4.5 (5.3)
Average Pain Intensity	Mean (SD)	4.0 (2.7)

SD: standard deviation.

Results

Descriptive statistics

Sample characteristics. One-hundred thirty adults with SCD participated. The subject mean age was 35.0 ± 11.4 years. Nearly all of the participants were African American, 66% were female, and 76% had the HgbSS genotype. The other demographic characteristics of the sample appear in Table 1.

API and utilization. The mean API was 4.0 ± 2.7, and patients had on average 4.5 ± 5.3 acute care visits for SCD pain treatment during the 12 month period after enrolling in the study.

COMT and DRD3 genotyping and haplotype construction. Based on pairwise LD for *COMT* and *DRD3* SNPs, they were partitioned into seven and eight haploblocks, respectively, each consisting of 1-4 SNPs. The 14 *COMT* SNPs (rs2075507, rs737865, rs4646312, rs4633, rs6269, rs165656, rs165728, rs165774, rs174697, rs740602, rs769224, rs4646310, rs4646316, and rs9332377) and 16 DRD3 SNPs (rs167770, rs167771, rs2087017, rs2399504, rs3773679, rs7611535, rs1394016, rs324023, rs324026, rs324029, rs905568, rs1800828, rs2134655, rs963468, rs3732783, and rs9817063) are described in Table 2, and haplotypes within each haploblock are described in Table 3 (*COMT*) and Table 4 (*DRD3*) (including frequencies). Haplotype frequencies are not significantly different from that of the 1000 Genomes project for Southwestern US African Americans, the only US African American population in that project (chi-square $p > 0.05$). Some of the allele frequencies differ between the cohort and dbSNP for African Americans, which is most likely due to regional differences, cohort size, and genetic admixture.

COMT haplotype analysis

Regression analysis of haplotypes with API and utilization revealed no significant associations with API and utilization for *COMT* blocks 2–7. For the first haploblock (rs2075507,

Table 2. *COMT* and *DRD3* SNPs and Haploblock assignments.

SNP ID	Gene	Chromosome position (build 38)	Detail	Minor allele (*also dbSNP ref. allele)	Minor allele freq. (Afr. Amer. dbSNP)	SCD cohort minor allele frequency	Haploblock
rs2075507	COMT	22:19940569	Upstream	G*	0.398	0.324	1
rs4646310	COMT	22:19941283	Upstream	A	0.049	0.050	1
rs737865	COMT	22:19942598	Upstream	G	0.140	0.159	1
rs4646312	COMT	22:19960814	Intron	C	0.163	0.154	2
rs165656	COMT	22:19961340	Intron	C	0.418	0.465	2
rs6269	COMT	22:19962429	5'UTR	G	0.366	0.369	3
rs4633	COMT	22:19962712	His62=	T	0.334	0.364	3
rs740602	COMT	22:19962745	Gln73=	A	0.178	0.191	3
rs769224	COMT	22:19964281	Pro199=	A	0.087	0.050	4
rs4646316	COMT	22:19964609	Intron	T	0.197	0.148	4
rs165774	COMT	22:19965038	Intron	A	0.216	0.248	5
rs174697	COMT	22:19966309	Intron	A*	0.185	0.143	6
rs9332377	COMT	22:19968169	Intron	T	0.273	0.325	7
rs165728	COMT	22:19969500	3'UTR	C*	0.060	0.031	7
rs2087017	DRD3	3:114123166	dnstream	A	0.438	0.491	1
rs9817063	DRD3	3:114128261	3'UTR	C	0.379	0.342	2
rs2134655	DRD3	3:114139354	Intron	T	0.028	0.060	2
rs963468	DRD3	3:114144040	Intron	A	0.089	0.093	2
rs3773679	DRD3	3:114150488	Intron	T	0.124	0.115	2
rs167771	DRD3	3:114157428	Intron	A	0.218	0.273	3
rs167770	DRD3	3:114160715	Intron	A	0.377	0.421	4
rs324029	DRD3	3:114162776	Intron	G	0.281	0.389	4
rs324023	DRD3	3:114166548	Intron	C	0.279	0.310	4
rs3732783	DRD3	3:114171942	Ala17=	C	0.119	0.116	5
rs324026	DRD3	3:114172195	Intron	T	0.266	0.315	6
rs1800828	DRD3	3:114172702	Intron	G	0.140	0.165	6
rs1394016	DRD3	3:114191042	Intron	G*	0.167	0.170	7
rs7611535	DRD3	3:114205296	Upstream	T	0.103	0.151	7
rs2399504	DRD3	3:114219388	Upstream	T	0.081	0.087	7
rs905568	DRD3	3:114232449	Upstream	G	0.411	0.243	8

