HHS Public Access

Author manuscript

*Gene Rep*. Author manuscript; available in PMC 2024 September 01.

Published in final edited form as:

*Gene Rep*. 2024 September ; 36: . doi:10.1016/j.genrep.2024.101983.

# Genetic contributions to pain modulation in sickle cell: A focus on single nucleotide polymorphisms

**Katrina R. Hamilton**[a], **Lakeya S. McGill**[b], **Claudia M. Campbell**[a], **Sophie M. Lanzkron**[c], **C. Patrick Carroll**[a], **Alban Latremoliere**[d], **Jennifer A. Haythornthwaite**[a], **Olga A. Korczeniewska**[e,*]

[a]Department of Psychiatry and Behavioral Sciences, Johns Hopkins University, School of Medicine, Baltimore, MD, USA

[b]Department of Medicine, University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA

[c]Department of Medicine, Johns Hopkins University, School of Medicine, Baltimore, MD, USA

[d]Department of Neurosurgery, Johns Hopkins University, School of Medicine, Baltimore, MD, USA

[e]Center for Orofacial Pain and Temporomandibular Disorders, Department of Diagnostic Sciences, Rutgers School of Dental Medicine, Newark, NJ, USA

## Abstract

**Background:** Despite recent advances in our knowledge of genetic contributions to the highly variable sickle cell disease (SCD) phenotype, our understanding of genetic factors associated with pain sensitivity in SCD remains limited. Previous studies investigated specific variants in single candidate genes and their association with SCD pain variability. The primary aim of the current study was to expand the genes and polymorphisms tested to discover new risk genes (polymorphisms) associated with central sensitization for individuals with SCD.

**Methods:** Adults with sickle cell disease ($n$ = 59, Mage = 36.8 ± 11.5, 65.8 % female) underwent quantitative sensory testing to examine central sensitization and general pain sensitivity. Participants reported average crisis and non-crisis pain intensities weekly using a 0–100 scale, and provided salivary samples for genotyping. The Hardy-Weinberg equilibrium was verified for controls, and allele distributions were tested with chi-square and odds ratio tests. The Benjamini-Hochberg procedure was used to control for false discovery rate. Regression analyses and Wilcoxon tests were used to test associations for normally distributed and skewed data, respectively.



*Corresponding author at: Center for Orofacial Pain and Temporomandibular Disorders, Department of Diagnostic Sciences, Rutgers School of Dental Medicine, Rutgers the State University of New Jersey, Room D-837, 110 Bergen Street, Newark, NJ 07101, USA. olga.korczeniewska@rutgers.edu (O.A. Korczeniewska).

CRediT authorship contribution statement

**Katrina R. Hamilton:** Writing – review & editing, Writing – original draft. **Lakeya S. McGill:** Writing – review & editing, Writing – original draft. **Claudia M. Campbell:** Writing – review & editing, Writing – original draft, Funding acquisition. **Sophie M. Lanzkron:** Writing – review & editing. **C. Patrick Carroll:** Writing – review & editing. **Alban Latremoliere:** Writing – review & editing. **Jennifer A. Haythomthwaite:** Writing – review & editing, Project administration, Investigation, Funding acquisition, Conceptualization. **Olga A. Korczeniewska:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization.

**Results:** Central sensitization and general pain sensitivity were not associated with hemoglobin genotype (Ps > 0.05). Of 4145 SNPs tested, following false discovery rate adjustments, 11 SNPs (rs11575839, rs12185625, rs12289836, rs1493383, rs2233976, rs3131787, rs3739693, rs4292454, rs4364, rs4678, rs6773307) were significantly associated with central sensitization, and one SNP (rs7778077) was significantly associated with average weekly non-crisis pain. No SNPs were associated with general pain sensitivity.

**Conclusions:** These findings provide insights into genetic variants association with average non-crisis pain and central sensitization for individuals with SCD, and may provide support for genetic predictors of heightened pain experience within SCD.

### Keywords

Sickle cell; Pain; Single nucleotide polymorphism; Hemoglobin genotype; Central sensitization; Quantitative sensory testing

## 1. Introduction

Individuals with sickle cell disease (SCD) have a reduced life expectancy and high disease burden (Lubeck et al., 2019). They experience episodes of severe pain known as vaso-occlusive crises, when sickled red blood cells occlude small blood vessels. In addition to these acute pain episodes, the majority of adults with SCD experience persistent pain on most days and have significantly reduced quality of life from their condition (Matthie et al., 2020; Osunkwo et al., 2021). The four main genotypes of SCD are caused by co-inheriting a set of β-globin variants with the hemoglobin S gene (CDC, 2022). Hemoglobin SS (Hb SS) is the most common type, in which two copies of the hemoglobin S (Hb S) gene were inherited. When the Hb S gene is co-inherited with a beta thalassemia gene, severity of sickling pathology depends on the beta thalassemia gene. Hemoglobin Sβ° disease is similar in phenotype to Hb SS disease, as these people also produce only hemoglobin S. Hemoglobin Sβ + disease produces a mix of normal and sickle hemoglobin, and is typically less severe. Hemoglobin SC (Hb SC) is the second most common type, in which genes for hemoglobins S and C are co-inherited. This illness is typically less severe than HbSS or Hbsβ° disease.

The pain associated with SCD is highly variable. Although hemoglobin genotype can partially explain this variability, there are wide inter-individual differences in pain experience among individuals with the same hemoglobin genotype (i.e., SS or SC). The determinants of variation in SCD pain experience remain poorly understood, which is likely due to the complex nature of pain, with multiple biopsychosocial contributors (e.g., immune, brain, socioenvironmental factors). Hyper-sensitivity and hyperexcitability of the peripheral and central nervous systems, known as peripheral and central sensitization, are believed to contribute to clinical SCD pain (Campbell et al., 2016b). Recurrent vaso-occlusion in SCD is thought to result in persistent inflammation, and therefore- nociceptive input, which in turn may lead to pain sensitization (Gupta et al., 2018; Tran et al., 2017). With central sensitization (CS), input from peripheral nociceptors activate the central nervous system and alter the spinal cord and brain processing, resulting in a chronic amplification of pain sensations (Woolf, 2011). Individuals with SCD who have heightened central sensitization

profiles report more clinical pain, vaso-occlusive crises, catastrophizing, negative mood, and poorer sleep quality over an 18-month follow-up period (Campbell et al., 2016b). Although past work has demonstrated a relationship between hypersensitivity and high disease burden in SCD, the connection of SCD hypersensitivity to genetic contributions is not well elucidated.

Several genes have been associated with clinical characteristics of SCD. A meta-analysis of microarray and genome-wide association studies focused on inflammatory responses revealed a common molecular signature of individuals with SCD (Ben Hamda et al., 2018). The authors identified 335 differentially expressed genes and used genome-wide association studies to identify regulatory single nucleotide polymorphisms (SNPs) in the promoter regions of these genes that could contribute to their differential expression (Ben Hamda et al., 2018). However, in this meta-analysis, the authors focused on transcriptional changes contributing to pathogenesis of SCD, not pain modulation. Recent work examined the relationship between average SCD pain intensity and 11 functional SNPs in 9 pain-related genes, and found the minor allele in *ICAM1* rs1799969 was associated with lower average pain intensity (Knisely et al., 2023). Previously, Jhun et al. analyzed associations between SCD phenotypes and Val158Met (rs4680) SNP in the *COMT* gene and Ser9Gly (rs6280) SNP in dopamine D3 receptor gene (Jhun et al., 2014). They concluded that because these polymorphisms were associated with different rates of SCD related acute care utilization, they might contribute to acute pain crisis heterogeneity in SCD. Additionally, polymorphisms in glucocorticoid receptor gene NR3C1 (Jhun et al., 2018b), GCH1 gene (Sadhu et al., 2018), and the transient receptor potential A gene (TRPA1) (Jhun et al., 2018a) were shown to be associated with acute care visits for pain. More recently, contributions of beta2-adrenergic receptor (*ADRB2*) polymorphisms to chronic pain severity and heterogeneity observed in SCD were investigated (Jhun et al., 2019). The authors reported significant associations between chronic pain and seven SNPs in *ADRB2* and concluded that variations in *ADRB2* might contribute to chronic pain severity and heterogeneity in SCD (Jhun et al., 2019).

