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Patients with Sickle Cell Disease Have Increased Sensitivity to Cold and Heat

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Abstract

Sickle Cell Disease (SCD) pain is associated with colder temperatures and touch and described as “cold”, “hot” and “shooting” suggesting hypersensitivity to tactile stimuli. Sickle mice exhibit hypersensitivity to thermal (cold, heat) and mechanical stimuli compared to controls. It is unknown whether humans experience this same hypersensitivity. Thus, we quantified thermal and mechanical sensitivity differences between SCD patients and controls. Our primary hypothesis was that SCD patients will exhibit hypersensitivity to thermal and mechanical stimuli compared to race-matched controls. Our secondary hypothesis was this hypersensitivity will be associated with older and female subjects, and with frequent pain and hemolysis in SCD patients. A total of 55 patients and 57 controls 7 years completed quantitative sensory testing. SCD patients detected the sensation of cold and warm temperatures sooner as seen in their significantly lower median cold and heat detection thresholds [29.5°C vs. 28.6°C, $p=0.012$ and 34.5°C vs. 35.3°C, $p=0.02$] and experienced cold and heat pain sooner as seen in their significantly lower median cold and heat pain thresholds [21.1°C vs. 14.8°C, $p=0.01$ and 42.7°C vs. 45.2°C, $p=0.04$]. We found no mechanical threshold differences. Older age was associated with lower cold, heat, and mechanical pain thresholds in both groups. No association with pain, gender, or hemolysis was found. SCD patients exhibit hypersensitivity to thermal stimuli suggesting peripheral or central sensitization may exist and could contribute to SCD pain.

Keywords

sickle cell disease; pain; sensitivity

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Authorship Contributions

A.M.B. designed research, performed research, analyzed data, and wrote the manuscript; C.L.S. offered advice regarding basic mechanisms of pain and contributed to writing and editing of the manuscript; C.A.H. offered advice regarding basic mechanisms of sickle cell pain and critically reviewed the manuscript; R.G.H. analyzed data; J.A.P. designed research and contributed to writing and critically reviewing the manuscript.

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Introduction

Pain, the hallmark of sickle cell disease (SCD), is the leading cause of emergency department visits and hospitalizations and is associated with increased mortality in adults.[1] The frequency of pain increases with age and in adults a chronic pain syndrome can develop with associated daily pain.[2, 3] Thus, SCD pain can be acute or chronic. Despite extensive SCD basic research leading to the understanding of the effects of sickle hemoglobin on the red blood cell, the underlying mechanisms contributing to the onset of acute pain or the development and maintenance of chronic pain are poorly understood in humans.

SCD pain is reported to be provoked by increased wind speed and barometric pressure, colder temperatures, and touch.[4–6] Patients also describe SCD pain using neuropathic pain descriptors including “aching”, “cold”, “hot”, “penetrating”, “shooting”, “stabbing”, “burning”, “tingling”, “numb”, and “lancinating”.[7, 8] The precipitating factors and pain descriptors suggest SCD patients have hypersensitivity to tactile stimuli, a characteristic of neuropathic pain. Neuropathic pain is defined by the International Association for the Study of Pain as pain initiated or caused by a primary lesion or dysfunction of the nervous system that ultimately affects the somatosensory system.[9] In contrast to inflammatory or nociceptive pain which is caused by actual tissue damage or tissue damaging stimuli, neuropathic pain is produced by damage to or pathological change in the peripheral or central nervous system. Classical components of neuropathic pain include hyperalgesia, increased pain in response to a stimulus that is normally painful, and allodynia, pain due to a stimulus that does not normally provoke pain.[10, 11]

