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Neuronal transient receptor potential (TRP) channels and noxious sensory detection in sickle cell disease

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Abstract

Pain is the leading cause for hospitalization in patients with sickle cell disease (SCD). While the characteristics of SCD pain can vary widely between patients and between phases of the disease (e.g. vasoocclusive crisis pain vs. chronic pain), similar neuronal mechanisms likely underlie the various aspects of nociceptive processing. In the peripheral nervous system, small unmyelinated C fibers and lightly-myelinated Aδ fibers detect and transmit noxious stimuli. Both classes of neurons express members of the transient receptor potential (TRP) family, a group of ligand gated ion-channels that are activated by thermal, chemical, and mechanical stimuli. Promiscuous TRP channel family members are activated by a wide range of stimuli, many of which are dysregulated in patients with SCD and transgenic SCD mouse models. In 2011, our lab published the first report of TRP channel contributions to rodent SCD pain. Since that time, additional basic and clinical research efforts have investigated the genetic and biochemical status of TRP channels in SCD, placing particular focus on TRPV1. This review will discuss these advances and highlight the clinical SCD presentations that have not yet been studied, but which may be mediated by TRP channel activity.

Keywords

sickle cell disease; pain; TRPV1; TRPM8; TRPA1

2. Introduction

The detection and transmission of sensory stimuli are phylogenetically conserved processes critical to species survival. In eukaryotes, two broad classes of proteins have evolved to complete these activities: ion channels and G-protein coupled receptors. Varying biochemical and biophysical properties delineate the unique sensory relays in which each protein class participates and the downstream signals generated by activation of each protein class. Five decades of research have established ion channels, and particularly members of the transient receptor potential (TRP) superfamily, as critical detectors and transmitters of the signals induced by mechanical, thermal, or chemical perturbation of cellular membranes.

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Originally discovered in mutant *Drosophila*, “transient receptor potential” channels were named for the mutant fly phenotype; photoreceptors from *trp* gene mutants demonstrated abnormal transient changes in membrane potential following bright light stimulation¹. To date, 28 mammalian TRP channels have been identified (reviewed in^{2,3}). Each TRP channel consists of a tetramer of 6 transmembrane-spanning subunits organized around a central pore that non-specifically conducts cations and is highly permeable to Ca²⁺. Based on amino acid sequence and topological similarities, these mammalian proteins are classified into the following six sub-families: vanilloid (TRPV), ankyrin (TRPA), melastatin (TRPM), canonical (TRPC), polycystin (TRPP), and mucolipin (TRPML). TRPN (no mechanoreceptor potential C) is a seventh sub-family that has only been identified in *C. elegans* and *Drosophila*. While aberrant expression or sensitization of any one of these classes could contribute to altered somatosensation in pathological conditions, activity of TRPV, TRPA, and TRPM family members is most directly related to nociceptive transmission, an omnipresent process in sickle cell disease (SCD).

Pain is the leading reason for hospitalization and emergency department visits in patients with SCD⁴. For the purposes of this review, SCD pain will be broadly characterized into two categories: acute vaso-occlusive crisis (VOC) pain and chronic pain. VOC pain is hypothesized to result from tissue ischemia/reperfusion⁵. During a painful crisis, red blood cells (RBCs) morph into the disease namesake sickle shape. Sickled RBCs stick to each other, circulating immune cells, and endothelial cells lining blood vessels, eventually blocking small blood vessels all over the body (Figure 1). In the absence of blood flow, tissues are exposed to ischemic conditions (i.e. low oxygen, low pH) which revert to normal only when tissue occlusion subsides. The reperfused tissue is bathed in an inflammatory milieu generated by infiltrating immune cells, necrotic tissue, platelets, and factors released from neurons (neurogenic inflammation) via antidromic signaling⁶. VOC are often unpredictable, abrupt in onset, and require patients to be hospitalized for several days. Patients describe the pain accompanying these crises as widespread and continuous throbbing, stabbing, or cutting⁵. Unlike acute SCD pain, the underlying pathology of chronic SCD pain is far less understood. Occasionally, sources of pain including bone infarcts or leg ulcers are identified using tissue histology or *in vivo* imaging, but often the source of chronic SCD pain cannot be determined. Patients and mouse models with SCD are in a chronic state of inflammation which undoubtedly drives at least part of the reported chronic hypersensitivity⁷. Neuronal mechanisms may also contribute to chronic SCD pathology; peripheral sensory neurons from SCD mice have altered electrophysiological parameters and increased levels of spontaneous firing compared to neurons from control mice^{8,9}. Altered connectivity and activity of central nervous system circuits have also been reported in both SCD patients and mouse models^{10,11}.

