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Original Article

Evaluating Associations between Average Pain Intensity and Genetic Variation in People with Sickle Cell Disease: An Exploratory Study

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ABSTRACT

Background: Pain is one of the most common and deleterious symptoms experienced by individuals with sickle cell disease (SCD). There is a paucity of studies identifying potential genetic mechanisms of pain in this population.
Aim: Examine associations between 11 functional single nucleotide polymorphisms in 9 candidate genes with reports of average pain intensity in individuals with sickle cell disease.
Method: Cross-sectional analyses were performed on data and blood samples collected through the Duke SCD Implementation Consortium Registry. Participants were asked to rate their pain "on the average" using an 11-point numeric rating scale (0 = no pain; 10 = pain as bad as you can imagine). We genotyped 11 single nucleotide polymorphisms in 9 pain-related genes using TaqMan® Genotyping Assays. Associations between each polymorphism and reports of average pain were evaluated.
Results: The 86 participants (mean age: 28.7 years; 64% female) included in this study reported moderate pain on average (Mean = 4, Standard Deviation = 2.4). *ICAM1* rs1799969 was the only genetic polymorphism that was significantly associated with pain ($p = .01$). Individuals with one or more minor alleles had lower average pain (Mean = 1.25, Standard Deviation = 1.50) than individuals without a minor allele (Mean = 4.13, Standard Deviation = 2.25). The effect size for *ICAM1* rs1799969 was 1.30, which is considered large. The effect sizes for all other single nucleotide polymorphisms ranged from small to medium (range: 0–0.3).
Conclusions: Our findings provide preliminary evidence that the minor allele in *ICAM1* rs1799969 had protective effects against experiencing more severe pain in sickle cell disease.
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Individuals with sickle cell disease (SCD), a group of inherited chronic blood cell disorders, experience severe pain throughout their lifespan. SCD has a complex pathophysiology that involves a genetic variation in the hemoglobin beta globin gene. This genetic variation results in misshapen red blood cells, often referred to as sickled cells due to their crescent shape, which are likely to clump and stick to blood vessel walls, causing multifaceted processes such as vaso-occlusion, ischemia-reperfusion injury, endothelial dysfunction of the vasculature, and chronic inflammation (Conran & De Paula, 2020; Odievre et al., 2011). These processes ultimately result in significant medical complications such as vascular and organ damage. Although severe acute pain episodes that correspond with vaso-occlusive events (VOEs) are the hallmark of SCD, research highlights the occurrence of persistent pain in this population; a majority of adults with SCD report experiencing pain on most days in addition to acute episodes (Matthie et al., 2020; Smith et al., 2008).

Hyperexcitability and hypersensitivity of the central and peripheral nervous systems, known as central and peripheral sensitization, are thought to contribute to development of SCD pain. It is

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suspected that recurrent vaso-occlusion leads to persistent inflammation and nociceptive input, which promotes pain sensitization (Gupta et al., 2018; Tran et al., 2017). The molecular basis for this alteration in pain processing in SCD is not well understood; therefore, studying genetic variations known to influence inflammatory processes and neurotransmission can help delineate underlying biologic mechanisms of pain in this population.

Inflammation is a putative mechanism of pain in individuals with SCD. Dysregulation of cytokines (proteins that mediate inflammatory and immune processes and can serve as excitatory mediators in pain modulation) has been implicated in the development of persistent pain syndromes (e.g., fibromyalgia, neuropathic pain) (Ji et al., 2018; Kraychete et al., 2009; Ramesh et al., 2013; Sturgill et al., 2014). Research has shown that individuals with SCD have elevated cytokine levels that increase further during acute VOEs (Francis & Haywood, 1992; Graido-Gonzalez et al., 1998; Hibbert et al., 2005; Pathare et al., 2004; Qari et al., 2012). Furthermore, a study evaluating associations between experimentally induced pain and cytokines in adults with SCD identified that cytokines, such as interleukin-4 (IL-4), interleukin-8 (IL-8), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α), were all associated with pain severity (Campbell et al., 2016).