Despite recent advances in our knowledge of genetic contributions to the highly variable SCD phenotype, our understanding of genetic factors associated with pain sensitivity in SCD remains limited. Previous studies investigated specific variants in single candidate genes, and their association with pain variability. The primary aim of the current study was to expand the genes and polymorphisms tested to discover new risk genes (polymorphisms) associated with central sensitization for adults with SCD. To address this aim, we conducted a secondary analysis association study of 4900 SNPs in 553 genes with the Algynomics Pain Research Panel, specialized to genes pertinent to elements of the pain experience (i.e., nociception, pain perception, affect, mood, inflammation) (Kutlar et al., 2014). In this secondary analysis we leverage the extensive pain phenotyping collected in the parent study, including assessment of clinical pain and quantitative sensory testing to assess sensitivity to heat and pressure pain, as well as conditioned pain modulation and central sensitization parameters for individuals with SCD (Campbell et al., 2016b). We hypothesized that genetic variability in genes associated with pain perception and modulation would be associated with central sensitization measures for adults with SCD.

## 2. Materials and methods

Fifty-nine adults with sickle cell disease were included in this secondary analysis, recruitment and methods have been previously reported (Campbell et al., 2016b; Mathur et al., 2016; Moscou-Jackson et al., 2015). Demographic and clinical characteristics of the included SCD participants are summarized in Table 1.

### 2.1. Genotyping

Saliva samples were collected in Oragene Discover Series self-collection kits (OG-500, DNA Genotek, Ontario, Canada). Collection kits contained a tube with funnel pre-attached. Participants were asked to spit into the funnel until optimal volume was obtained (approximately 2 mL). The technician then closed the funnel cap to release sample stabilizing fluid, replaced the lid, and inverted the tube to ensure mixing. Tubes were labeled with a participant ID and stored at room temperature for batch analyses. Samples were sent to the University of Florida for DNA extraction and genotyping using the Algynomics Pain Research Panel (Chapel Hill, NC). The Panel is a chip-based platform manufactured by Illumina. It assesses 4900 SNPs representing 553 genes known to play a role in pain perception. Specifically, these genes mediate pain perception through the central nervous system via sensory nerve fibers (Slade et al., 2013). Genotypes were detected using the Illumina platform. The data were exported to GenomeStudio Software (Illumina Inc., Hayward, CA) for allele calling.

### 2.2. Quantitative sensory testing (QST) – central sensitization and general sensitivity

Quantitative Sensory Testing (QST) refers to a set of standardized procedures to test sensory sensitivity; and has been to used reliably in healthy and chronic pain samples, including in those with SCD (Dyal et al., 2020; Middlebrook et al., 2020; Miller et al., 2019; O'Neill and O'Neill, 2015). As previously discussed in detail (Campbell et al., 2016a; Campbell et al., 2016b; Carroll et al., 2016), QST was employed during an in-person laboratory visit for the current study. To ensure the accuracy of QST, all individuals who administered testing underwent training and practice sessions before interacting with participants. Additionally, the lab provided a standardized protocol with a detailed script for QST assessments and a data sheet for recording outcomes and assessment timing. Laboratory standards were followed that are typical to QST, including thermal pain testing on the underside of the ventral dominant forearm, a 2-minute time limit for cold water testing, and a maximum of 45 °C for warm water testing. Central sensitization (CS) was operationalized as an average of the participant's *Z*-scored mechanical temporal summation, thermal temporal summation, and after sensation of lingering post-temporal summation and hot water test pain. The current analyses used previously divided data where SCD participants were categorized into no/low CS and high CS. We compared genotype frequencies of the 4145 SNPs between the two types of SCD participants and found that after FDR adjustment genotype frequencies did not differ significantly between the two groups. Therefore, all the SCD participants were combined in further analyses. A non-CS QST index score was created as a measure of general sensitivity using average Z-scored conditioned pain modulation, pressure pain threshold, heat pain threshold, heat pain tolerance, hot water bath hand withdraw latency, intensity, and water bath temperature.

### 2.3. Weekly crisis and non-crisis pain

Vaso-occlusive pain (i.e., crisis pain) and non-vaso-occlusive pain (i. e., non-crisis pain) were determined by averaging daily participant pain reports over 3 months (Campbell et al., 2016b). At bedtime, participants indicated their daily pain level on a scale of 0 “no pain” to 100 “pain as bad as you can imagine”. If the participant indicated that they had experience a crisis, they indicated their crisis pain the same 0–100 scale used for daily pain.

### 2.4. Statistical analysis

Data were analyzed with JMP Genomics 8.1 (*SAS* Institute Inc., Cary, NC, 1989–2007). The Hardy-Weinberg equilibrium (HWE) was verified for control subjects. The distribution of SNPs (alleles) was compared with chi-square tests and odds ratios (OR) with 95 % confidence intervals (CI) estimated. False discovery rate (FDR) was controlled by applying the Benjamini-Hochberg procedure. The significance level was FDR corrected and population stratification was accounted for in analyses. To measure the effects of SNP on pain phenotype, marker-trait association testing was conducted. Pain phenotype variables (CS and crisis/non-crisis pain) were tested for normality using Goodness of Fit Tests. Normally distributed data were analyzed using regression analysis and nonparametric Wilcoxon test was used for not normally distributed variables. Results are presented as mean ± standard deviation unless otherwise specified.

All subjects were genotyped for a total of 4900 SNPs (Kutlar et al., 2014; Slade et al., 2013). Monomorphic and SNPs with no calls (i.e., allele (s) were not determined during genotyping and therefore could not be reported, $n = 229$) were excluded from the analyses. Twenty-eight healthy controls with the available SNP genotype data were used to identify SNPs that deviated from Hardy Weinberg Equilibrium (HWE). SNPs that deviated from HWE in control subjects ($n = 16$) were excluded to avoid false positive results. SNP minor allele frequency (MAF) cutoff value was set at 0.05 and proportion of missing genotypes at 0.1. Based on the specified MAF cut off and proportion of missing genotypes, a total of 526 markers were further excluded leaving a total of 4145 SNPs included in the analyses.

### 2.5. Pathway/biological process analyses

The PANTHER (Protein ANalysis THrough Evolutionary Relationships) Classification System (Mi and Thomas, 2009) was used to perform pathway and biological process analyses of genes. The PANHER knowledgebase was designed to classify proteins (and their genes) in order to facilitate high-throughput analysis. The PANTHER Classifications are the result of human curation as well as sophisticated bioinformatics algorithms. Details of the methods can be found in Mi et al. NAR 2013 (Mi et al., 2013) and Thomas et al., Protein Science 2022 (Thomas et al., 2022).

## 3. Results

### 3.1. Hemoglobin genotypes in SCD and pain variables

Central sensitization was not significantly associated with SCD hemoglobin genotype in our study (Wilcoxon test $\chi^2 = 2.1$, $P$-value = 0.6). Among 59 SCD participants included in this study, 38 (64 %) participants had homozygous hemoglobin S (Hb SS) genotype, 11

(19 %) had Sickle hemoglobin C (hemoglobin S and hemoglobin C, Hb SC) genotype, 7 (12 %) had hemoglobin S beta thalassaemia (Hb S-β(+)-thal) genotype, and 3 (5 %) had hemoglobin S beta 0 thalassemia (S-β(0)-thal) hemoglobin genotype. QST index reflecting general sensitivity was not associated with the hemoglobin genotype in SCD participants (ANOVA F-Ratio = 0.6, *P*-value = 0.6).

### 3.2. Single nucleotide polymorphism (SNP) analyses

Association tests were carried out between SNPs and parameters of quantitative sensory testing (QST). To measure the association between CS scores and markers' genotypes in SCD, genotype association testing was run on 4145 SNPs (Kutlar et al., 2014; Slade et al., 2013). Out of the 4145 SNPs tested, 197 SNPs showed significant association with QST CS scores in SCD participants and 11 SNPs remained significant following FDR adjustment (Table 2). The summary of QST CS scores and the associated SNPs are provided in Fig. 1 and Table 3.