To better study the underlying mechanisms of acute and chronic SCD pain, two transgenic SCD mouse models were developed. Both the Berkley (Berk) and Townes mouse models express the human sickle β globin gene which contains the single point mutation responsible for SCD. In Berk mice, the murine globin genes are “knocked out” and normal human α globin and sickle β globin genes are maintained via transgene. In the Townes model, the human α globin and sickle β globin genes are knocked into the same locus as the murine globin genes. Regardless of strain, mice homozygous for the sickle β globin gene (e.g. Berk

SS, Townes SS) recapitulate many qualities of the severe SCD patient phenotype including sickled red blood cells, hemolytic anemia, hepatic, cardiac, and pulmonary pathologies, and chronic hyperalgesia to mechanical, cold, and heat stimuli that increases with age^{12–15}. Notably, Berk SS mice are more sensitive to all somatosensory modalities than Townes SS mice¹⁴. This phenotype may result from more severe underlying hematological issues or decreased relative abundance of fetal hemoglobin (HbF), the form of hemoglobin that is expressed for a short period following birth and that is associated with decreased pain in patients with SCD^{12,13,16}. While the majority of experiments discussed in this manuscript were completed in “steady state” mice (i.e. testing chronic hypersensitivity), it should be noted that both mouse strains also experience VOC when placed into hypoxic environments. These characteristics make both mouse models useful tools for studying pain mechanisms in a sensitive and underrepresented patient population.

Despite their underlying differences in pathology, both acute and chronic SCD pain are multimodal. Patients and transgenic SCD mice experience mechanical allodynia and thermal hyperalgesia to evoked or environmental stimuli^{7,17,18}. Primary afferent neurons of the peripheral nervous system detect and transmit all types of sensory information to the central nervous system. In the context of SCD, these neurons are sensitized to mechanical, thermal (particularly cold), and chemical stimuli; in other words, lower mechanical forces, temperatures, or endogenous agonist concentrations are required for signal initiation (Figure 1). Members of the TRP family are well positioned to be the initial sensory detectors in this signaling process since they are activated or sensitized by a dynamic range of physical and chemical stimuli, many of which are present and/or increased in SCD tissue. In 2011, our lab published the initial report on TRP channel activity in SCD mouse pain¹⁹. This review will summarize the additional TRP-specific knowledge advances made in SCD pain research over the past seven years. Information regarding the expression patterns, preclinical therapeutic efficacy, and translational relevance will be presented for select TRP channels. Channel activity will be specifically discussed with regards to the following SCD clinical presentations: heat/cold hypersensitivity, mechanical allodynia, VOC pain, chronic deep tissue pain, and spontaneous pain.

3. TRPV subfamily

Members of the mammalian TRPV (vanilloid) subfamily were the first to be verified as noxious signal transducers. Cloned during a functional search for channels activated by capsaicin, the vanilloid compound in hot peppers which gives spicy foods their characteristic “heat”, TRPV1 was identified as a noxious heat transducer in the peripheral nervous system^{20,21}. Additional TRPV family members include TRPV2, 3, and 4, all of which are also activated by innocuous or noxious heat and a range of additional endogenous ligands^{2,3}. As the original nociceptive TRP channel, TRPV1 is the most extensively studied family member with regards to both normal somatosensation and the impact that diseases like SCD have on this process.

3.1 TRPV1 expression and activation profiles

TRPV1 is primarily expressed in unmyelinated C fibers (approximately 50%²²) and a smaller percentage of lightly myelinated A δ fibers^{21,22} within the peripheral nervous system. TRPV1 is also expressed in the CNS in post-synaptic neurons of lamina I and II within the spinal cord²³ and in several brain regions²⁴. Non-neuronal sources of TRPV1 include smooth muscle cells of the heart and pulmonary artery²⁵, mononuclear cells circulating in the blood²⁶, and keratinocytes within the epidermis²⁷. Although capsaicin is a highly potent activator of TRPV1, it is not an endogenous ligand of the channel. Rather, the main sources of TRPV1 activation are heat/warmth, protons, and endogenous proteins, carbohydrates, and lipids^{2,3}. Some endogenous chemicals including the endocannabinoid anandamide²⁸ and protons²⁹ can directly activate TRPV1. Other endogenous compounds including proinflammatory signaling molecules like eicosanoids and cytokines sensitize TRPV1 rather than directly activating the channel. When sensitized, the membrane voltage required for TRPV1 activation via noxious chemical and heat activation is decreased, such that significantly lower chemical concentrations and temperatures are required to induce cation flow through the channel. This change is conferred via phosphorylation or conformational changes brought on by other post-translational modifications at the channel residues responsible for heat or capsaicin sensitivity^{2,3}.