SNP: single-nucleotide polymorphism; SCD: sickle cell disease.

rs4646310, and rs737865), haplotypes A-G-A, G-G-A, A-G-G, and A-A-A occurred with 47%, 32%, 16%, and 5% frequencies, respectively. Relative to A-G-A, the A-G-G haplotype was associated with higher API ($p = 0.02$), whereas the A-A-A haplotype was associated with higher acute care utilization ($p = 0.02$) (Table 3).

DRD3 haplotype analysis. There were no significant associations with API or utilization for *DRD3* blocks 1, 3, 5–8 (Table 4). For the second block (rs9817063, rs2134655, rs963468, and rs3773679), haplotypes T-C-G-C, C-C-G-C, C-C-A-T, T-T-G-C, C-C-G-T, and C-C-A-C occurred at 60%, 21%, 8%, 6%, 3%, and 1% frequencies, respectively. Relative to T-C-G-C, the T-T-G-C haplotype was associated with higher utilization ($p = 0.01$) (Table 4). In the fourth block (rs167770, rs324029, and rs324023), haplotypes G-A-T, A-G-C, A-G-T, and A-A-T occurred with 58%, 31%, 8%, and 3% frequencies, respectively. Relative to reference G-A-T, the A-G-T haplotype was associated with higher API ($p = .04$) and higher utilization ($p < .001$), whereas A-A-T was associated with higher utilization ($p = .01$).

Discussion

Individual SNPs do not represent the contribution of the linked interacting variants; thus, haplotype analysis has the potential to reveal a greater genetic effect. Accordingly, based on our promising previous single-SNP studies of COMT and DRD3 in a SCD cohort, we performed the first analysis of *COMT* and *DRD3* haplotypes in SCD to test for associations with the two pain outcomes (average pain intensity and pain-related acute care) in this 98% African American cohort.

We found haplotypes in one *COMT* haploblock and two *DRD3* haploblocks that were significantly associated with one or both pain outcomes. The first *COMT* haploblock was significantly associated with both pain outcomes. This block maps to the *COMT* promoter, which suggests that the underlying effect might be related to level of gene expression (and presumably, protein level). *DRD3* block 3 was associated with utilization, and block 4 was associated with API and utilization. These blocks flank exon 6 in the middle of the gene. Thus, effects within the haplotypes could arise from cis coding sequences and/or regulatory intronic sequences. Both *COMT* and *DRD3* genes are associated with chronic and acute pain in patients who have SCD. These haplotypes are important for understanding the genomic contributions to SCD pain. This study is the basis for future work to validate the findings, which will provide further understanding of genomic contributions of pain in patients with SCD.

To our knowledge, there are no studies of acute and chronic pain in African American adults, including the *COMT* SNPs rs5075507, rs464310, and rs737865. For SNP

Table 3. COMT association analysis of haplotypes with patient outcomes (REF = reference haplotype).