We used LDLink, an Interactive Web Tool for Exploring Disequilibrium (Mathur et al., 2016), to measure linkage disequilibrium between the identified SNPs that were significantly associated with QST CS scores in SCD participants. Two of the eleven identified SNPs were in complete linkage disequilibrium with each other (rs3131787 T/C and rs4678 G/A; $D' = 1.0$, $R^2 = 1.0$; Correlated Alleles C = A, T = G). Prior to FDR adjustment, 193 SNPs were significantly associated with general sensitivity (i.e., non-CS QST index); however, none remained significant following FDR adjustment.

One SNP - rs7778077 - located in *PRKAG2* gene was significantly associated with average weekly non-crisis pain reported by SCD participants during weekly calls (FDR-adjusted *P*-value = 0.001). SCD participants with G/G genotype at rs7778077 SNP reported significantly more average weekly pain (5.12 ± 1.4) than those with either A/G (2.1 ± 1.5, $P < 0.0001$) or A/A (1.3 ± 1.2, $P < 0.0001$) genotypes (Fig. 2).

### 3.3. Sex specific effects

To identify sex specific associations, we analyzed 86 X-chromosome SNPs separately in males ($n = 19$) and females ($n = 40$). In males, four SNPs (rs1155215, rs11796093, rS2229963, and rs6630811) were significantly associated with QST CS scores, two SNPs (rs1040398 and rs3829708) were associated with general sensitivity, five SNPs (rs11796093, rs12838742, rs3761555, rs508865 and rs6630811) were significantly associated with average pain, eight (rs11796093, rs12838742, rs2497510, rs35609266, rs3761555, rs4911878, rs508865, and rs6318) with average combined diary pain, and four (rs1624766, rs6651806, rs2229963, and rs6651806) with average pain during crisis. In females, one SNP (rs17146226) was significantly associated with QST CS score, nine SNPs (rs1152187, rs1160198, rs12011733, rs2188931, rs2497515, rs3027379, rs3027935, rs34834543, and rs35609266) were significantly associated with general sensitivity, one (rs5980064) with average combined diary pain and three SNPs (rs3027449, rs5201, and rs5906729) were significantly associated with mean pain during crisis. These results suggest that sex chromosome SNPs could play a role in pain modulation in SCD, however these results should be replicated in a larger sample size to confirm these associations.

### 3.4. Pathway and biological processes analyses

**3.4.1. Pathway analyses—**Genes with SNPs associated with QST central sensitization did not converge on the same pathway. Nine of 11 genes with SNPs associated with QST central sensitization scores (C6orf15, SFTA2, GHR, ACTL7A, TRPM8, VARS1, NCR3, ACE, AND VARS2) had no PANTHER pathway category assigned. Transcription factor p65 (RELA) gene belongs to four pathways which included: apoptosis signaling pathway (P00006), gonadotropin-releasing hormone receptor pathway (P06664), inflammation mediated by chemokine and cytokine signaling pathway (P00031) and toll receptor signaling pathway (P00054). Histamine H1 receptor (HRH1) gene belongs to two pathways including heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway (P00026), as well as histamine H1 receptor mediated signaling pathway (P04385). Finally, glutamate receptor 1 (GRIA1) gene was identified to belong to ionotropic glutamate receptor pathway (P00037) and metabotropic glutamate receptor group III pathway (P00039).

**3.4.2. Biological process analyses—**Eight out of 11 genes with SNPs associated with QST central sensitization belonged to cellular processes (GO: 0009987) and these included: C6orf15, GHR, RELA, HRH1, TRPMS, GRIA1, VARS1, and VARS2 genes. Five genes (RELA, GRIA1, GHR, ACE, and HRH1) belonged to biological regulation (GO: 0065007), three genes (GHR, RELA, and HRH1) to stimulus response (GO: 0050896) biological process, two genes (VARS1 and VARS2) belonged to metabolic process (GO: 0008152), the ACE gene belonged to multicellular organismal process (GO: 0032501), and TRMP8 gene belonged to localization (GO: 0051179) biological process. The RELA gene was identified to belong to two biological processes, including interspecies interaction between organisms (GO: 0044419) and immune system process (GO: 0002376).

## 4. Discussion

Individual differences in pain processing, including genetic variability in the central processing of nociceptive stimuli, may affect the presentation of SCD pain. However, the molecular basis for the alterations in pain processing in SCD is not well understood. Knowledge of the factors associated with increased SCD pain sensitivity could provide clinically relevant information. Accordingly, the current study examined the association of genetic variations in 553 genes on clinical non-crisis pain and central sensitization in individuals with SCD. Our findings indicate a significant relationship for twelve SNPs to the pain experience for individuals with SCD. These relationships have not been previously reported in the literature and may provide an avenue for genetic identification of those at risk for greater SCD pain and insight into potential clinical targets for reduced suffering.

This study is the first to report the associations of polymorphisms in *NCR3* (rs11575839), *TRPM8* (rs12185625), *RELA* (rs12289836), *GRIA1* (rs1493383), *C6orf15* (rs2233976), *SFTA2* (rs3131787), *IKBKAP* (rs3739693), *GHR* (rs4292454), *ACE* (rs4364), *VARS2* (rs4678), and *HRH1* (rs6773307) genes and central sensitization measures for individuals with SCD. Only one SNP - rs7778077 - located in *PRKAG2* gene was significantly associated with average weekly non-crisis pain.

Of the eleven SNPs associated with central sensitization in our SCD cohort, three SNPs (rs11575839, rs2233976, and rs4678) have been previously reported to be associated with other chronic conditions that may provide important directions for future investigations. The synonymous variant is located in the major histocompatibility complex (MHC) class 1 area that includes HLA-A, HLA-C and HLA-B genes. The rs11575839 is located close to HLA-C and has been associated with complement C4 levels in Chinese participants (Yang et al., 2012). The complement members C3 and C4 exert their powerful roles as host defense proteins (Inoue et al., 2008). In addition to evidence of association with hemoglobin A1C (HbA1C) levels (Urbanek et al., 2012), deficiencies or over-expression of these complement system members are associated with pathogenesis of many inflammatory and autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, and asthma (Unsworth, 2008; Walport, 2001). Also associated with immune responses, we found an association between *C6orf15* (rs2233976) and central sensitization in SCD. This non-synonymous SNP was found to be in absolute linkage disequilibrium with human leukocyte antigen (HLA) allele C*04:01 in a Japanese sample (Middlebrook et al., 2020). HLA class I and II molecules play a central role in T cell differentiation in thymus and in immune responses to foreign antigens in the peripheral lymphoid organs (Kitajima et al., 2012). Genes encoding HLA class I and II molecules are located on the short arm of chromosome 6 in the MHC, where many immune-related genes reside (Kitajima et al., 2012). And finally, in addition to increased risk for lung cancer, (Kazma et al., 2012) the non-synonymous variant (rs4678) in VARS2 gene was previously associated with rheumatoid arthritis, though it should be noted that the clinical significance for this variant is predicted by Clin-Var to be benign (Vignal et al., 2009). This prior work may point to an inflammatory correlate in SCD related to adequate immune functioning that is associated with these variants, and may indicate an avenue for future work in this area.

We did not find significant associations between hemoglobin genotype and central sensitization or general pain sensitivity as measured with quantitative sensory testing, which could in part be due to a limited study population. To our knowledge, only one published study has reported on quantitative sensory testing and hemoglobin genotype associations in SCD previously (Kidwell et al., 2021). In this work, lower pressure pain threshold only for the ulna testing site was found for high utilizers (i.e., ≥6 emergency department visits or hospitalizations a year), which was largely made up of those with Hb SS genotype. Our work took a novel approach by incorporating composite QST measures that are reflective of central sensitization and general pain sensitivity, factors that when elevated have been associated with multiple clinical factors (e.g., vaso-occlusive crises, pain catastrophizing, poor sleep) for individuals with SCD over an extended follow-up of one and half years (Campbell et al., 2016b). Perhaps of note, the current study did not conduct pressure pain testing on the ulna.