3.2 Canonical TRPV1-mediated sensations are dysregulated in SCD

Both patients with SCD and transgenic SCD mice exhibit heat hypersensitivity. Patients with SCD have lower heat pain and heat detection thresholds (i.e. heat hypersensitivity) than race-matched controls at baseline.¹⁸ Interestingly, heat pain hypersensitivity is not further exacerbated during episodes of acute pain, which is consistent with patient use of innocuous heat as analgesia during these pain events³⁰. Like patients, the Berk^{19,31–33} and Townes^{14,34} transgenic animal models of severe SCD and less severe models of sickle cell trait (SCT; Berk AS)³⁵ all exhibit heat hyperalgesia during steady state health as measured by decreased paw withdrawal latencies to heat stimulation. *In vivo* recordings of C fibers from Berk mice further demonstrate heat hypersensitivity on the neuronal level; despite having similar heat thresholds, C fibers from naïve Berk mice fire more action potentials than C fibers from naïve control mice over the course of a warm ramp stimulation (36°C to 50°C)⁸. Berk mice also display increased sensitivity to other TRPV1 agonists in addition to heat. Following intraplantar injection of 160 ng of capsaicin, Berk mice spend significantly more time exhibiting nociceptive behaviors (i.e. flicking, licking, biting, attending to hind paw) than control mice⁷. Neurons isolated from naïve Berk dorsal root ganglia (DRG) also exhibit larger calcium transients than neurons isolated from control mice DRG during extracellular exposure to capsaicin, suggesting that activity at calciumpermeant TRPV1 channels is increased in SCD⁷.

To determine if increased TRPV1 expression underlies these persistent changes in thermosensation and TRPV1 activity, microarray analyses were carried out on lumbar DRG from Berk SCD mice. DRG contain the cell bodies of sensory neurons that project to peripheral tissues and are the site where the majority of gene transcription and translation occur for the entire sensory neuron. *TRPV1* mRNA expression levels in Berk DRG did not differ from levels in control mouse DRG¹⁵. Several notable considerations exist for these

data, however. Firstly, gene expression in ganglia consists of levels within the sensory neurons and any residual blood cells which are also known to express TRPV1²⁶. Secondly, gene expression at the level of the cell body does not always correlate with protein expression at the DRG membrane or at the peripheral or central terminals. In fact, pro-inflammatory mediators including interleukin-6 (which is upregulated in SCD³⁶ and further increased during VOC³⁷) are capable of initiating axonal translation of many nociceptive channels³⁸. Thirdly, sub-cellular localization and membrane trafficking of the TRPV1 protein which can also be mediated by pro-inflammatory factors, may differ between control and SCD sensory neurons, thus rendering differential channel activity^{39,40}. Considering these points, additional molecular analyses should be conducted to mechanistically assess TRPV1 expression and function in SCD tissues as both can be disease-dependent.

3.3 Molecular sensitization of TRPV1 and chronic SCD heat hypersensitivity

Many of the stimulation temperatures required to elicit noxious responses in SCD patients are lower than the TRPV1 activation threshold ($>42^{\circ}\text{C}$)¹⁸. While it is possible that other TRP family members mediate this sensation, it is also possible that posttranslational modifications alter TRPV1 gating so that lower temperatures and agonist concentrations can open the channel. Targeted phosphorylation and/or conformational changes induced by proton binding alter TRPV1 activity so that lower temperatures ($\sim 35\text{--}37^{\circ}\text{C}$, i.e. body temperature) can initiate cation flow through the pore⁴¹. Many enzymes are capable of phosphorylating TRPV1 including protein kinase C (PKC) and activated (i.e. phosphorylated) calcium/calmodulin-dependent kinase II α (pCamKII α)⁴². Notably, CamKII α is significantly upregulated in both the spinal cord dorsal horn and DRG of Berk SCD mice³³. Furthermore, siRNA mediated knockdown of CamKII α in Berk mice spinal cords reverses the heat hyperalgesia displayed by these animals prior to treatment³³. Decreases in TRPV1 activity following CamKII α knockdown have not been specifically assessed in SCD tissue, but may explain some of the reductions in behavioral hypersensitivity observed in these studies. Activity at the endothelin A receptor (ET_AR) can also sensitize TRPV1 via PKC phosphorylation⁴³. The endogenous agonist of ET_AR is endothelin-1 (ET-1), a vasoconstrictive peptide primarily released from endothelial cells. In SCD, sickled red blood cells adhere to and subsequently damage endothelial cells, causing the release of intracellular contents including ET-1 into the blood stream. Both patients and mice with SCD have higher levels of circulating ET-1 as compared to healthy controls^{9,44,45}. ET-1 and the corresponding ET_AR are also increased in SCD DRG, suggesting a role for this agonist/receptor complex in disease-related neuronal sensitization⁹. Similar to CamKII α manipulations, pharmacological inhibition and sensory neuron-specific knockdown of ET_AR significantly decrease basal heat hypersensitivity in SCD mice⁹. The impact of these manipulations on TRPV1 activity was not specifically studied in SCD mice, however it is highly likely that decreased ET_AR activity subsequently decreased TRPV1 sensitivity and resulting behavioral correlates.