The complex inflammatory processes in SCD are influenced by activation of the endothelium during VOEs. The endothelium is the lining of blood vessel walls; it consists of endothelial cells that become adhesive and recruit platelets and leukocytes to the area when activated (Conran & Belcher, 2018; Conran & De Paula, 2020). These interactions induce pro-inflammatory mediators, including cytokines (e.g., TNF- α, IL-1β) and other inflammatory molecules (e.g., chemokines, growth factors), further increasing the expression of endothelial surface adhesion molecules (e.g., intercellular cell adhesion molecule-1 [ICAM-1], E-selectin, and P-selectin) that lead to clot formation and/or leukocyte diapedesis (Conran & De Paula, 2020; Harjunpää et al., 2019). In our previous pilot study of 74 adults with SCD, we identified that plasma concentration of soluble E-selectin was elevated in individuals who reported more frequent occurrence of severe persistent pain (Knisely et al., 2022).

Alterations in neurotransmitters such as brain-derived neurotrophic factor (BDNF) and the reduced activity of catecholamines (epinephrine and norepinephrine) breakdown the enzyme catechol-O-methyltransferase (COMT) and contribute to neuroinflammation, the molecular process that induces and maintains central sensitization (Ji et al., 2018). Decreased COMT activity is associated with increased risk of orofacial pain (Slade et al., 2015) and increased pain sensitivity in individuals with fibromyalgia (Barbosa et al., 2012; Cohen et al., 2009). In a study evaluating associations between genetic variation and pain related to SCD, investigators found that two genetic polymorphisms in the *COMT* gene were associated with increased frequency in pain-related emergency room visits (Zhang et al., 2018). Furthermore, preclinical studies in BDNF knockout mice suggest that BDNF may play an important role in acute to chronic pain transition (Sikandar et al., 2018).

Although evidence suggests that several biologic mechanisms likely contribute to the pain experiences of individuals with SCD, few studies have explored genetic variations associated with pain in this population. Determining functional polymorphisms associated with pain is an essential step in elucidating potential mechanisms and biomarkers of pain. Functional polymorphisms are variations in an individual's DNA which are thought to influence the structure, function, or level of a gene product (Albert, 2011). The purpose of this exploratory study was to examine associations between functional single nucleotide polymorphisms (SNPs) in candidate genes and reports of average pain intensity in individuals with SCD. This hypothesis-generating study can advance nursing science and practice by: (a) improving the ability to identify mechanisms that place patients at greater risk for high pain burden, and (b) identifying potential new targets for interventions for pain relief in people with SCD.

Methods

Design & Setting

This exploratory, cross-sectional, genetic ancillary study leveraged recruitment and data collected for the Sickle Cell Disease Implementation Consortium (SCDIC) Research Registry at Duke University. This comprehensive research registry includes prospective, longitudinally collected patient-reported and electronic health record (her) data (DiMartino et al., 2018; Glassberg et al., 2020). Data were collected at enrollment in the registry and yearly thereafter. A subset of participants also provided blood specimens.

Sample

Inclusion criteria for the registry were the following: (a) aged 15–45 years; (b) live in North Carolina; and (c) a genetically confirmed SCD diagnosis. This study used patient-reported data and biospecimens collected at the 1-year follow-up assessment for the research registry. This study was reviewed and approved by the Duke University institutional review board and participants provided written informed consent.

Data Collection & Management Procedures

Demographic & disease-related characteristics

Demographic and clinical data were used to describe the sample. Demographic data included age, sex, marital status, education, and employment. Clinical characteristics included SCD genotype, whether participant was taking pain medications daily, number of pain attacks (VOEs) in past year, and time since most recent pain attack.

Assessment of pain

The primary outcome for the current study was average pain intensity. Participants were asked to rate their pain "on the average" using an 11-point numeric rating scale (0 = no pain; 10 = pain as bad as you can imagine) from the National Institutes of Health's Toolbox that is commonly used to measure pain intensity in clinical trials (Cook et al., 2013). The rating for this item was used as the outcome in the data analysis.