Although the 11 SNP-associated genes that were related to central sensitization in the current analyses did not converge on the same pathway, we found overlap for SNP-associated genes that were related to biological processes, including those that may be most important to consider in regards to SCD pain and crisis (e.g., multicellular organismal process, stimulus response, localization). We offer the following overview of relevant connections to three of these genes to SCD, but relevance to the current results should be

taken with caution as many of the SNPs related to pain sensitivity in the current sample were located in non-coding regions. The ACE gene, which belonged to multicellular organismal process, controls ACE enzyme production; and significantly lower levels of ACE enzyme have been associated with low blood pressure in both human and mouse SCD (Brito et al., 2022). The authors concluded that blood pressure regulation in SCD may be greatly impacted by ACE depletion and should be studied further (Brito et al., 2022. In the broader context of pain, HRH1 serves an important role in histamine-induced itch and mechanical sensitization in humans (Tavares-Ferreira et al., 2022). TRPM8 plays a major role in cold detection (McKemy, 2007); however, even given the cold allodynia and hypersensitivity in SCD, especially during VOC, and our results, there has yet to be strong evidence for TRPM8 mRNA differential expression between SCD and healthy samples (Sadler and Stucky, 2019; Zappia et al., 2014). Sadler and Stucky suggest that TRPM8 should be further studied to better understand possible contributions to SCD cold sensitivity (Sadler and Stucky, 2019).

Genetic, biological, psychological and environmental factors have all been implicated in the complex experience of pain. Interestingly, healthy twin studies suggest that 22–55 % of the variability for pain sensitivity could be attributed to genetic contributions (Norbury et al., 2007). A number of genes and polymorphisms have been investigated in the context of pain sensitivity in humans, but relationships are not always clear-cut. For example, some mutations in the gene encoding Na+ channel Nav1.7 (*SCN9A*) result in extreme pain conditions (e.g., familial erythromelalgia, paroxysmal extreme pain disorder), others produce congenital insensitivity to pain, and polymorphisms have detectable effects on pain without causing chronic pain (Catterall et al., 2008; Fischer and Waxman, 2010). In one of the most studied genes in pain work, catechol-*O*-methyl-transferase (*COMT*), variations are associated with experimental pain as well as clinical pain phenotypes (Diatchenko et al., 2005; Korczeniewska et al., 2021). A recent meta-analysis indicated that findings across chronic pain and healthy individuals did not find an association between COMT SNPs (rs6269, rs4633, rs4818) when examined independently and pain threshold; however, COMT haplotypes did affect pain sensitivity in combined sample (Vetterlein et al., 2023. Additional work examining pain sensitivity has shown decreased pressure pain threshold for temporomandibular disorder with TNFα-308 SNP rs1800629 (Furquim et al., 2016), and increased pressure pain sensitivity associated with SNP IL6-174 (CC and CG genotypes) and SNP COMT Val158Met (AA and GA genotypes) (Pinto Fiamengui et al., 2020).

Other genes that show association with experimental pain sensitivity include OPRM1 (Fillingim et al., 2005), GCH1 (Tegeder et al., 2006), SLC6A3, SLC6A4 (Mogil, 2012), OPRD1 (rs2234918C) with heightened thermal pain sensitivity in hip osteoarthritis, and an A allele in a common polymorphism (rs6746030) in *SCN9A* gene (Reimann et al., 2010). The latter has also been associated with osteoarthritis and pancreatitis pain reports (Reimann et al., 2010). In the context of SCD, previous work identified that common SNPs at the BCL11A, HBS1L-MYB and hemoglobin subunit beta loci accounted for >20 % of the variation in fetal hemoglobin levels and were associated with the rate of painful SCD crises (Lettre et al., 2008). Our work did not replicate these findings, nor did we replicate an early clinical poster (2014) (Kutlar et al., 2014) that reported associations between pressure pain threshold (masseter, ulna, and trapezius) and 6 SNPs (ESR2, KCNJ11, DBH,

ATP1A1, CACNA2D2) using the Algynomics Pain Research Panel. Moreover, past work has shown that the relationship between the GCH1 haplotype with SCD pain crisis was only significant for females, indicating a need for sex-specific analyses. Although we report sex-specific effects, the limited sample size precludes the ability to draw strong conclusions from these results. Additional work that replicates these findings in a large sample is necessary. (Belfer et al., 2014). Of additional interest, past work has included indirect measures of painful crises while the current study is reflective of weekly patient reports, this may explain incongruences in findings. Nevertheless, these inconsistencies suggest more work is necessary to fully understand genomic considerations for central sensitization and hypersensitivity in SCD. We recommend that future work takes into account multiple factors, such as pain intensity during crisis, crisis frequency, and crisis duration to better elucidate these relationships.

The primary goal of this investigation was to expand polymorphisms testing in SCD, with the addition of variables reflective of nociceptive and peripheral hypersensitivity. The addition of these variables provided great novelty; and the genomic analyses using the Algynomics Pain Research Panel (Kutlar et al., 2014; Slade et al., 2013) resulted in a targeted analysis with a pain-specific focus which was a strength of the current study. Nevertheless, the findings presented herein should be interpreted with caution, as this investigation was exploratory in nature. Future work should aim to have a larger sample size to allow for reproduction and expansion of the current results; and in addition to particular SNPs of interest, investigators should consider the contribution of whole haplotypes.

### 4.1. Limitations

Due to a modest sample size, only individual SNP analyses were performed to identify significant associations with QST sensitization scores and other parameters. It is possible that multiple SNPs would have an additive or synergistic effects on QST sensitization scores and other parameters, however due to a small sample size and therefore limited power we were unable to perform these types of analyses and therefore identify potential synergistic effects. A larger sample size would be required to identify synergistic effects of multiple SNPs, especially if they have modest individual effects.

### 4.2. Conclusions

These findings provide insights into genetic variants associated with central sensitization in sickle cell disease (SCD) and may provide genetic predictors of heightened pain experience within SCD. Individuals with SCD experience acute and chronic pain influenced by biopsychosocial factors, such as psychological distress, racism-based discrimination, and sleep disturbances (Lubeck et al., 2019; Matthie et al., 2020; McGill et al., 2023; Osunkwo et al., 2021). The current study focused on polymorphisms associated with heightened pain sensitivity, an expansion of previous work that examined associates with worse pain outcomes. Thereby the current work may support genetic predictors of heightened pain experience within SCD. Moreover, this investigation into the genetic predictors of pain sensitivity may provide valuable insight into potential clinical targets for central sensitization for individuals with SCD that may ultimately lead to reduced disease burden.

## Declaration of competing interest

Declarations of interest: none. All authors contributed significantly to this work. JAH conceptualized the parent study, obtained funding, and performed the research. OAK conceptualized the current work and analyzed the data. KRH and OAK interpreted the findings and led paper writing, with significant support from LSM and CMC. All authors (KRH, LSM, CMC, SML, CPC, AL, JAH, OAK) provided feedback on analyses, data interpretation, and manuscript drafting. This work was supported by the National Institutes of Health NHLBI R01HL098110 (JAH), NINDS T32NS070201 (KRH), NICHD T32HD007414 (LSM), NINDS K12NS130673 (LSM), NIAMS K24AR081143 (CMC), and NHLBI R01HL133327 (CMC, JAH). Note that funders were not involved in project creation, management, or outcome reporting. The authors report no conflicts of interest.

## Data availability

Data will be made available on request.