In addition to phosphorylation by CamKII α and PKC, TRPV1 activity is also sensitized by proinflammatory cytokines. Chemokine (c-c motif) ligand 2 (CCL2; also known as MCP-1) is significantly upregulated in patients with SCD and Berk SCD mice^{7,46}. Increased presence of this chemokine and many others could arise from several sources, but 5-day

administration of the mast cell inhibitor imatinib significantly decreased CCL2 levels in Berk mouse serum in addition to behavioral heat hyperalgesia⁴⁷. Additionally, blocking CCL2 activity at the cognate CCR2 receptor significantly decreased both Berk mouse behavioral responses to intraplantar capsaicin and Berk mouse neuronal responses to extracellular capsaicin administration⁷. While inhibition of CCR2 and CamKII α , sufficiently decreased TRPV1-mediated activities in steady state SCD mice, the therapeutic potential of these targets in acute VOC pain, a condition saturated with even more known TRPV1 sensitizers, is unknown but likely significant.

3.4 TRPV1 and VOC pain

The proton sensitivity of TRPV1 makes it a logical candidate for the transmission of VOC pain. Protons are capable of sensitizing TRPV1 (pH>6.0) or directly activating the channel when extracellular pH drops below 6.0⁴⁸. While the local pH in tissues and sub compartments of tissue is quite challenging to accurately measure, the exact pH of hypoxic tissue likely varies from site to site (i.e bone, muscle, blood vessel, and microdomains within these tissues). Furthermore, it is likely that during VOC, extracellular pH decreases in all tissues when the highly acidic contents of sickled red blood cells leak out of the compromised membrane and into circulating blood⁴⁹. Through this process, TRPV1 channels which are highly expressed on perivascular primary sensory neurons^{25,50,51} can be sensitized. In addition to acidic modification, TRPV1 is also sensitized by the aptly named hypoxia-inducible factor-1 α (HIF-1 α) transcription factor, via PKC recruitment and phosphorylation⁵². In addition to TRPV1 sensitization, levels of *TRPV1* mRNA are significantly increased in DRG following an ischemia/reperfusion event like that which happens during a VOC⁵³. Notably, these increases are only observed in tissue from female rodents and not males, despite channel sensitization being observed in the latter⁵⁴.

VOC further exacerbates TRPV1-mediated behaviors in SCD mice. Heat thresholds are significantly lower in Berk mice immediately following hypoxia/reoxygenation. These low thresholds persist for >18 hours following hypoxia/reoxygenation and can be immediately reinstated following a second vaso-occlusive event^{32,47}. Like its effects on chronic heat hyperalgesia, prophylactic administration of the mast cell inhibitor imatinib also prevents VOC-induced exacerbation of heat hypersensitivity in Berk mice⁴⁷, perhaps as a result of preventing mast cell degranulation and subsequent TRPV1 sensitization. Interestingly, heat pain thresholds do not decrease in patients with SCD following VOC³⁰. Additionally, a recent genetic analysis revealed no association between TRPV1 polymorphisms in patients and hospital utilization rates for VOC⁵⁵. These data represent one of the first known disparities between the SCD patient and mouse model pain phenotype. The translational relevance of the robust VOC-induced heat hypersensitivity in rodent models and subsequent modulation by TRPV1 should be carefully evaluated before progressing into patient-centered studies.

3.5 TRPV1 and deep tissue pain in SCD

TRPV1 is expressed in many different tissues that are anecdotally symptomatic in SCD patients including bone, muscle, viscera, and skin (Figure 1). Bone pain is one of the most common complaints of patients with SCD; in one report, over 80% of patients with SCD

suffered from at least one bone pain crisis in a year-long period⁵⁶. TRPV1 is uniquely positioned to contribute to bone pain as it is highly expressed on peripheral sensory neurons that innervate both mineralized bone tissue and marrow⁵⁷. In marrow, TRPV1 expressing afferents are in close proximity to the narrow blood vessels that vascularize the tissue⁵⁷. During VOC, these narrow vessels can be readily blocked by sickled red blood cells, resulting in conditions known to activate TRPV1 (i.e. acidic, pro-inflammatory). Repeated damage of these vessels can eventually lead to avascular necrosis (AVN), a condition that occurs in 20–30% of patients with SCD⁵⁸. Pain associated with AVN significantly contributes to the decreased quality of life reported by many SCD patients⁵⁶. VOC and AVN withstanding, conditions within SCD bone are well-suited for frequent TRPV1 activation. Pediatric⁵⁹ and adult SCD patients⁶⁰ exhibit significant decreases in bone mineral density; this finding is recapitulated in transgenic SCD animal models and partially explained by significant increases in osteoclast number⁶¹. Osteoclasts degrade and absorb mineralized components of bone by maintaining a highly acidic extracellular environment⁶² which may subsequently activate or potentiate TRPV1 channel activity. Bone-specific studies centering on TRPV1 should be completed in preclinical animal models to determine the therapeutic efficacy of this target. No such studies currently exist, most likely because of the ambiguous methods for stimulating and assessing “deep” (i.e. muscle and/or bone) pain in rodents.