Despite the suggestion of neuropathic pain in SCD, there are little data on this in humans. However, there is strong evidence that sickle mice exhibit marked baseline hypersensitivity to mechanical, heat, and cold tactile stimuli, suggesting altered peripheral neural mechanisms and a potential mechanism for neuropathic pain.[12, 13] In addition, female sickle mice and older sickle mice have increased sensitivity and pain related behaviors to peripheral tactile stimuli.[12] Importantly, it is not known whether humans with SCD experience similar hypersensitivity to these same tactile stimuli that the mice exhibited. Previous research in humans without SCD reveals females experience greater sensitivity to experimentally induced pain (thermal, pressure, ischemic) than males.[14, 15] In addition, these data reveal older age is associated with increased frequency and severity of pain, suggesting that age may impact pain processing.[16, 17] Furthermore, in recurrent pain syndromes such as migraines, chronic regional pain syndrome, and juvenile rheumatoid arthritis, patients experience hypersensitivity to tactile stimuli possibly related to repeated painful events leading to sensitization of primary afferent neurons.[10, 18–20] It is not known whether females or older humans with SCD similarly experience increased pain sensitivity to experimentally induced pain and whether a prior history of SCD painful events impacts pain sensitivity to experimentally induced pain.

In order to further explore the neurobiology of human SCD pain, we sought to determine whether humans with SCD have altered sensitivity to cutaneous stimuli. Our primary objective was to quantify the difference in sensitivity to thermal (cold, heat) and mechanical stimuli between SCD patients and healthy African American controls. We hypothesized that SCD patients will exhibit hypersensitivity to mechanical and thermal stimuli compared to healthy race-matched controls as measured by decreased cold, heat, and mechanical pain thresholds and detection thresholds. Our secondary objectives were to determine the impact of gender and age on pain thresholds in SCD patients and controls. We hypothesized that females and older patients would have increased pain sensitivity to thermal and mechanical stimuli. Lastly, in SCD patients we examined the impact of a prior history of pain and increased hemolysis on pain and detection thresholds. We hypothesized that those with a

history of more painful events would have increased pain sensitivity and that hemolytic rate would be correlated with thermal and mechanical thresholds.

Methods

Study Setting and Subjects

This cross-sectional study was conducted between January 2010 and June 2011. Children and adults with SCD were recruited from the Wisconsin Sickle Cell Center during routine clinic visits. African American controls were recruited from siblings (biologic, adoptive, half), parents/legal guardians, and friends/relatives of SCD patients. Inclusion criteria for SCD patients were 1) ≥ 7 years (lowest validated age limit for testing methodology) 2) SCD (all genotypes with a pain phenotype defined as: ever requiring admission or emergency department care for pain and/or ever requiring opioids for pain at home) and for controls was ≥ 7 years. Race-matched controls were vital since data indicate African Americans have lower tolerance to experimentally-induced pain and increased sensitivity to thermal and mechanical testing.[21, 22] All subjects were excluded for the following: 1) Other chronic disease resulting in pain phenotype (i.e. Juvenile Rheumatoid Arthritis, Chronic Regional Pain Syndrome), 2) Overt stroke based on known history and available neuroimaging since neurologic deficits may alter testing results, 3) Analgesics 24 hours before or on day of testing (including neuroleptics), 4) Acute SCD painful event defined as pain severe enough to require opioids in the inpatient or outpatient setting within 2 weeks of testing. This was not a convenience sample. All SCD patients who met eligibility criteria were approached.

The Institutional Review Board of the Children's Hospital of Wisconsin approved the study and informed consent was obtained from the parent/legal guardian and assent from the child when appropriate. Stipends were given to compensate subjects for their time and transportation was provided if testing occurred on a non-clinic day.

Measurements and Outcomes

The primary outcome was sensitivity to thermal (cold, heat) and mechanical stimuli as defined by: 1) Cold Pain Threshold (°C), 2) Heat Pain Threshold (°C), and 3) Mechanical Pain Threshold (g). Secondary outcomes were detection of thermal (cold, heat) and mechanical stimuli as defined by: 1) Cold Detection Threshold (°C), 2) Heat Detection Threshold (°C), and 3) Mechanical Detection Threshold (g).