## Abbreviations:

| | |
|---|---|
| **SCD** | sickle cell disease |
| **Hb SS** | Hemoglobin SS |
| **Hb S** | Hemoglobin S |
| **Hb SC** | Hemoglobin SC |
| **CS** | central sensitization |
| **SNPs** | single nucleotide polymorphisms |
| **TRP** | transient receptor potential |
| **ADRB2** | beta2-adrenergic receptor |
| **QST** | quantitative sensory testing |
| **OR** | odds ratio |
| **CI** | confidence intervals |
| **FDR** | false discovery rate |
| **HWE** | Hardy Weinberg Equilibrium |
| **MAF** | minor allele frequency |
| **ANOVA** | analysis of variance |
| **MHC** | major histocompatibility complex |
| **HLA** | human leukocyte antigen |
| **COMT** | catechol-*O*-methyl-transferase |

## References

Belfer I, Youngblood V, Darbari DS, Wang Z, Diaw L, Freeman L, Desai K, Dizon M, Allen D, Cunnington C, Channon KM, Milton J, Hartley SW, Nolan V, Kato GJ, Steinberg MH, Goldman D,

Taylor VIJG, 2014. A GCH1 haplotype confers sex-specific susceptibility to pain crises and altered endothelial function in adults with sickle cell anemia. Am. J. Hematol 89, 187–193. [PubMed: 24136375]

Ben Hamda C, Sangeda R, Mwita L, Meintjes A, Nkya S, Panji S, Mulder N, Guizani-Tabbane L, Benkahla A, Makani J, Ghedira K, 2018. A common molecular signature of patients with sickle cell disease revealed by microarray meta-analysis and a genome-wide association study. PloS One 13, e0199461. [PubMed: 29979707]

Brito PL, dos Santos AF, Chweih H, Favero ME, Gotardo EMF, Silva JAF, Leonardo FC, Franco-Penteado CF, de Oliveira MG, W.A.F. Jr, Zaidan BC, Billis A, Baldanzi G, Mashima DA, Antunes E, Saad STO, Costa FF, Conran N, 2022. Reduced blood pressure in sickle cell disease is associated with decreased angiotensin converting enzyme (ACE) activity and is not modulated by ACE inhibition. PloS One 17, e0263424. [PubMed: 35113975]

Campbell CM, Carroll CP, Kiley K, Han D, Haywood C, Lanzkron S, Swedberg L, Edwards RR, Page GG, Haythornthwaite JA, 2016a. Quantitative sensory testing and pain-evoked cytokine reactivity: comparison of patients with sickle cell disease to healthy matched controls. Pain 157, 949–956. [PubMed: 26713424]

Campbell CM, Moscou-Jackson G, Carroll CP, Kiley K, Haywood C, Lanzkron S, Hand M, Edwards RR, Haythornthwaite JA, 2016b. An evaluation of central sensitization in patients with sickle cell disease. J. Pain 17, 617–627. [PubMed: 26892240]

Carroll CP, Lanzkron S, Haywood C, Kiley K, Pejsa M, Moscou-Jackson G, Haythornthwaite JA, Campbell CM, 2016. Chronic opioid therapy and central sensitization in sickle cell disease. Am. J. Prev. Med 51, S69–S77. [PubMed: 27320469]

Catterall WA, Dib-Hajij S, Meisler MH, Pietrobon D, 2008. Inherited neuronal ion channelopathies: new windows on complex neurological diseases. J. Neurosci 28, 11768–11777. [PubMed: 19005038]

CDC, 2022. What is Sickle Cell Disease? CDC. Centers for Disease Control and Prevention. Available: https://www.cdc.gov/ncbddd/sicklecell/facts.html (Accessed 28 Sep 2023).

Diatchenko L, Slade GD, Nackley AG, Bhalang K, Sigurdsson A, Belfer I, Goldman D, Xu K, Shabalina SA, Shagin D, Max MB, Makarov SS, Maixner W, 2005. Genetic basis for individual variations in pain perception and the development of a chronic pain condition. Hum. Mol. Genet 14, 135–143. [PubMed: 15537663]

Dyal BW, Ezenwa MO, Yoon SL, Fillingim RB, Yao Y, Schlaeger JM, Suarez ML, Wang ZJ, Molokie RE, Wilkie DJ, 2020. A QST-based pain phenotype in adults with sickle cell disease: sensitivity and specificity of quality descriptors. Pain Pract 20, 168–178. [PubMed: 31553824]

Fillingim RB, Kaplan L, Staud R, Ness TJ, Glover TL, Campbell CM, Mogil JS, Wallace MR, 2005. The A118G single nucleotide polymorphism of the μ-opioid receptor gene (OPRM1) is associated with pressure pain sensitivity in humans. J. Pain 6, 159–167. [PubMed: 15772909]

Fischer TZ, Waxman SG, 2010. Familial pain syndromes from mutations of the Nav1.7 sodium channel. Ann. N. Y. Acad. Sci 1184, 196–207. [PubMed: 20146699]

Furquim BD, Flamengui LMSP, Repeke CEP, Cavalla F, Garlet GP, Conti PCR, 2016. Influence of TNF-α-308 G/A gene polymorphism on temporomandibular disorder. Am. J. Orthod. Dentofacial Orthop 149, 692–698. [PubMed: 27131251]

Gupta K, Jahagirdar O, Gupta K, 2018. Targeting pain at its source in sickle cell disease. Am. J. Physiol. Regul. Integr. Comp. Physiol 315, R104–R112. [PubMed: 29590553]

Inoue H, Mashimo Y, Funamizu M, Shimojo N, Hasegawa K, Hirota T, Doi S, Kameda M, Miyatake A, Kohno Y, Okamoto Y, Tamari M, Hata A, Suzuki Y, 2008. Association study of the C3 gene with adult and childhood asthma. J. Hum. Genet 53, 728–738. [PubMed: 18566738]

Jhun E, He Y, Yao Y, Molokie RE, Wilkie DJ, Wang ZJ, 2014. Dopamine D3 receptor Ser9Gly and catechol-O-methyltransferase Val158Met polymorphisms and acute pain in sickle cell disease. Anesth. Analg 119, 1201–1207. [PubMed: 25102390]

Jhun EH, Hu X, Sadhu N, Yao Y, He Y, Wilkie DJ, Molokie RE, Wang ZJ, 2018a. Transient receptor potential polymorphism and haplotype associate with crisis pain in sickle cell disease. Pharmacogenomics 19, 401–411. [PubMed: 29620434]

Jhun EH, Sadhu N, Yao Y, He Y, Molokie RE, Wilkie DJ, Wang ZJ, 2018b. Glucocorticoid receptor single nucleotide polymorphisms are associated with acute crisis pain in sickle cell disease. Pharmacogenomics 19, 1003–1011. [PubMed: 30079801]

Jhun EH, Sadhu N, Hu X, Yao Y, He Y, Wilkie DJ, Molokie RE, Wang ZJ, 2019. Beta2-adrenergic receptor polymorphisms and haplotypes associate with chronic pain in sickle cell disease. Front. Pharmacol 10. Available: https://www.frontiersin.org/articles/10.3389/fphar.2019.00084. (Accessed 28 September 2023).

Kazma R, Babron M-C, Gaborieau V, Génin E, Brennan P, Hung RJ, McLaughlin JR, Krokan HE, Elvestad MB, Skorpen F, Anderssen E, Vooder T, Välk K, Metspalu A, Field JK, Lathrop M, Sarasin A, Benhamou S, 2012. Lung cancer and DNA repair genes: multilevel association analysis from the International Lung Cancer Consortium. Carcinogenesis 33, 1059–1064. [PubMed: 22382497]

Kidwell K, Albo C, Pope M, Bowman L, Xu H, Wells L, Barrett N, Patel N, Allison A, Kutlar A, 2021. Characteristics of sickle cell patients with frequent emergency department visits and hospitalizations. PloS One 16, e0247324. [PubMed: 33617594]

Kitajima H, Sonoda M, Yamamoto K, 2012. HLA and SNP haplotype mapping in the Japanese population. Genes Immun. 13, 543–548. [PubMed: 22914434]

Knisely MR, Yang Q, Stauffer N, Kenney M, Ashley-Koch A, Myers J, Walker JKL, Tanabe PJ, Shah NR, 2023. Evaluating associations between average pain intensity and genetic variation in people with sickle cell disease: an exploratory study. Pain Manag. Nurs 24, 12–18. [PubMed: 36096903]

Korczeniewska OA, Kuo F, Huang C, Nasri-Heir C, Khan J, Benoliel R, Hirschberg C, Eliav E, Diehl SR, 2021. Genetic variation in catechol-O-methyltransferase is associated with individual differences in conditioned pain modulation in healthy subjects. J. Gene Med 23, e3374. [PubMed: 34156736]

Kutlar A, Wells LG, Xu H, Bowman L, Bora P, Chand AR, Clair B, Meiler SE, Wang X, Natrajan K, Patel N, Fillingim RB, 2014. Genetic association studies of pain phenotypes in adult SCD patients. Blood 124, 2708.