Candidate gene selection & data collection

Eleven functional SNPs in nine genes of interest (candidate genes) involved in pathways that likely influence pain (inflammation, immunoregulation, neuroregulation) were identified through a review of the literature and findings from our previous studies (Knisely et al., 2019; Knisely et al., 2022) and included in this study (Table 1). We included polymorphisms (genetic variations) if they had a known functional consequence on the gene and/or gene product or had been associated previously with pain in other chronic conditions (e.g., cancer).

DNA samples from whole blood were collected as part of the SCDIC research registry. DNA was extracted from whole blood in the Duke University School of Nursing Biomarker Laboratory using the protocol and reagents from the Gentra Puregene blood kit (Qiagen, Germantown, MD). Genotypes were determined in the Molecular Genomics Core at the Duke Molecular Physiology Institute using the TaqMan allele discrimination platform (Thermo Fisher Scientific Inc., Waltham, MA). Duplication of a subset of samples

Table 1
Selected Pain-related Candidate Genes and Functional Polymorphisms

Pain-related pathway	Gene(*gene symbol*)	Functional SNP	Minor allele	MAF[a]	Functional consequence
Inflammation/ Immunoregulation	FKBP prolyl isomerase 5 (*FKBP5*)	rs3800373	C	0.419	Intron variant associated with greater FKBP5 induction by cortisol and decreased glucocorticoid-receptor sensitivity (Fudalej et al., 2015; Tatro et al., 2009)
	E-selectin (*SELE*)	rs5361	G	0.034	Missense variant associated with higher levels of plasma E-selectin levels (Mlekusch et al., 2004)
	Intercellular adhesion molecule 1 (*ICAM1*)	rs5498	G	0.204	Missense variant associated with increased levels of soluble ICAM-1 (Bielinski et al., 2011)
	Intercellular adhesion molecule 1 (*ICAM1*)	rs1799969	A	0.026	Missense variant associated with ICAM-1 concentrations (Albert et al., 2009; Schnabel et al., 2009)
	Interleukin 1 beta (*IL-1β*)	rs1143634	A	0.142	Synonymous variant with some evidence the T allele is associated with increased production of IL-1β (Pociot et al., 1992)
	Interleukin 6 (*IL-6*)	rs1800795	C	0.069	Intron variant with evidence C allele is associated with decreased IL-6 levels (Bull et al., 2009; Paul-Samojedny et al., 2010)
	Interleukin 8 (*IL-8*)	rs4073	T	0.210	2KB upstream variant with evidence A allele is associated with increased IL-8 production (Hull, 2000; Taguchi et al., 2005)
	Tumor necrosis factor alpha (*TNF-α*)	rs1799964	C	0.164	2KB upstream variant with evidence C allele is associated with increased production of TNF-α (Nourian et al. 2017; Sandoval-Pinto et al., 2016)
	Tumor necrosis factor alpha (*TNF-α*)	rs1800629	A	0.124	2KB upstream variant with evidence A allele is associated with an increase in the binding of nuclear factors and heightened transcription of the gene (Kroeger et al., 1997; Wilson et al., 1997)
Neuroregulation	Brain derived neurotrophic factor (*BDNF*)	rs6265	T	0.045	Missense variant linked to impairment of BDNF secretion in nervous system (Chen et al., 2004; Egan et al., 2003)
	Catechol-O-methyltransferase (*COMT*)	rs4680	A	0.309	Missense variant that reduces the function of the COMT enzyme, resulting in higher dopamine levels (Stein et al, 2006)

[a] As reported in Black populations in dbSNP database (https://www.ncbi.nlm.nih.gov/snp/).SNP = single nucleotide polymorphism; MAF = minor allele frequency.

(n = 3) and standard controls (n = 2) was completed for validation of genotyping processes. Genotypes were coded as two doses of major allele versus one or two doses of minor allele present for each functional SNP. The major allele, also considered the common allele, is present more frequently than the minor allele in a population of interest.