Testing Procedures

Quantitative Sensory Testing (QST)—QST was the procedure used to collect data for our primary and secondary outcomes, pain and detection thresholds. QST is used to detect sensory loss (hyposensitivity) or gain (hypersensitivity) to cold, heat, or mechanical stimuli. QST is a valid, reliable, and reproducible methodology for evaluating the sensitivity of the peripheral nervous system in humans and has been used in other pain syndromes.[18–20, 23–28]

Testing was completed in the Pediatric Translational Research Unit of the Children's Hospital of Wisconsin. QST was conducted by one of three research personnel. To minimize bias, the principal investigator did not conduct testing. QST was completed on the thenar eminence of the non-dominant hand and lateral dorsum of the foot (randomized to right/left using a table of random numbers). These locations were chosen since they have served as reference sites in other QST studies.[24, 26] All testing was completed without a parent, guardian, or observer in the room. Subjects were given 10–15 minutes to acclimate to room temperature that was kept constant between 68–72°C. During this time, scripted instructions were read to each subject to ensure standardized methodology. The order of the testing was:

1) mechanical detection threshold, 2) mechanical pain threshold, 3) cold detection threshold, 4) heat detection threshold, 5) cold pain threshold, 6) heat pain threshold. The order of thermal testing was important because cold/heat pain thresholds tested before cold/heat detection thresholds could influence the outcome.[28]

Thermal (cold, heat) Testing

Cold and heat stimulation was performed with a Thermal Sensory Analyzer (TSA-II) (Medoc; Israel)[29], an FDA approved computer-assisted QST device used in QST studies in other childhood and adult diseases (i.e. chronic regional pain syndrome, migraines, juvenile rheumatoid arthritis, and normal healthy controls).[10, 19, 20, 26] This device delivers warm or cold stimuli through a computer-driven thermode attached to the skin. The baseline temperature was 32°C, the stimulus range was 0–50°C, and the stimulus temperature did not exceed that on either side of this range. The following thresholds were determined utilizing the “method of limits” where participants were directed to push a button when the specified sensation was first perceived.[26] These thresholds were chosen based on the murine studies and other prior QST studies.[19, 26]

Cold and Heat Pain Thresholds—The probe temperature decreased/increased linearly from baseline at 1.5°C/sec and the subject was asked to press a button when the stimulus was perceived as uncomfortable. This was defined as the *cold or heat pain threshold*. The change in temperature was then halted and the thermode temperature was reset to baseline at a rate of 10°C/sec. This was repeated three times with an interstimulus interval of 10 seconds. The final *cold or heat pain threshold* was determined by calculating the mean of the three threshold temperatures (°C). [10, 25, 26, 30]

Cold and Heat Detection Thresholds—The probe temperature decreased/increased linearly from baseline at 1°C/sec and the participant was asked to press a button when the sensation of cold or heat was first perceived. This was the *cold or heat detection threshold*. The change in temperature was then halted and the thermode temperature was reset to baseline at a rate of 1°C/sec. This was repeated four times with an interstimulus interval of 6 seconds. The final *cold or heat detection threshold* was determined by calculating the mean of the four threshold temperatures (°C).[10, 25, 26, 30]

Mechanical Testing

Mechanical Sensitivity Threshold was measured using a standardized set of von Frey filaments that exert forces of pressure upon bending between 0.026 grams (g) and 110 g. Subjects' hand and foot were placed behind a screen so he/she could not see the filament applied to the skin. Starting with the lowest intensity, von Frey filaments were applied in five ascending series with a 1–2 second contact time. The “method of limits”[26] was used, where the subject was asked to reply “yes” if the stimulus was “painful”. Five threshold determinations (subject answered “yes”) were done and the final threshold was the mean of the varying forces (g) of these five series.[25, 30]

Mechanical Detection Threshold was measured using the above procedure except that the end point was the patient giving a verbal signal when the mechanical stimulus was first felt. [25, 31]

Covariates

Covariates of interest were age, gender, number of prior pain events (SCD patients), and laboratory markers of hemolysis (SCD patients). Pain events were defined as events requiring health care utilization in the emergency department or inpatient unit and these data were extracted from the comprehensive sickle cell center database, medical record, or self-

report. Laboratory markers of hemolysis (hemoglobin, reticulocyte count, lactate dehydrogenase (LDH), total bilirubin, and aspartate aminotransferase (AST), were averaged from the three most recent clinic visits while in baseline health.