Notably, TRPV1 is also expressed in muscle tissue⁶³; small diameter sensory neurons in skeletal muscle respond to capsaicin and decreases in pH⁶⁴. Like in bone, repeated VOC events can also lead to myonecrosis⁶⁵, a condition characterized by decreased vascularization⁶⁶ and death of muscle tissue⁶⁷. While the prevalence of this condition is likely under reported, less severe muscle pathology has been documented in both patients with SCD and transgenic animal models. SCD patients have decreased exercise tolerance⁶⁸. Similarly, SCD mice exhibit decreased muscle strength in the grip force assay^{14,31,32,47,69,70} and use exercise running wheels less than non-SCD control mice^{69,70}. Notably, grip force strength is further decreased in SCD mice following VOC^{14,32,47}. While decreased red blood cell oxygen capacity may underlie some of these diseasespecific muscle impairments, the possibility also exists that TRPV1 activity and subsequent nociceptor activation may decrease skeletal muscle usage.

In addition to muscle and bone, many visceral organs including the small intestine⁷¹, bladder⁷², colon⁷³, and pancreas⁷⁴ are innervated by sensory neurons that express high levels of TRPV1. In fact under naïve conditions, TRPV1 expression and activity is significantly higher in certain visceral afferents than in afferents innervating skin^{73,75}. Thus, much of the visceral pain that occurs in SCD patients may be mediated by TRPV1 activity. VOC pain withstanding, splenic and hepatic infarctions and cholelithiasis (i.e. gallstones) are the leading causes of visceral pain in SCD⁷⁶. Because of the difficulties in differentiating acute ischemic tissue crises from other visceral organ pathophysiologies, visceral pain is not well described in patients with SCD⁷⁶. The scarcity of reliable, easily implemented visceral pain assays in rodents further complicates this issue. To our knowledge no assessments of generic or organ-specific visceral hypersensitivity have been made in either the Townes or Berk SCD mouse model.

3.6 TRPV1 and mechanical hypersensitivity in SCD

In our initial 2011 report and most recent 2018 study, we describe a role for TRPV1 in the mechanical hypersensitivity observed in SCD mice^{7,19}. Many labs have documented chronic mechanical allodynia in transgenic SCD and SCT mouse models as assessed by von Frey filament responsiveness^{7,9,10,14,15,19,31,32,34,47,77,78}. Like heat, sensitivity to this modality is further amplified following VOC in both mouse models and SCD patients^{14,19,30,32,47}. Patients with SCD also exhibit mechanical brush allodynia in the absence of VOC¹⁷ and are more likely to be hospitalized for VOC on windy days, presumably due to increased mechanical stimulation⁷⁹. Pronounced mechanical sensitivity is also present on the neuronal level. Multiple types of nociceptive and non-nociceptive A β , A δ , and C fibers from SCD mice exhibit sensitization to mechanical stimulation; all fiber types fire more action potentials during mechanical stimulation of their cutaneous terminals than matched fibers from non-sickle controls^{8,19,78}. Patch clamp experiments also reveal that the membranes of SCD sensory neuron somata are sensitized to mechanical stimulation. There are more mechanically-responsive small-diameter neurons in SCD DRG than in control DRG¹⁹. Together, these data indicate that there is a markedly enhanced drive from mechanically-responsive primary afferents in SCD that likely contributes to mechanical sensitization at the behavioral levels.

In our 2011 report, fewer small-diameter DRG somata from Berk animals responded to mechanical stimulation when exposed to A-425619, a TRPV1 antagonist¹⁹. Similarly, C fibers from Berk mice fired fewer action potentials during mechanical stimulation when A-425619 was present in the recording bath¹⁹. Intraperitoneal injection of this inhibitor also reduced mechanical allodynia in SCD mice^{7,19}. Inhibition of molecules related to TRPV1 sensitization including CCR2⁷, ET_AR⁹, and CamKII α ³³ similarly decrease the mechanical hypersensitivity observed in SCD mice. These results were initially puzzling since TRPV1 is not intrinsically gated by mechanical stimulation and non-injured, naïve TRPV1 knockout mice do not display any deficits in mechanical sensitivity⁸⁰. Accumulating evidence however, suggests that TRPV1 may be involved in mechanical sensitization after tissue injury and inflammation. In addition to our findings in SCD mice, TRPV1-related mechanical hypersensitivity has now been noted in several other inflammatory models including carrageenan⁸¹, Complete Freud's Adjuvant (CFA)^{82,83}, nerve growth factor⁸⁴, and parathyroid hormone-related peptide⁸⁵ pain. The precise mechanism through which inflammation confers mechanical sensitivity to TRPV1 is unknown, but may involve one of the following: 1) constitutive activity of TRPV1 by PKC or other modulators that sensitize the channel to mechanical stimuli⁸⁶, 2) altered composition of the plasma membrane lipid bilayer and novel gating/modulation of TRPV1 activity, or 3) recruitment and coupling of a bona fide mechanically-gated ion channel (e.g. Piezo2) to TRPV1.