Gene and haploblock	First SNP in haplotype (allele below)	Next SNP in haplotype (allele below)	Next SNP in haplotype (allele below)	Cohort Haplo-type freq	LDhap freq (S.W. Afr-Amer.)	API *p* value relative to reference haplotype	Utilization *p* value relative to reference haplotype
COMT 1	rs2075507	rs4646310	rs737865	–	–	–	–
	A	A	A	0.05	0.06	0.06	**0.02**
	A	G	G	0.16	0.16	**0.02**	0.07
	A	G	A	0.47	0.42	REF	REF
	G	A	A	0.00	0	–	–
	G	G	G	0.00	0	–	–
	G	G	A	0.32	0.36	0.08	0.21
COMT 2	rs4646312	rs165656	–	–	–	–	–
	C	C	–	0.00	0	–	–
	C	G	–	0.15	0.15	0.76	0.11
	T	G	–	0.47	0.45	REF	REF
	T	C	–	0.38	0.40	0.14	0.10
COMT 3	rs6269	rs4633	rs740602	–	–	–	–
	A	C	G	0.27	0.34	0.07	0.32
	A	T	G	0.36	0.32	REF	REF
	G	C	A	0.20	0.14	0.54	0.14
	G	C	G	0.17	0.20	0.37	0.07
	G	T	A	0.00	0	–	–
	G	T	G	0.00	0	–	–
COMT 4	rs769224	rs4646316	–	–	–	–	–
	A	C	–	0.00	0	–	–
	A	T	–	0.05	0.09	0.37	0.39
	G	C	–	0.85	0.76	REF	REF
	G	T	–	0.10	0.15	0.60	0.75
COMT 5	rs165774	–	–	–	–	0.71	0.23
COMT 6	rs174697	–	–	–	–	0.40	0.74
COMT 7	rs9332377	rs165728	–	–	–	–	–
	C	T	–	0.64	0.57	REF	REF
	C	C	–	0.03	0.10	0.74	0.69
	T	C	–	0.00	0	–	–
	T	T	–	0.32	0.34	0.94	0.51

SNP: single-nucleotide polymorphism; API: average pain intensity.

rs2075507, pain-related studies were focused primarily on European populations with lower back pain.[26] No pain studies focused on African Americans that examined SNP rs4646310, and for SNP rs737865, only pain studies of brain function were found[27] but did not focus on African Americans. In a literature search for our *DRD3* SNPs of interest in African American populations that were associated with pain-related phenotypes, we found no studies. SNPs rs9817063, rs9817063, rs2134655, rs963468, rs3773679, rs167770, and rs324029 were reported in studies involving Parkinson's disease, depression, obsessive compulsive disorder, tardive dyskinesia, attention deficit hyperactivity disorder, and schizophrenia.[28–35] No previously reported studies were found for rs324023.

The relevance of our findings to pain research and practice is related to the known functions of the COMT and DRD3 genes. The COMT protein is involved in the degradation of catecholamine neurotransmitters (epinephrine, norepinephrine, and dopamine) and catecholestrogens (e.g. estrogen). It inactivates extracellular dopamine levels.[22,36,37] Although the sample size is too small to reliably evaluate results by gender, there is precedent for potential gender differences,[38–43] albeit not in SCD. *COMT* haplotype HPS (coding for low-COMT activity) was found associated with increased experimental pain perception (capsaicin) in women, but not men.[42] In addition, *COMT* expression is lower in women compared to men, affecting baseline COMT-dependent sensitivity to pain.[4,43] The DRD3-encoded dopamine receptor affects the degree to which dopamine can trigger downstream signaling.[44] Thus, variants affecting levels or function of either protein can impact dopamine transmission, affecting nociception.

Limitations of this study include its small sample that was limited to a cohort in the US Midwest. Further, three subjects were not African American and could have different haploblocking. The allele frequency data support the presence of genetic heterogeneity in the African American population, likely both from ancestors tracking to different parts of the African continent, and variable admixture from other populations. However, this is unlikely to affect the overall analysis since they only represent 2% of the subjects. Similarly, haplotypes involving heterozygous SNPs were assigned based on highest probability of phase, but if any of those subjects had rarer recombinant haplotypes, that could introduce a small degree of error. It is also possible that other variables, such as type of SCD or gender, could have an underlying effect on pain or utilization, but the sample size has insufficient power to test for effects of these co-variates. We believe that our data, despite being exploratory, are novel and may be pointing to

Table 4. DRD3 association analysis of haplotypes with patient outcomes (REF = reference haplotype).