Statistical Analysis

Descriptive statistics were calculated for all demographic and outcome variables using appropriate methods. Differences in the primary outcomes (pain thresholds) and secondary outcomes (detection thresholds) between SCD patients and controls were determined using Mann-Whitney tests since data were non-normally distributed. A sub-analysis of the same outcomes comparing HbSS patients to controls was also done using the same statistical methods. Medians with IQR and non-parametric p-values are presented. Spearman rank correlation was used to determine the association between age and cold, heat, and mechanical pain thresholds. Spearman rank partial correlation was used to determine the differential impact of age in the SCD group versus the control group. Within the SCD group, Spearman rank correlation was also used to determine the association between prior history of pain over the lifetime of the patient adjusted for age and the primary and secondary outcomes. The same methods were used to evaluate the association between the number of pain events in the 3 years prior to study enrollment and the same outcomes. Within the SCD group, Spearman rank correlation was used to determine the association between the laboratory parameters and the primary and secondary outcomes. A sub-analysis of the association of pain events and markers of hemolysis in the HbSS patients only was also completed using the same methods. All statistical analyses were conducted with STATA version 11.2 (STATA, College Station, TX). In general, a p-value ≤ 0.05 was considered statistically significant. Bonferroni correction was applied when appropriate.

The study was powered based on our primary objective using preliminary data. For an alpha of 0.05 and a power of 0.80, a projected sample size of 50 subjects per group would allow detection of a difference of 0.6 standard deviations, a moderate effect size. Heat and cold pain thresholds exceeded this in our pilot sample (0.89 and 0.72, respectively), while observed mechanical sensitivity did not (0.15, an extremely small effect size using Cohen's criteria). Thus, it was not used as part of the planning process for the study.

Results

Study Population

A total of 78 SCD patients were approached during routine clinic visits and verbal consent was obtained from 76 to arrange for them to return on a separate day to give full written consent and complete testing. Of those that agreed to testing, 55 patients returned and completed testing during the study period. A total of 37 eligible controls were approached during a clinic visit of a sibling with SCD and more than 20 were recruited outside clinic. Of these, 57 controls completed testing during the study period.

Demographic Data and Baseline Characteristics

Table I displays the demographics, baseline characteristics, and laboratory parameters of the study population. The demographics of the SCD patients that did not return for full consent and testing were overall similar to the study population [mean age 14.8 (± 4.0) years; (vs 15.4 (± 6.3) years; $p=0.69$, student's t-test); 76% were female (vs 60%; $p=0.19$, chi-square test); genotypes were 67% HbSS, 23.8% HbSC, 9.5% HbS β +thal].

Outcomes

Patients with SCD experience hypersensitivity to cold and heat stimuli—We determined the threshold at which subjects perceived the cold or warm stimulus to be uncomfortable. SCD patients have increased sensitivity to cold and heat in the hand compared to controls as reflected in their significantly lower cold pain thresholds and lower heat pain thresholds ([21.1°C (IQR 11.9–24.2) vs. 14.8°C (IQR 5.5–22.6), $p=0.01$ and 42.7°C (IQR 38.6–46.1) vs. 45.2°C (IQR 40.8–48.1), $p=0.04$]; Figure 1). In other words, patients with SCD felt cold pain and heat pain at a temperature closer to the starting temperature of 32°C than controls. A sub-analysis of HbSS patients compared to controls revealed similar differences for the cold pain threshold ([20.8°C (IQR 11.6–24.1) vs. 14.8°C (IQR 5.5–22.6), $p=0.03$]) and heat pain threshold ([41.8°C (IQR 38.6–45.9) vs. 45.2°C (IQR 40.8–48.1), $p=0.03$]). In the tested foot, there were no significant differences in the cold or heat pain thresholds between SCD patients and controls or between HbSS patients and controls.