3.7 TRPV1 and spontaneous pain in SCD

Spontaneous pain is one of the chief symptoms accompanying neuropathic injury. Verbal descriptions by and quantitative sensory testing of patients with SCD suggest that this quality can also be ascribed to chronic SCD pain that is not related to VOC⁸⁷. It is perhaps not surprising then that spontaneous activity is reported in electrophysiological recordings from SCD mice. *In vivo* recordings reveal increased spontaneous action potential firing in

A δ and C fibers from Berk mice when compared to matched fiber types from control animals⁸. Patch clamp recordings of medium and small diameter DRG neurons from Townes mice also reveal increased spontaneous firing frequencies and prevalence in these cell types⁹. Behavioral assessments of spontaneous pain in rodents are notoriously challenging due to the non-verbal nature of these mammals. However, several notable attempts have been made at assessing this measure in transgenic SCD mice. SCD mice have significantly higher grimace scores than control mice. This non-evoked measure collates several key facial expressions that are observed during evoked pain measures⁸⁸. SCD mice also develop a preference for the lidocaine-paired chamber in the analgesic conditioned place preference (CPP) paradigm^{33,34}. Based on Pavlovian conditioning principles, this assay trains injured rodents to associate a specific environment with the pain relieving (or lack thereof) effects of a specific drug. Since SCD mice develop a preference for a setting associated with intrathecal lidocaine injections and control mice do not, it can be inferred that SCD mice are receiving some type of ongoing pain relief from this treatment. SCD mice also develop a preference for the CamKII α inhibitor-paired chamber in this assay³³, and knockdown of ET_AR in SCD DRG prevents the establishment of a lidocaine place preference⁹. These latter experiments, both of which inhibit mechanisms through which TRPV1 activity is sensitized, suggest a possible role for this channel activity in spontaneous SCD pain^{9,33}. The term “spontaneous” is somewhat misleading with regards to nociceptive processing, however. Nociceptors still fire because of activation via a subthreshold endogenous agonist or altered electrophysiological properties. Supporting the latter, small and medium diameter DRG neurons from SCD mice have higher resting membrane potentials and lower rheobase currents than DRG neurons from control mice⁹. As outlined in previous sections, many endogenous chemicals that sensitize TRPV1 are also increased in SCD mouse and human serum, thus potentially altering the activation parameters of the channel at the level of the DRG or nerve. No experiments have directly inhibited TRPV1 and examined the effects on spontaneous pain-like behaviors or neuronal activity.

3.8 Therapeutic potential of molecules targeting TRPV1 activity

When considering the numerous ways in which TRPV1 activity could drive SCD pain, it is perhaps surprising that relatively few preclinical experiments have assessed the therapeutic effects of TRPV1 antagonists. One reason for this may be the declining enthusiasm surrounding TRPV1 inhibitors that resulted from negative side-effects reported during initial clinical trials. In one small molecule TRPV1 inhibitor trial, participants withdrew after developing hyperthermia and in a second trial, noxious temperature detection thresholds were blunted to that point that participants were at risk for serious burn injuries⁸⁹. Second generation TRPV1 antagonists that are in development are modifying first generation compound structures to interact with different residues on the channel, thereby limiting the temperature-related side effects. While these new drug formulations hold therapeutic potential, pharmaceutical companies are also exploring inhibitors that target TRPV1-sensitizing molecules including CCR2 and CamKII α . In addition to these molecules, TRPV1 activity can also be modulated by many lipids including eicosanoids. Prostaglandin E₂ (PGE₂) is an eicosanoid that is generated when arachidonic acid (AA) is metabolized by cyclooxygenase 1 or 2 (COX-1, COX-2) enzymes. Notably, patients and mice with SCD have significantly higher levels of both PGE₂⁴⁵ and AA⁹⁰ in serum than age and race

matched controls, suggesting increased activity of the COX-1 and -2 enzymes. These enzymes are inhibited by non-steroidal anti-inflammatory drugs (NSAIDs; e.g. aspirin, ibuprofen, etc.), some of the most commonly used pain medications throughout the world. Because of their accessibility, NSAIDs are the mainstay treatment for SCD pain in parts of Africa and the Middle East⁹¹, and are often used as adjuvants during opioid therapy⁹². While PGE2 most likely drives SCD pain through a variety of pathways, it is possible that NSAID efficacy in this disease may be partially due to decreased eicosanoid activation of TRPV1.