Lettre G, Sankaran VG, Bezerra MAC, Araújo AS, Uda M, Sanna S, Cao A, Schlessinger D, Costa FF, Hirschhorn JN, Orkin SH, 2008. DNA polymorphisms at the BCL11A, HBS1L-MYB, and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. Proc. Natl. Acad. Sci. U. S. A 105, 11869–11874. [PubMed: 18667698]

Lubeck D, Agodoa I, Bhakta N, Danese M, Pappu K, Howard R, Gleeson M, Halperin M, Lanzkron S, 2019. Estimated life expectancy and income of patients with sickle cell disease compared with those without sickle cell disease. JAMA Netw. Open 2, e1915374. [PubMed: 31730182]

Mathur VA, Kiley KB, Haywood C, Bediako SM, Lanzkron S, Carroll CP, Buenaver LF, Pejsa M, Edwards RR, Haythornthwaite JA, Campbell CM, 2016. Multiple levels of suffering. Clin. J. Pain 32, 1076–1085. [PubMed: 26889615]

Matthie N, Jenerette C, Gibson A, Paul S, Higgins M, Krishnamurti L, 2020. Prevalence and predictors of chronic pain intensity and disability among adults with sickle cell disease. Health Psychol. Open 7, 2055102920917250. [PubMed: 32426150]

McGill LS, Hamilton KR, Letzen JE, Finan PH, Lanzkron SM, Smith MT, Haythornthwaite JA, Campbell CM, 2023. Depressive and insomnia symptoms sequentially mediate the association between racism-based discrimination in healthcare settings and clinical pain among adults with sickle cell disease. J. Pain 24, 643–654. [PubMed: 36414154]

McKemy DD, 2007. TRPM8: the cold and menthol receptor. In: Liedtke WB, Heller S (Eds.), TRP Ion Channel Function in Sensory Transduction and Cellular Signaling Cascades, Frontiers in Neuroscience. CRC Press/Taylor & Francis, Boca Raton (FL). Available: http://www.ncbi.nlm.nih.gov/books/NBK5238/. (Accessed 20 June 2024).

Mi H, Thomas P, 2009. PANTHER pathway: an ontology-based pathway database coupled with data analysis tools. Methods Mol. Biol 563, 123–140. [PubMed: 19597783]

Mi H, Muruganujan A, Thomas PD, 2013. PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. Nucleic Acids Res. 41, D377–D386. [PubMed: 23193289]

Middlebrook N, Heneghan NR, Evans DW, Rushton A, Falla D, 2020. Reliability of temporal summation, thermal and pressure pain thresholds in a healthy cohort and musculoskeletal trauma population. PloS One 15, e0233521. [PubMed: 32469913]

Miller RE, Brown DS, Keith SW, Hegarty SE, Setty Y, Campbell CM, McCahan SM, Gayen-Betal S, Byck H, Stuart M, 2019. Quantitative sensory testing in children with sickle cell disease: additional insights and future possibilities. Br. J. Haematol 185, 925–934. [PubMed: 30924134]

Mogil JS, 2012. Pain genetics: past, present and future. Trends Genet. 28, 258–266. [PubMed: 22464640]

Moscou-Jackson G, Finan PH, Campbell CM, Smyth JM, Haythornthwaite JA, 2015. The effect of sleep continuity on pain in adults with sickle cell disease. J. Pain 16, 587–593. [PubMed: 25842346]

Norbury TA, MacGregor AJ, Urwin J, Spector TD, McMahon SB, 2007. Heritability of responses to painful stimuli in women: a classical twin study. Brain 130, 3041–3049. [PubMed: 17932101]

O'Neill S, O'Neill L, 2015. Improving QST reliability—more raters, tests, or occasions? A multivariate generalizability study. J. Pain 16, 454–462. [PubMed: 25683899]

Osunkwo I, Andemariam B, Minniti CP, Inusa BPD, El Rassi F, Francis-Gibson B, Nero A, Trimnell C, Abboud MR, Arlet J-B, Colombatti R, de Montalembert M, Jain S, Jastaniah W, Nur E, Pita M, DeBonnett L, Ramscar N, Bailey T, Rajkovic-Hooley O, James J, 2021. Impact of sickle cell disease on patients' daily lives, symptoms reported, and disease management strategies: results from the international Sickle Cell World Assessment Survey (SWAY). Am. J. Hematol 96, 404–417. [PubMed: 33264445]

Pinto Fiamengui LMS, Furquim BD, De la Torre Canales G., Fonseca Carvalho Soares F, Poluha RL, Palanch Repeke CE, Bonjardim LR, Garlet GP, Rodrigues Conti PC, 2020. Role of inflammatory and pain genes polymorphisms in temporomandibular disorder and pressure pain sensitivity. Arch. Oral Biol 118, 104854. [PubMed: 32763472]

Reimann F, Cox JJ, Belfer I, Diatchenko L, Zaykin DV, McHale DP, Drenth JPH, Dai F, Wheeler J, Sanders F, Wood L, Wu T-X, Karppinen J, Nikolajsen L, Männikkö M, Max MB, Kiselycznyk C, Poddar M, Te Morsche RHM, Smith S, Gibson D, Kelempisioti A, Maixner W, Gribble FM, Woods CG, 2010. Pain perception is altered by a nucleotide polymorphism in SCN9A. Proc. Natl. Acad. Sci. U. S. A 107, 5148–5153. [PubMed: 20212137]

Sadhu N, Jhun EH, Yao Y, He Y, Molokie RE, Wilkie DJ, Wang ZJ, 2018. Genetic variants of GCH1 associate with chronic and acute crisis pain in African Americans with sickle cell disease. Exp. Hematol 66, 42–49. [PubMed: 30031848]

Sadler KE, Stucky CL, 2019. Neuronal transient receptor potential (TRP) channels and noxious sensory detection in sickle cell disease. Neurosci. Lett 694, 184–191. [PubMed: 30508569]

Slade GD, Smith SB, Zaykin DV, Tchivileva IE, Gibson DG, Yuryev A, Mazo I, Bair E, Fillingim R, Ohrbach R, Greenspan J, Maixner W, Diatchenko L, 2013. Facial pain with localized and widespread manifestations: separate pathways of vulnerability. Pain 154, 2335–2343. [PubMed: 23867732]

Tavares-Ferreira D, Shiers S, Ray PR, Wangzhou A, Jeevakumar V, Sankaranarayanan I, Cervantes AM, Reese JC, Chamessian A, Copits BA, Dougherty PM, Gereau RW, Burton MD, Dussor G, Price TJ, 2022. Spatial transcriptomics of dorsal root ganglia identifies molecular signatures of human nociceptors. Sci. Transl. Med 14, eabj8186. [PubMed: 35171654]

Tegeder I, Costigan M, Griffin RS, Abele A, Belfer I, Schmidt H, Ehnert C, Nejim J, Marian C, Scholz J, Wu T, Allchorne A, Diatchenko L, Binshtok AM, Goldman D, Adolph J, Sama S, Atlas SJ, Carlezon WA, Parsegian A, Lotsch J, Fillingim RB, Maixner W, Geisslinger G, Max MB, Woolf CJ, 2006. GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence. Nat. Med 12, 1269–1277. [PubMed: 17057711]

Thomas PD, Ebert D, Muruganujan A, Mushayahama T, Albou L-P, Mi H, 2022. PANTHER: making genome-scale phylogenetics accessible to all. Protein Sci. 31, 8–22. [PubMed: 34717010]