Patients with SCD have lower cold and heat detection thresholds—We determined the threshold at which subjects first perceived the sensation of cold or heat. SCD patients detected the sensation of cold and heat in the hand earlier than controls ([29.5°C (IQR 27.9–30.4) vs. 28.6°C (IQR 26.7–29.7), $p=0.012$ and 34.5°C (IQR 33.8–35.4) vs. 35.3°C (IQR 34.3–36.7), $p=0.02$]; Figure 2). In other words, SCD patients detected the sensation of cold at a higher temperature and the sensation of heat at a lower temperature compared to controls with both stimuli detected closer to the starting temperature of 32°C. When comparing HbSS patients to controls, we found differences in the cold detection thresholds ([29.5°C (IQR 27.9–30.4) vs. 28.6°C (IQR 26.7–29.7); $p=0.03$]), however, there were no significant differences in the heat detection thresholds ([34.9°C (IQR 33.9–35.4) vs. 35.3°C (IQR 34.3–36.7); $p=0.10$]). This may be due to a smaller sample size used when completing the sub-analysis. There were no differences in cold or heat detection thresholds in the tested foot in the SCD group as a whole compared to controls or in the sub-analysis of HbSS patients compared to controls.

Patients with SCD are not hypersensitive to mechanical stimuli—We did not detect differences in the mechanical sensitivity of SCD patients compared to controls as reflected by the absence of significant differences in their mechanical pain thresholds in either the hand or the foot (Figure 1). There were also no differences in mechanical detection thresholds between SCD patients and controls in the tested hand or foot (Figure 2). A sub-analysis of HbSS patients compared to controls revealed no differences in mechanical pain thresholds or detection thresholds.

Older age is associated with increased sensitivity to cold, heat and mechanical stimuli in SCD patients and controls—Age was analyzed as a continuous variable. In the SCD group as a whole, median age was 14 (IQR 11–19) and in controls median age was 12 (IQR 9–18); $p=0.42$. With the sample size of 55 SCD subjects there was 80% power to find a correlation of 0.35 (R-square of 12%) or greater with age. Older age was associated with increased sensitivity to cold, heat, and mechanical stimuli in both SCD patients and controls (Table II). There was no significant difference in the association of age with cold, heat and mechanical pain thresholds in the SCD patients and controls. The difference in the age adjusted (partial correlation) with unadjusted correlation with age was less than 0.014 (median 0.006; IQR 0.003–0.009).

Prior history of pain, gender, and laboratory markers of hemolysis do not have a generalized effect on pain or detection thresholds—The number of SCD pain events requiring emergency department care or inpatient hospitalization over a patient's

lifetime and in the immediate 3 years before study entry are displayed in Table I. We found no association between the number of lifetime pain events adjusted for age and the cold, heat, and mechanical pain and detection thresholds (all r values < 0.2 and $p > 0.05$). There was also no association between the number pain events in the immediate 3 years before study enrollment and the same outcomes (all r values < 0.2 and $p > 0.05$). In a sub-analysis of HbSS patients, there were no significant associations between the number of pain events and the same outcomes (all r values < 0.2 and $p > 0.05$). The SCD group was 60% female and controls were 56% female; $p = 0.68$. Significant differences in pain thresholds in males versus females in the group as a whole (SCD patients and controls) were not detected. Within the SCD patient group, the only significant differential impact of gender on pain thresholds was for the mechanical pain threshold in the hand (females = 16.7 g vs. males = 2.9 g; $p = 0.039$). With the sample size of 55 SCD subjects there was 80% power to find a difference of 20% or greater by gender. When evaluating associations with markers of hemolysis in the SCD group as a whole, the cold detection threshold in the hand was significantly correlated with total bilirubin ($r = 0.42$; $p = 0.01$), otherwise we did not find significant correlations between other markers of hemolysis and pain or detection thresholds in SCD patients (all other r values < 0.2 and $p > 0.05$). A sub-analysis of HbSS patients revealed the mechanical pain threshold in the hand was significantly correlated with AST ($r = 0.38$; $p = 0.04$), heat pain threshold in the hand was significantly correlated with hemoglobin ($r = 0.4$; $p = 0.02$), and mechanical detection threshold in the hand was significantly negatively correlated with reticulocyte count ($r = -0.39$; $p = 0.03$). After adjusting for multiple testing using Bonferroni Correction ($p < 0.004$), none of the above significant correlations were significant.