4. TRPM8 and SCD pain

Following the identification and characterization of TRPV1 as a molecular heat sensor, similar experiments were carried out to identify the molecular cold sensor. Activated by innocuous and noxious cool temperatures (8–26 °C), TRPM8 was identified as one of the primary cold detectors in DRG and trigeminal ganglia neurons⁹³. In addition to cool temperatures, TRPM8 is also activated by the compounds menthol and icilin and sensitized by polyunsaturated fatty acids and lysophospholipids. Upon binding to these compounds, the temperature activation range of TRPM8 increases so that the channel can be opened by body temperature^{94,95}. Like many neuropathic pain patients, individuals with SCD exhibit both cold allodynia and hypersensitivity during steady state health¹⁸. Furthermore, cold weather exacerbates SCD pain intensity and is a risk factor for VOC^{96,97}. Recently, it was also shown that patients with SCD have increased cold hypersensitivity following VOC³⁰. Transgenic mouse models of SCD and SCT also exhibit increased cold sensitivity. SCD/T mice have shorter paw withdrawal latencies to cold stimulation than control mice and spend significantly less time in cold environments during two-temperature choice assays^{7,14,15,19,32,33,77}, a behavior that is further exacerbated following VOC^{14,15,32}. Similar temperature sensitivity is noted on the cellular level. Despite having similar activation temperatures, a larger proportion of SCD DRG neurons exhibit calcium transients during a cold ramp stimulus than control DRG populations⁷. In *ex vivo* recordings, C fibers from SCD and control mice have similar firing frequencies during cold stimulation of the receptive field, however the temperature threshold for firing is significantly higher (i.e. more sensitive to cold) in SCD preparations¹⁵. No differences in *TRPM8* mRNA expression or menthol sensitivity have been observed between SCD and control DRG¹⁵, but additional protein expression and sensitization mechanisms of TRPM8 should continue to be studied. Like mechanical sensitivity, it is possible that pathological TRPM8 expression or function in non-neuronal tissues (e.g. keratinocytes) may contribute to altered thermosensation in SCD. TRPM8 expression and activity may also contribute to enhanced cold sensitivity following VOC, but no studies have specifically assessed this to date. Alternatively, cold hypersensitivity may be mediated by other TRP channels (e.g. TRPC5⁹⁸ or TRPA1 discussed below) or additional cold-sensitive ion channels that are not members of the TRP family (i.e. TREK-1, TRAAK, Na_v1.8 reviewed in⁹⁹).

5. TRPA1 and SCD pain

Shortly after the identification of TRPM8, TRPA1 was described as an additional cold sensor in trigeminal and dorsal root ganglia neurons¹⁰⁰. Named for the numerous ankyrin

repeats at the N-terminus of the protein, members of the TRPA family also have debated roles in mechanical, heat, and chemical sensation in the peripheral nervous system. While the original experiments describing these functions utilized global genetic or pharmacological manipulations, the advent of cell-type specific technologies has brought some clarity to the specific role of TRPA1 in acute and pathological somatosensation. Under naïve conditions, TRPA1 sensory neuron specific knockout animals demonstrate decreased sensitivity to mechanical stimuli but have no deficits in heat or cold sensation¹⁰¹. When expressed in heterologous systems, rat and mouse TRPA1 channels are sensitive to cold, but monkey and human TRPA1 are not¹⁰². Furthermore, TRPA1 is required for noxious heat detection in the absence of TRPV1 and TRPM3¹⁰³. While these studies clearly highlight TRP channel redundancy and perhaps diminish the role of TRPA1 in acute somatosensation, this channel's sensitivity to chemical and inflammatory mediators positions it as a key sensory detector following injury. Reactive oxygen and nitrogen species and various prostaglandin metabolites¹⁰⁴, all of which are dysregulated in SCD patients^{45,105} and further increased following VOC¹⁰⁶, sensitize TRPA1 activity¹⁰⁷. Interestingly, pharmacological experiments with the TRPA1 antagonist HC-030031 do not support the involvement of this channel in chronic SCD hypersensitivity. Application of HC-030031 has no effect on mechanically induced firing of SCD C fibers in *ex vivo* recordings¹⁹ and administration of this compound to transgenic SCD mice does not alleviate behavioral cold hypersensitivity⁷. Likewise, DRG neurons from SCD and control mice are activated to the same extent by cinnamaldehyde, a TRPA1 agonist and furthermore, express similar levels of *TRPA1* mRNA¹⁵. SCD and control mice also exhibit similar nociceptive behavior times during the first phase of the formalin test, a popular noxious inflammatory assay that is mediated in part by TRPA1 activation^{33,108}. Collectively, these data suggest that TRPA1 activity does not contribute to chronic pain in SCD. Like TRPV1, human TRPA1 (but not rodent) is activated by protons¹⁰⁹. As previously mentioned, TRPA1 is also activated by ROS¹⁰⁷. These attributes make TRPA1 a logical candidate for mediating VOC pain. To date, the relative expression and activity of TRPA1 following VOC in mouse has not been examined. However a recent genetic analysis in patients with SCD revealed an association between a TRPA1 single nucleotide polymorphism and emergency department/acute care visits for VOC pain⁵⁵. Future heterologous expression studies are needed to validate these associations and provide additional reasons for the continued exploration of TRPA1 VOC and steady state SCD pain mediation.