Tran H, Gupta M, Gupta K, 2017. Targeting novel mechanisms of pain in sickle cell disease. Blood 130, 2377–2385. [PubMed: 29187376]

Unsworth DJ, 2008. Complement deficiency and disease. J. Clin. Pathol 61, 1013–1017. [PubMed: 18495794]

Urbanek M, Hayes MG, Lee H, Freathy RM, Lowe LP, Ackerman C, Jafari N, Dyer AR, Cox NJ, Dunger DB, Hattersley AT, Metzger BE, Lowe WL, 2012. The role of inflammatory pathway genetic variation on maternal metabolic phenotypes during pregnancy. PloS One 7, e32958. [PubMed: 22479352]

Vetterlein A, Monzel M, Reuter M, 2023. Are catechol-O-methyltransferase gene polymorphisms genetic markers for pain sensitivity after all? – a review and meta-analysis. Neurosci. Biobehav. Rev 148, 105112. [PubMed: 36842714]

Vignal C, Bansal AT, Balding DJ, Binks MH, Dickson MC, Montgomery DS, Wilson AG, 2009. Genetic association of the major histocompatibility complex with rheumatoid arthritis implicates two non-DRB1 loci. Arthritis Rheum. 60, 53–62. [PubMed: 19116923]

Walport MJ, 2001. Complement. N. Engl. J. Med 344, 1058–1066. [PubMed: 11287977]

Woolf CJ, 2011. Central sensitization: implications for the diagnosis and treatment of pain. Pain 152, S2–S15. [PubMed: 20961685]

Yang X, Sun J, Gao Y, Tan A, Zhang H, Hu Y, Feng J, Qin X, Tao S, Chen Z, Kim S-T, Peng T, Liao M, Lin X, Zhang Z, Tang M, Li L, Mo L, Liang Z, Shi D, Huang Z, Huang X, Liu M, Liu Q, Zhang S, Trent JM, Zheng SL, Xu J, Mo Z, 2012. Genome-wide association study for serum complement C3 and C4 levels in healthy Chinese subjects. PLoS Genet. 8, el002916.

Zappia KJ, Garrison SR, Hillery CA, Stucky CL, 2014. Cold hypersensitivity increases with age in mice with sickle cell disease. Pain 155, 2476–2485. [PubMed: 24953902]

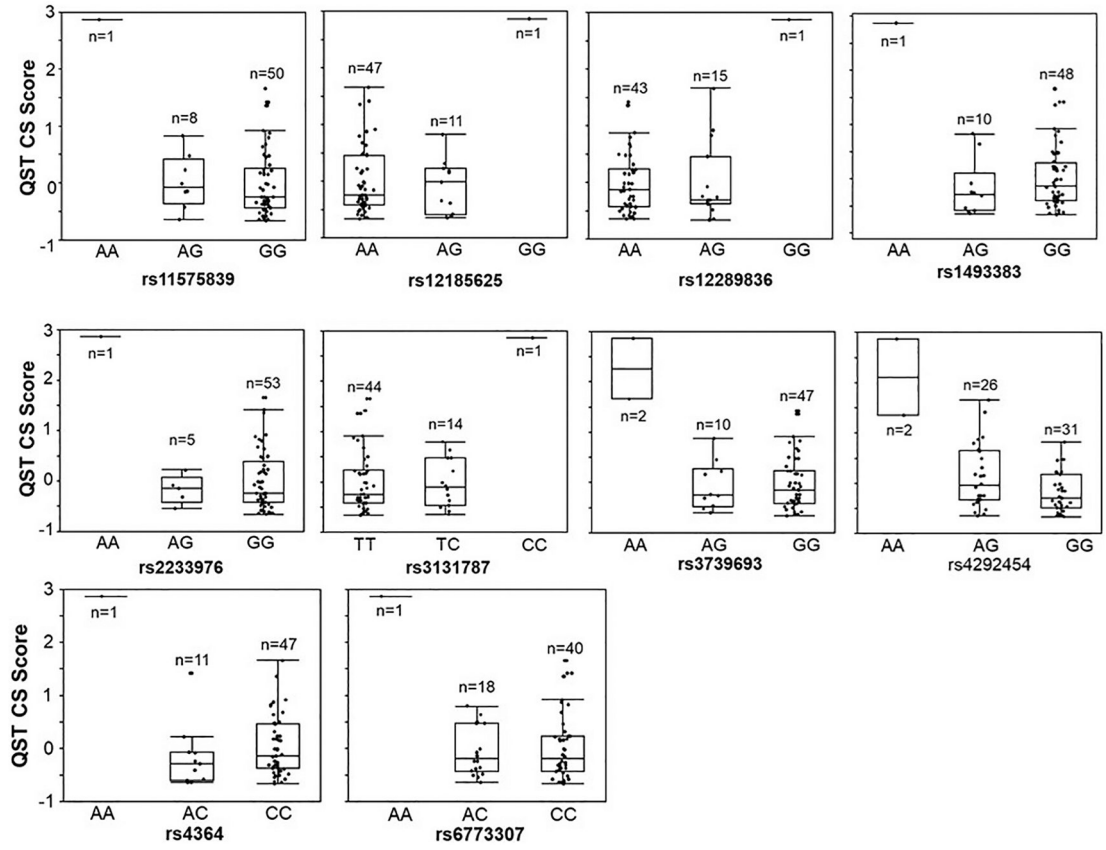


**Fig. 1.**
Central sensitization by allele frequency in significant SNPs.

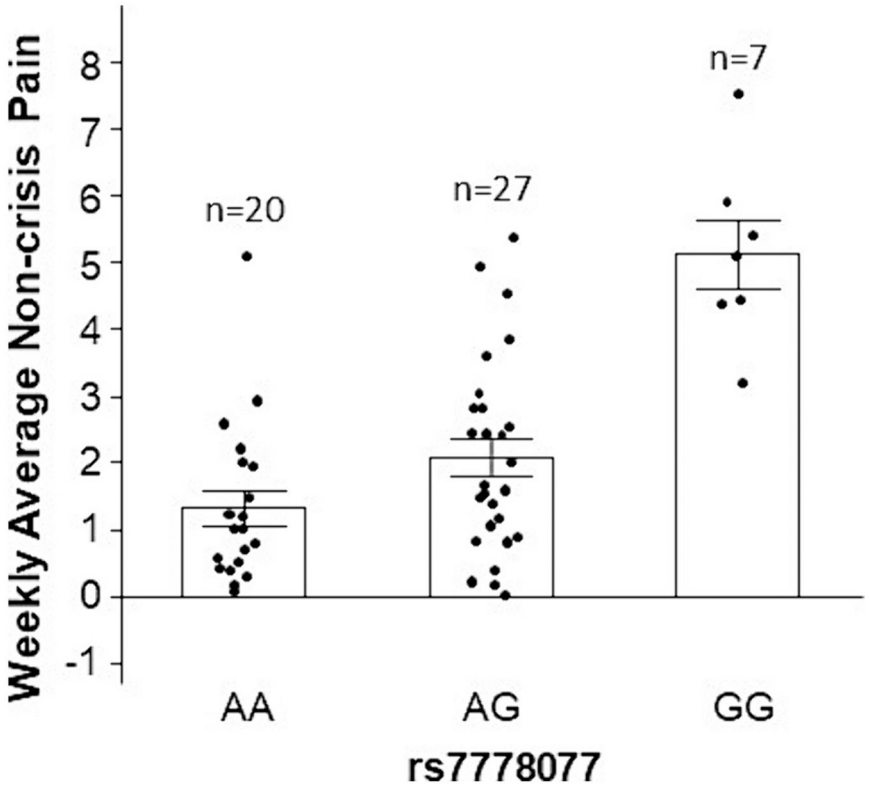


**Fig. 2.**

Average non-crisis pain by allele for rs7778077. Note that this figure includes an $n = 54$, as 5 individuals had missing average non-crisis pain.