Discussion

Using the well-established methodology of QST, we report for the first time that SCD patients have enhanced thermal pain sensitivity. Our data reveal that SCD patients have increased pain sensitivity to both cold and heat compared to healthy race-matched controls (i.e. decreased cold and heat pain thresholds). In addition, our study found that SCD patients detect the sensation of cold and heat sooner than controls (i.e. have decreased cold and heat detection thresholds), providing further evidence suggesting altered peripheral or central neural mechanisms may mediate pain sensitivity in SCD. Further, older age was associated with decreased cold and heat pain thresholds in both SCD patients and controls.

Epidemiologic studies reveal that SCD pain is associated with cold temperatures.[4–6] The multicenter study of hydroxyurea found that lower temperatures were associated with higher pain frequency and intensity.[5] Murine studies reveal that sickle mice have enhanced sensitivity to cold stimuli compared to control mice.[12, 13] Our study supports, for the first time, similar findings in humans with SCD and provides objective evidence supporting prior epidemiological data.

Our finding of heat hypersensitivity in SCD patients also parallels the finding of heat sensitivity found in the SCD murine model.[12, 13] Although the finding of heat pain sensitivity suggests altered neural mechanisms (peripheral or central), this finding is interesting clinically since many SCD patients use heat as an analgesic measure when in pain and heat has been recommended for SCD pain.[32]

Our finding of decreased cold and heat detection thresholds (i.e. perceived sensation of cold or warm temperatures) in SCD patients suggests increased sensitivity to cooling and warming. This finding may be linked to specific receptors at the level of the peripheral neuron that are thought to be responsible for both cooling and warming.[33]

We did not find differences in mechanical pain or detection thresholds between SCD patients and controls. This was in contrast to what has been shown in the murine model.[12, 13] The reason for this difference in humans may in part be due to the controlled genetic background of the sickle mice.

The mechanisms underlying our overall findings are unknown and require further investigation. The findings of increased cold and heat sensitivity in SCD patients raise the possibility that sensitization at the level of either the peripheral neurons and/or brain and spinal cord may occur in SCD and this peripheral or central sensitization may be a potential contributing mechanism for pain.[34] Peripheral or central sensitization can clinically result in hypersensitivity and/or allodynia and present as increased sensitivity to cold, heat, or mechanical stimuli.[35, 36] We cannot delineate from our data whether the underlying mechanism for the increased thermal sensitivity we observed is due to peripheral or central sensitization or from some other mechanism. Thus, further research into the underlying neurobiological mechanisms for our findings is warranted. Alternatively, it may be possible that the sensitivities observed have no mechanistic link to pain.

Members of the family of thermal Transient Receptor Potential (TRP) ion channels (thermoTRPs) located on nociceptors are sensitive to thermal stimuli and implicated in the detection of noxious thermal stimuli.[33, 37] The thermal hypersensitivity we observed in SCD patients may in part be due to sensitization of the thermal TRP receptors by endogenous or exogenous stimuli.[33, 38] Interestingly, the mechanical hypersensitivity in the Berkley HbSS mouse has been shown to be mediated via a Transient Receptor Potential Vanilloid 1 (TRPV1) mechanism.[13] TRPV1 is a receptor located on unmyelinated nociceptors and is traditionally known as a heat receptor.[39, 40] Therefore, one candidate mechanism underlying the heat sensitization in SCD patients is TRPV1. However, since human SCD patients do not exhibit mechanical sensitization, TRPV1 in humans may not function in the same role(s) in mechanical sensitization as in rodents. Future studies will need to determine whether TRPV1 contributes to heat hypersensitivity in SCD patients. In addition, the TRP receptors responsible for the detection of both noxious cold stimuli and cooling should be investigated in SCD.[33] Although the underlying mechanisms of thermal hypersensitivity in SCD patients are not yet known, we are the first to report data supporting thermal hypersensitivity exists in SCD patients.

Differences in pain and detection thresholds between SCD patients and controls were observed in the tested hand but not in the tested foot. This may be due to biologic differences between the types of skin that was tested. The hand was tested on the thenar eminence which is glabrous skin and the foot was tested on the lateral dorsum which is hairy skin. It may also be possible that the pathophysiology of pain in SCD is fundamentally different than other pain syndromes and these findings may be a clue to these differences. Alternatively, it may be possible that the sensitivities observed have no mechanistic link to pain. The reason for these differences requires further investigation.