Gene and haploblock	First SNP in haplotype (allele below)	Next SNP in haplotype (allele below)	Next SNP in haplotype (allele below)	Next SNP in haplotype (allele below)	Cohort Haplo-type freq	LDhap freq (S.W. Afr-Amer.)	API p value relative to reference haplotype	Utilization p value relative to reference haplotype
DRD3 1	rs2087017	–	–	–	–	–	0.94	0.33
DRD3 2	rs9817063	rs2134655	rs963468	rs3773679	–	–	–	–
	C	T	G	C	0	0	–	–
	C	C	A	T	0.08	0.15	0.24	1
	C	C	A	C	0.01	0	–	–
	C	C	G	T	0.03	0	–	–
	C	C	G	C	0.21	0.16	0.88	0.98
	T	T	G	C	0.06	0.07	0.85	**0.01**
	T	C	G	C	0.60	0.62	REF	REF
DRD3 3	rs167771	–	–	–	–	–	.63	1
DRD3 4	rs167770	rs324029	rs324023	–	–	–	–	–
	A	A	T	–	0.03	0.04	0.51	**0.01**
	A	G	T	–	0.08	0.07	**0.04**	**< .001**
	A	G	C	–	0.31	0.25	0.66	0.86
	G	A	T	–	0.58	0.64	REF	REF
	G	A	C	–	0.00	0	–	–
DRD3 5	rs3732783	–	–	–	–	–	0.11	0.64
DRD3 6	rs324026	rs1800828	–	–	–	–	–	–
	C	G	–	–	0.17	0.19	0.98	0.95
	C	C	–	–	0.52	0.56	REF	REF
	T	G	–	–	0.00	0	–	–
	T	C	–	–	0.32	0.25	0.78	0.54
DRD3 7	rs1394016	rs7611535	rs2399504	–	–	–	–	–
	A	C	T	–	0.00	0	–	–
	A	C	C	–	0.70	0.58	REF	REF
	A	T	T	–	0.09	0.14	0.88	0.11
	A	T	C	–	0.05	0.07	0.19	0.10
	G	C	C	–	0.15	0.20	0.13	0.29
	G	T	C	–	0.02	0	–	–
DRD3 8	rs905568	–	–	–	–	–	0.93	0.54

SNP: single-nucleotide polymorphism; API: average pain intensity.

important underpinnings of SCD pain and will be of utility for future SCD and other genetics research. Thus, future studies to validate these findings should include a larger cohort, which would also allow co-variates to be studied, to define the highest pain-risk profile among SCD patients. Laboratory studies of the African American haplotypes may also shed light on functional effects at the protein level.

Conclusions

Haplotype analyses of *COMT* and *DRD3* genes found evidence for genetic contribution to SCD average pain intensity and acute care utilization. This work complements previous studies of individual SNPs of these genes in pain-related phenotypes, illustrating how genotyping data can be mined for additional information. Future studies might consider measuring and analyzing admixture as a co-variate as well as gathering control subjects from the same region for comparison. To gain a further understanding of the genomic contributions to SCD pain and acute care utilization for pain, future studies are needed to confirm these observations and consider biological effects. In addition, these COMT haplotypes may be compared in future pain studies of pain that also investigate gender, including in African American populations.

AUTHORS' CONTRIBUTIONS

All authors participated in the design, interpretation of the studies, analysis of the data and review of the manuscript. Y.Y., S.C., D.J.W., and M.R.W. analyzed and interpreted statistical analysis. K.P.R., M.R.W., and D.J.W. wrote and/or reviewed the manuscript. Y.Y., Y.C.A., S.C., E.J., R.E.M., Z.J.W. reviewed and edited the manuscript. D.J.W., R.E.M., and Z.J.W. conceived the idea and provided the design. All authors read and approved the final manuscript.

DECLARATION OF CONFLICTING INTERESTS

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

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was made possible by grants 1R01 HL078536 and K01HL153210-02 from the National Institutes of Health (NIH); National Heart, Lung, and Blood Institute (NHLBI); and T32AG049673 Integrative and Multidisciplinary Pain and Aging Research Training in conjunction with the National Institute on Aging (NIA). These contents are solely the responsibility of the authors and do not necessarily represent the official views of

the NIH, NHLBI, NIA, or Veteran's Administration. The final peer-reviewed manuscript is subject to the National Institutes of Health Public Access Policy.

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(Received October 20, 2021, Accepted January 30, 2022)