## Table 1

Demographic and clinical characteristics for study participants with sickle cell.

| ***Variable*** | | **Full sample *n* = 59** | **Hb SS *n* = 38 (64 %)** | **S-β(0)-thal *n* = 3 (5 %)** | **Hb S-p(+)-thal *n* = 7(12 %)** | **Hb SC *n* = 11 (19 %)** |
|---|---|---|---|---|---|---|
| Age (years) | *M* (SD) | 38.37 (12.08) | 36.8 (11.46) | 34.00 (13.89) | 48.00 (12.73) | 38.73 (12.05) |
| Sex (female) | % (*N*) | 67.80 % (40) | 65.8 % (25) | 100 % (3) | 85.7 % (6) | 54.5 % (6) |
| Race (Black or African American) | % (*N*) | 94.9 % (56) | 94.7 % (36) | 100 % (3) | 100 % (7) | 90.9 % (10) |
| Marital Status (single) | % (*N*) | 55.9 % (33) | 63.2 % (24) | 0.00 % (0) | 28.6 % (2) | 36.4 % (4) |
| Education (college degree or higher) | % (*N*) | 39.0 % (23) | 36.8 % (14) | 0.00 % (0) | 42.9 % (3) | 54.6 % (6) |
| Employment (part- or full-time) | % (*N*) | 44.1 % (26) | 47.3 % (18) | 33.33 % (1) | 28.6 % (2) | 45.5 % (5) |
| Ethnicity (non-Hispanic) | % (*N*) | 93.2 % (55) | 92.1 % (35) | 100 % (3) | 100 % (7) | 90.9 % (10) |
| BMI | *M* (SD) | 25.55 (5.30) | 24.66 (5.24) | 27.28 (3.61) | 26.25 (7.77) | 27.68 (3.69) |
| Long-acting Opioid Medication (Yes) | % (*N*) | 32.2% (19) | 34.2 % (13) | 33.3 % (1) | 42.9 % (3) | 18.2 % (2) |
| Total Daily Morphine Equivalent | *M* (SD) | 52.25 (107.8) | 62.39 (119.2) | 10.00 (17.32) | 35.71 (52.24) | 41.00 (113.18) |

*Notes*. HbSS: Homozygous hemoglobin S, thal: Thalassemia, Hb SC: Sickle hemoglobin C (hemoglobin S and hemoglobin C).

**Table 2**

SNPs significantly associated with QST central sensitization scores.

| Gene | SNP | Chr | Variant Type | Major/Minor Allele | MAF | Genotype *P*-value | FDR adj. Genotype P-value |
|---|---|---|---|---|---|---|---|
| NCR3 | rs11575839 | 6 | Synonymous | G/A | 0.07 | 2.743E–05 | 0.01 |
| TRPM8 | rs12185625 | 2 | Intron | A/G | 0.09 | 2.527E–05 | 0.01 |
| RELA | rs12289836 | 11 | Intron | A/G | 0.14 | 2.784E–05 | 0.01 |
| GRIA1 | rs1493383 | 5 | Intron | G/A | 0.09 | 1.623E–05 | 0.01 |
| C6orf15 | rs2233976 | 6 | Missense | G/A | 0.08 | 2.234E–05 | 0.01 |
| SFTA2 | rs3131787 | 6 | Missense | A/G | 0.13 | 2.754E–05 | 0.01 |
| IKBKAP | rs3739693 | 9 | Synonymous | G/A | 0.17 | 8.318E–07 | 0.002 |
| GHR | rs4292454 | 5 | Intron | G/A | 0.28 | 8.553E–07 | 0.002 |
| ACE | rs4364 | 17 | Missense | C/A | 0.10 | 1.566E–05 | 0.01 |
| VARS 2 | rs4678 | 6 | Missense | G/A | 0.13 | 2.754E–05 | 0.01 |
| HRH1 | rs6773307 | 3 | Intron | C/A | 0.18 | 2.438E–05 | 0.01 |

A = adenine; C = cytosine; G = guanine; T = thymine; Chr = chromosome; FDR adj. P-value = significance following false discovery rate (FDR) adjustment; SCD = sickle cell disease.

*NCR3* = Natural cytotoxicity triggering receptor 3; *TRPM8* = Transient receptor potential cation channel subfamily M member 8; *RELA* = RELA proto-oncogene, NF-kB subunit; *GRIA1* = Glutamate ionotropic receptor AMPA type subunit 1; *C6orf15* = Chromosome 4 open reading Frame, human C6orf15; *SFTA2* = Surfactant associated 2; *IKBKAP* = Elongator complex protein 1; *GHR* = Growth hormone receptor; *ACE* = Angiotensin I converting enzyme; *VARS2* = Valyl-tRNA synthetase 2, mito-chondrial; *HRH1* = Histamine receptor H1.

**Table 3**

Mean QST central sensitization (CS) scores by SNP genotypes.

| SNP | Genotype | N (%) | CS score mean | Tukey P-value (95 % CI) |
|---|---|---|---|---|
| rs11575839 | AA | 1 (1.69) | 2.87 ± . | |
| | AG | 8 (13.56) | 0.02 ± 0.48 | <0.0001 (1.4, 4.3) |
| | GG | 50 (84.75) | −0.01 ± 0.58 | <0.0001 (1.5, 4.3) |
| rs12185625 | AA | 47 (79.66) | 0.01 ± 0.58 | <0.0001 (1.5, 4.2) |
| | AG | 11 (18.64) | −0.08 ± 0.47 | <0.0001 (1.5, 4.4) |
| | GG | 1 (1.69) | 2.87 ± . | |
| rs12289836 | AA | 43 (72.88) | −0.01 ± 0.53 | <0.0001 (1.5, 4.3) |
| | AG | 15 (25.42) | −0.01 ± 0.67 | <0.0001 (1.5, 4.3) |
| | GG | 1 (1.69) | 2.87 ± . | |
| rs1493383 | AA | 1 (1.69) | 2.87 ± . | |
| | AG | 10 (16.95) | −0.18 ± 0.52 | <0.0001 (1.6, 4.5) |
| | GG | 48 (81.36) | 0.03 ± 0.57 | <0.0001 (1.5, 4.2) |
| rs2233976 | AA | 1 (1.69) | 2.87 ± . | |
| | AG | 5 (8.47) | −0.17 ± 0.28 | <0.0001 (1.6, 4.5) |
| | GG | 53 (89.83) | 0.01 ± 0.58 | <0.0001 (1.5, 4.2) |
| rs3131787 | TT | 44 (74.58) | −0.003 ± 0.59 | <0.0001 (1.5, 4.3) |
| | TC | 14 (23.73) | −0.03 ± 0.48 | <0.0001 (1.5, 4.3) |
| | CC | 1 (1.69) | 2.87 ± . | |
| rs3739693 | AA | 2 (3.39) | 2.27 ± 0.86 | |
| | AG | 10 (16.95) | −0.07 ± 0.48 | <0.0001 (1.3, 3.3) |
| | GG | 47 (79.66) | −0.03 ± 0.53 | <0.0001 (1.4, 3.2) |
| rs4292454 | AA | 2 (3.39) | 2.12 ± 1.07 | |
| | AG | 26 (44.07) | 0.13 ± 0.63 | <0.0001 (1.0, 2.9) |
| | GG | 31 (52.54) | −0.17 ± 0.39 | <0.0001 (1.4, 3.2) |
| rs4364 | AA | 1 (1.69) | 2.87 ± . | |
| | AC | 11 (18.64) | −0.17 ± 0.60 | <0.0001 (1.5, 4.3) |
| | CC | 47 (79.66) | 0.03 ± 0.55 | <0.0001 (1.5, 4.3) |
| rs4678 | AA | 1 (1.69) | 2.87 ± . | |
| | AG | 14 (23.73) | −0.03 ± 0.48 | <0.0001 (1.6, 4.5) |
| | GG | 44 (74.58) | −0.003 ± 0.59 | <0.0001 (1.5, 4.2) |
| rs6773307 | AA | 1 (1.69) | 2.87 ± . | |
| | AC | 18 (30.51) | −0.07 ± 0.45 | <0.0001 (1.5, 4.3) |
| | CC | 40 (67.80) | 0.02 ± 0.61 | <0.0001 (1.5, 4.3) |