When examining factors that may influence pain thresholds, we found older age was associated with decreased cold, heat, and mechanical pain thresholds in both SCD patients and controls. There was not a differential association between the two groups. These data are consistent with published literature revealing that older age is associated with increased pain sensitivity in non-SCD human pain conditions.[16, 17] The reason for this association is not known, but is an active area of research. Our data suggest that gender had no impact on our primary outcomes. These data are in contrast to our hypothesis and the prior published human pain literature where females have been shown to have increased pain sensitivity.[14, 15]

Surprisingly, a history of more frequent pain was not associated with increased pain sensitivity in this group of SCD patients. This is in contrast to prior literature in pain syndromes other than SCD where it has been hypothesized that more painful events result in neuro-remodeling and increased pain sensitivity.[10, 18–20] The reason for this unexpected finding may in part be related to the fact that we used health care utilization as a measure of prior acute pain in order to minimize recall bias. In using this as our measure, we likely underestimated the true amount of pain patients experience since the majority of pain is managed at home.[2, 41] Alternatively, it may be possible that the sensitivities measured by our study may have no mechanistic link to pain. Future studies should focus on prospective collection of both home pain and health care utilization in order to better answer this question.

We did not find a generalized association with baseline laboratory markers of hemolysis and pain and detection thresholds in SCD patients. We chose to look at the association of hemolysis and pain sensitivity since a lower hemolytic rate and higher blood viscosity (i.e. higher hemoglobin and lower reticulocyte count, LDH, AST and bilirubin) is associated with a SCD phenotype with more painful events.[42, 43] The lack of a generalized association of these laboratory parameters with our primary and secondary outcomes suggests hemolysis may not play a major role in the pain sensitivity as measured by our techniques. Other mechanisms may be linked to this increased pain sensitivity. For example, multiple inflammatory mediators including leukotrienes have been documented to sensitize nociceptors in non-SCD models.[11, 33] Other factors shown to be associated with peripheral nociceptor sensitization are substance P, calcitonin-gene related peptide, and other markers of inflammation known to be elevated in SCD (i.e., TNF- α , endothelin-1, PGE2, bradykinin).[11, 12, 44–46]

Our data are limited by the study's cross-sectional design where subjects were tested at one point in time. It is unknown if thresholds change over time so longitudinal evaluation is warranted. We were unable to blind the person conducting the testing as to whether subjects had SCD or were controls since the study's research personnel are the primary research assistants in our SCD clinic. Since thermal testing was computer-driven, this issue was less crucial. Although mechanical testing was not, we followed standardized testing procedures. Computer-driven mechanical testing does not currently exist for humans. The history of prior acute painful events was determined based on health care utilization, however, data reveal this underestimates the true amount of SCD pain since the majority of pain is managed at home.[2, 41] We used health care utilization as a marker of prior pain to minimize recall bias. The laboratory data analyzed were not obtained the day of the study but were rather obtained as a mean from the three most recent clinic visits when patient was in baseline health. This is a limitation, however, we believe the laboratory values are most representative of the patient's steady state. Urine drug testing was not performed to ensure subjects were not under the influence of analgesics at the time of testing which is also a limitation. All subjects were called 48 hours prior to testing to remind them to withhold analgesics within 24 hours of testing and if analgesics were taken testing was rescheduled. Patients with known silent cerebral infarcts were excluded. Screening every patient with MRI to rule out an undiagnosed silent infarct was not feasible. Females were likely at different phases of their menstrual cycle which may influence pain sensitivity, however, attempting to test all females during the same phase of their menstrual cycle would significantly limit the study's feasibility. Blood flow velocity of the tested sites was not performed. Approximately 60% of our SCD patients were on hydroxyurea which can decrease hemolytic rate. Thus, the secondary analyses of the associations of hemolytic rate may not be generalizable to the overall SCD population. Future work should attempt to evaluate those on and off hydroxyurea, however, this will be increasingly difficult as indications for hydroxyurea are expanded. Psychosocial functioning (depression, anxiety,

coping) may affect the outcomes. We have an ongoing study to evaluate the impact of psychosocial functioning on the outcomes.