Statistical Analysis

Initially a descriptive analysis of the demographic and clinical characteristics of the analytical sample was performed, in which means and standard deviations (SD) are reported for continuous variables and frequency and percentages reported for categorical variables. Independent-samples *t* test techniques were used to test for differences in pain scores between those with a minor allele present and those without any minor allele present for each specific gene of interest. For any specific gene, if the sample size was smaller than 20 in each group (or the data were non-Gaussian), Mann-Whitney *U* tests were performed instead. Effect size was assessed using Cohen's *d*. Given the exploratory nature of this study, $p < .05$ was considered statistically significant without adjusting for any multiple testing. All data analyses were performed using SPSS Version 24 (IBM, Armonk, NY).

Results

A total of 86 participants met the study inclusion criteria and were included for analysis. Table 2 summarizes the demographic and clinical characteristics of the sample. In summary, participants' mean age was 28.7 years, and 64% were female. A large majority (77.9%) had never been married, and most (72.1%) had at least some college education or more. The most severe genotypes of SCD, HbSS or Sβ^0 genotypes, were the most common. Nearly half (46.5%) of participants were taking pain medications daily. Most participants had experienced 4 or more pain attacks (VOEs) over the past year, with a majority (57%) having had a pain attack within the past 6 months.

For the overall sample (N = 86), there was an average pain rating of 4.0 (SD = 2.4; range 0–10). Table 3 shows the differences in average pain scores between participants with and without any minor allele for each of the SNPs. Pain scores were significantly higher for those with no minor allele present for *ICAM1* rs1799969 when compared with those with a minor allele present for the same SNP (4.13 [SD = 2.25] vs. 1.25 [SD = 1.50], $p = .01$). The effect size for *ICAM1* rs1799969 is 1.30, which is conventionally considered a large effect size. The effect sizes for all the other SNPs were smaller and would be considered small to medium (Cohen's *d* range: 0.0–0.3; Table 3).

Discussion

This exploratory study provides preliminary evidence of associations between functional SNPs with reports of pain among individuals with SCD. On average, participants reported moderate pain; however, of the candidate genes selected, only one SNP was identified as significantly associated with reports of pain. Specifically, individuals carrying one or two copies of the minor allele (A) for *ICAM1* rs1799969 reported lower levels of severe pain on the average. This association had a large effect size (*Cohen's d* >1). That is

Table 2
Sample Demographic and Clinical Characteristics

	Total sample(n = 86)
Demographic characteristics	
Age (y)	
Mean ± SD	28.7 ± 7.9
Range	17-45
	n (%)
Sex (female)	55 (64.0%)
Marital status (never married)	67 (77.9%)
Education	
High school graduate or less	24 (27.9%)
Some college	35 (40.7%)
College graduate	17 (19.8%)
Graduate/Professional degree	10 (11.6%)
Employment	
Student	13 (15.1%)
Employed	36 (41.9%)
Unemployed	32 (37.2%)
Other	5 (5.8%)
Clinical characteristics	
SCD genotype	
Hb SS or Sβ^0	63 (73.3%)
Hb SC	20 (23.3%)
Other (Hb Sβ^+, S/HPFH, SE, SO, SD)	3 (3.5%)
Taking pain medications every day (no)	40 (46.5%)
Number of pain attacks (VOEs) in past year	
0	10 (11.6%)
1	7 (8.1%)
2	11 (12.8%)
3	10 (11.6%)
4 or more	48 (55.8%)
Time since most recent pain attack (VOE)*	
<1 week ago	10 (11.6%)
1-3 weeks ago	17 (19.8%)
1-6 months ago	22 (25.6%)
7-11 months ago	24 (27.9%)
1-5 years ago	2 (2.3%)
5+ years ago	9 (10.5%)
Never had a pain attack	2 (2.3%)

SD = standard deviation; Hb = hemoglobin; SCD = sickle cell disease; VOE = vaso-occlusive events.

Table 3
Differences in Average Pain Score between Participants Individuals Without and With Presence of Minor Alleles

Functional SNP	No minor allele present			Minor allele present			*P*	Cohen's *d*
	N	Mean	SD	N	Mean	SD		
FKBP5 rs3800373	35	4.4	2.23	51	3.73	2.32	.18	0.30
SELE rs5361[a]	77	4.04	2.19	9	3.67	3.20	.77	0.16
ICAM1 rs5498	52	4.08	2.07	34	3.88	2.64	.70	0.08
ICAM1 rs1799969[a]	82	4.13	2.25	4	1.25	1.5	.01[b]	1.30
IL-1β rs1143634	57	4.09	2.08	29	3.83	2.7	.62	0.11
IL-6 rs1800795[a]	75	4.04	2.29	11	3.73	2.45	.75	0.14
IL-8 rs4073	57	3.93	2.37	29	4.14	2.12	.69	-0.09
TNF-α rs1799964	61	3.85	2.35	25	4.36	2.16	.36	-0.22
TNF-α rs1800629	65	4.06	2.33	21	3.81	2.23	.67	0.11
BDNF rs6265[a]	82	4	2.29	4	4	2.83	.79	0
COMT rs4680	39	3.77	2.35	47	4.19	2.26	.40	-0.18

[a] Mann-Whitney U test was used to test for differences between the two groups.
[b] significant at the .05 level.SNP = single nucleotide polymorphism; SD = stamdard deviation.

more than one standard deviation difference between groups. Despite the small number of participants in the group with one or two copies of minor alleles, this finding was consistent with the observed allele frequencies in previously published estimates for African Americans (see Table 1).

Our findings provide preliminary evidence that the minor allele in *ICAM1* rs1799969, a missense variant that changes amino acid 241 from glycine to arginine (G241R), may have protective effects against the likelihood of experiencing more severe pain in SCD. *ICAM1* is an encoding gene that stimulates the synthesis of ICAM-1. ICAM-1 is one member of a large family of adhesion molecules. Adhesion molecules are proteins that span the cell membrane and have an extracellular portion that can interact with similar regions of adhesion molecules on other cells. ICAM-1 is expressed on endothelial cells and immune cells. Expression of *ICAM1* is upregulated by inflammatory mediators (i.e., cytokines), resulting in the recruitment of leukocytes to sites of inflammation. The *ICAM1* rs1799969 variant has been associated with normal ICAM-1 adhesion properties (Bai et al., 2014), increased cell surface expression of ICAM-1 (Bai et al., 2014; Holder et al., 2008), and lower

levels of circulating ICAM-1 (Albert et al., 2009; Ponthieux et al., 2003; Zee et al., 2007). This combination of increased ICAM-1 expression, yet decreased soluble ICAM-1 (sICAM-1), is somewhat paradoxical since sICAM-1 is a degradation product of cell surface ICAM-1 (Ramos et al., 2014), and under normal regulation sICAM-1 levels parallel those of ICAM1 expression. However, sICAM-1 can also be produced from alternative splicing of ICAM-1 messenger RNA (Ramos et al., 2014), which may be altered by the gene variant. Others have shown that *ICAM1* rs1799969 is associated with increased susceptibility to some inflammatory diseases (Lee & Bae, 2016; Paré et al., 2011), but not others (Ponthieux et al., 2003). Furthermore, it is not definitively known how *ICAM1* genotype and disease type impact sICAM-1 levels.