Our findings of increased cold and heat pain sensitivity are the first step towards delineating the underlying neurobiology of pain in SCD patients and closely parallel the findings in the sickle cell murine model.[12, 13] Further dissection or resolution of the mechanism that leads to this hypersensitivity may reveal potential targets for novel therapeutics to treat and/or prevent SCD pain. Treatments that specifically target the TRP pain receptors are currently in pre-clinical[47, 48] and phase I-II trials in diseases other than SCD.[49] Finally, these findings may allow for better phenotyping of SCD pain and allow further investigation into the transition from acute to chronic pain in SCD.

In conclusion, pain significantly impacts the lives of SCD patients and the underlying neurobiology is poorly understood. We have shown that SCD patients experience increased pain sensitivity to cold and heat stimuli and have lower cold and heat detection thresholds. Both of these phenomena suggest that the underlying neurobiology of SCD pain may in part be due to this sensitivity that could be linked to the activation or sensitization of the thermal TRP receptors. Furthermore, our data support the concept that peripheral or central sensitization may exist at baseline in SCD patients and may contribute to SCD pain, thus warranting further exploration.

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Figure 1. Boxplots displaying differences in median cold, heat, and mechanical pain thresholds between SCD patients (n=55) and African American controls (n=57)

Significant differences were found between groups for both the cold and heat pain thresholds in the hand. No differences were seen in the tested foot. Significant differences were not seen in the mechanical pain thresholds in the tested hand or foot.

Detection Thresholds

Figure 2. Boxplots displaying differences in median cold, heat, and mechanical detection thresholds between SCD patients (n=55) and African American controls (n=57)
Significant differences were found between groups for both the cold and heat detection thresholds in the hand. No differences were seen in the tested foot. Significant differences were not seen in the mechanical detection thresholds in the tested hand or foot.

Table I

Demographics and baseline characteristics of SCD patients and controls

Variable	SCD Patients (n=55)	Controls (n=57)	p-value
Age (years)			
Mean ($\pm$ SD)	15.4 ($\pm$ 6.3)	16.3 ($\pm$ 10.2)	0.59
Median (IQR)	14 (11–19)	12 (9–18)	0.42
Gender			
Female	33 (60%)	32 (56%)	0.68
Sickle Cell Genotype			
HbSS	37 (67%)	N/A	
HbSC	10 (18%)		
HbS β +thal	6 (11%)		
Other	2 (4%)		
Trait Status			
Unknown	N/A	5 (9%)	
No		28 (49%)	
<u>Yes</u>		<u>24 (42%)</u>	
S trait		16 (28%)	
C trait		2 (4%)	
Thal trait		1 (2%)	
Unknown		5 (9%)	
Hydroxyurea			
Yes	23 (42%)	N/A	
Number of Prior Pain Events[#]			
Over Lifetime of patient		N/A	
7–12 years	4 (1–14)		
13–18 years	13.5 (5–30)		
19 years	15 (6.5–30)		
3 years before study entry			
7–12 years	2 (0–4)		
13–18 years	6 (3–10)		
19 years	1 (0–2)		
Markers of Hemolysis[#]			
Hemoglobin (g/dL)	9.92 (8.62 – 11.00)	N/A	
Reticulocyte count (%)	7.60 (3.08 – 11.17)		
Total Bilirubin (mg/dL)	2.05 (0.97–2.87)		
Lactate Dehydrogenase (LDH) (units/L)	855 (663 – 1100)		
Aspartate aminotransferase (AST) (units/L)	43 (35–59.2)		

Median (IQR)

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Table II

Association of age and pain thresholds in SCD patients and controls

Outcome	r*	p-value
Cold Pain Threshold Hand (°C)	0.22	0.02
Heat Pain Threshold Hand (°C)	0.27	0.004
Mechanical Pain Threshold Hand (°C)	0.21	0.03
Cold Pain Threshold Foot (°C)	0.07	0.48
Heat Pain Threshold Foot (°C)	0.17	0.08
Mechanical Pain Threshold Foot (°C)	0.18	0.05

* Spearman rank correlation