Insights into mechanisms by which *ICAM1* rs1799969 may protect SCD patients against experiencing more severe pain in SCD comes from a recent review by Müller (2019). This review examines the relationship between blood-brain-barrier permeability, microglial activation, and psychiatric disorders. Müller posited that an important aspect of ICAM-1 in psychiatric disorders may be through its regulation of the blood-brain-barrier and microglial activation (Müller, 2019). In a 2014 review examining pain disorders and the blood-brain-barrier, DosSantos et al. (2014) noted that peripheral models of pain increase ICAM-1 expression and microglial activation in brain regions (thalamus, frontal and parietal cortices) responsible for pain processing and modulation. Additionally, investigators evaluating the efficacy of simvastatin (a drug that lowers cholesterol and protects against vascular injury by decreasing inflammation) for treatment of vaso-occlusive pain in SCD found that this drug caused a significant decrease in acute vaso-occlusive pain events, oral analgesic use, and circulating ICAM-1 among other circulating vascular markers (i.e., soluble E-selectin, vascular cell adhesion protein 1, vascular endothelial growth factor) (Hoppe et al., 2017). The relationship between SCD pain and ICAM-1 is complex and could benefit from further study. However, the association between *ICAM1* rs1799969 and protection against experiencing more severe pain in SCD may be related to effects on the blood-brain-barrier, microglia, and/or the endothelium.

Although we did not identify significant associations between pain and the 10 other functional SNPs, *FKBP5* rs3800373 did demonstrate a medium effect size in this study. *FKBP5* is a gene that encodes a glucocorticoid receptor and plays an integral role in stress response and immune function (Zannas et al., 2016). Previous studies have shown that people with the minor allele are more susceptible to pain after exposure to trauma such as sexual assault or motor vehicle collision (Bortsov et al., 2014; Linnstaedt et al., 2018). However, in this study, participants with at least one dose of the minor allele had slightly lower pain scores compared with those with no minor alleles present. Further investigation with a larger sample size may delineate more clearly whether a statistically significant relationship between this functional SNP and pain exists in this population.

Limitations

These findings should be interpreted with attention to the limitations of this study. This study consisted of a small sample, leading to some small groups with the presence of minor alleles; findings for these groups were, however, consistent with published estimates of minor allele frequencies (reported in Table 1). Additionally, we assessed associations in only a small number of SNPs in pain-related candidate genes which were supported by the literature. Future studies should consider a broader selection of SNPs and genes, as well as other potential mechanisms (e.g., gene expression) of pain in this population. In alignment with the exploratory nature of this study, we did not correct for multiple testing. We also assessed associations with only one dimension of pain (i.e., average pain intensity), and did not distinguish between acute and chronic pain in our assessments, as there are currently no validated diagnostic criteria to distinguish between acute and chronic pain in the SCD population. Despite these limitations, our findings provide important preliminary evidence of associations between average pain rating and functional polymorphisms in pain-related candidate genes. Future studies in larger and more geographically diverse samples are needed to replicate these findings and extend our understanding of genetic variations and other biopsychosocial mechanisms that contribute to the complexity of pain in this population.

Conclusions

This exploratory study is among the first to provide preliminary evidence linking genetic variation in the *ICAM-1* gene to reports of severe pain in people with SCD. These results underscore the potential role played by endothelial function and inflammation in contributing to the variability in pain in this population. Further research with a larger, more geographically diverse sample is needed to extend and validate these findings. It is possible that further investigation will find functional genetic polymorphisms that can serve as biomarkers to assist the identification of individuals who are at risk for more severe pain and the development of strategies for personalized interventions.

Clinical Implications

Nurses are well positioned to lead the translation of genetic knowledge to pain management practice. Although further validation of our findings is needed, this study is an initial step in understanding how genetic variation contributes to experiences of pain, and it extends our knowledge of potential mechanisms of pain that can guide treatment approaches. For example, given that pain experiences are modifiable, it is plausible that, with further investigation, the *ICAM-1* rs1799969 polymorphism could serve as a biomarker for risk stratifying patients who are less likely to experience severe pain. Additionally, this marker could serve as a potential target for pharmacologic and non-pharmacologic interventions.

Declaration of Competing Interest

Dr. Shah reports receiving fees from Novartis, Global Blood Therapeutics, Forma, and Alexion. Dr. Kenney has received research funds from Global Blood Therapeutics. No other authors report any potential conflicts of interest.

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