6. Summary

TRP channels are highly promiscuous cation channels that are activated and sensitized by a wide range of endogenous and environmental stimuli, many of which are dysregulated in SCD. Patients and transgenic mice with SCD all display chronic mechanical, thermal, and chemical hypersensitivity which may be at least partially mediated by TRP channel signaling. Gene expression analyses have never detected changes in TRP channel expression between SCD and control mice, but increased activity at specific family members has been reported in SCD mice. Subsequent experiments have identified various SCD-related signaling events (e.g. channel phosphorylation) that result in sensitization of TRP channel family members, particularly TRPV1. TRPV1 was originally identified as a noxious heat

detector. Perhaps surprisingly, the effects of TRPV1 inhibition have never been investigated with regards to heat hypersensitivity in SCD, but rather have been more well defined in terms of SCD mechanical hypersensitivity. While not intrinsically gated by mechanical force, TRPV1 activity somehow contributes to mechanical allodynia in both SCD and other inflammatory pain models. The pro-inflammatory acidic environment that accompanies hypoxia and reoxygenation like that which occurs during a VOC also positions TRPV1 as a key player in acute SCD pain. Again, inhibition of many TRPV1 sensitizing molecules like ET_A R and $CamKII\alpha$ decreases VOC pain, but the effect of TRPV1 inhibition alone has never been assessed with regards to the prevention or alleviation of acute VOC pain in SCD mice. TRPV1 is highly expressed on afferents that innervate symptomatic tissues in SCD including bone, muscle, and visceral organs. The lack of accessibility to these tissues and difficulty in assessing tissue-specific nociception however, has precluded experiments examining the therapeutic efficacy of TRPV1 inhibition on disease related symptoms in these deeper pain sources. Targeting TRPV1 or the many agents that sensitize the channel may prove therapeutically advantageous for the aforementioned symptoms as well as the spontaneous pain that is relevant in SCD mouse models. However, therapeutic efficacy of other TRP subfamilies may be lower. Neither TRPM8 nor TRPA1 activity is documented as contributing to the chronic cold, mechanical, or heat hypersensitivity that exists in SCD mouse models, but additional experiments like those completed for TRPV1, should investigate sensitization of these channels. Notably, TRPV4 is also well suited to drive SCD pain as it is activated by heat ($>33^\circ\text{C}$), arachidonic acid metabolites¹¹⁰ which are significantly upregulated in transgenic SCD mouse blood⁹⁰, and mechanical pressure¹¹¹. To date, no study has examined expression of functional relevance of this channel in SCD pain. Since our original description in 2011, basic pain research has built a strong case for the involvement of TRPV1 in chronic SCD pain. However, attention must now be focused on the other 27 mammalian members of this superfamily in order to learn more about neuronal sensory transduction in both chronic and acute SCD pain states.

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References:

1. Cosens DJ & Manning A Abnormal electroretinogram from a *Drosophila* mutant. *Nature* 224, 285–287 (1969). [PubMed: 5344615]
2. Venkatachalam K & Montell C TRP Channels. *Annu. Rev. Biochem* 76, 387–417 (2007). [PubMed: 17579562]
3. Mickle AD, Shepherd AJ & Mohapatra DP Sensory TRP Channels. in *Progress in molecular biology and translational science* 131, 73–118 (2015). [PubMed: 25744671]
4. Yusuf HR, Atrash HK, Grosse SD, Parker CS & Grant AM Emergency Department Visits Made by Patients with Sickle Cell Disease. A Descriptive Study, 1999–2007. *American Journal of Preventive Medicine* 38, S536–41 (2010). [PubMed: 20331955]

5. Ballas SK, Gupta K & Adams-Graves P Sickle cell pain: a critical reappraisal. *Blood* 120, 3647–3656 (2013).
6. Manwani D Vaso-occlusion in sickle cell disease: *Blood* 122, 3892–3898 (2013). [PubMed: 24052549]
7. Sadler KE et al. Chemokine (c-c motif) receptor 2 mediates mechanical and cold hypersensitivity in sickle cell disease mice. *Pain* 159, 1 (2018). [PubMed: 28885455]
8. Uhelski ML, Gupta K & Simone DA Sensitization of C-fiber nociceptors in mice with sickle cell disease is decreased by local inhibition of anandamide hydrolysis. *Pain* 158, 1711–1722 (2017). [PubMed: 28570479]
9. Lutz BM et al. Endothelin type A receptors mediate pain in a mouse model of sickle cell disease. *Haematologica* 103, 1124–1135 (2018). [PubMed: 29545351]
10. Cataldo G, Rajput S, Gupta K & Simone DA Sensitization of nociceptive spinal neurons contributes to pain in a transgenic model of sickle cell disease. *Pain* 156, 722–730 (2015). [PubMed: 25630029]
11. Case M et al. Characterization of functional brain activity and connectivity using EEG and fMRI in patients with sickle cell disease. *NeuroImage Clin* 14, 1–17 (2017). [PubMed: 28116239]
12. Ryan TM, Ciavatta DJ & Townes TM Knockout-Transgenic Mouse Model of Sickle Cell Disease. *Science* (80-.) 278, 873–876 (1997).
13. Paszty C Transgenic Knockout Mice with Exclusively Human Sickle Hemoglobin and Sickle Cell Disease. *Science* (80-.) 278, 876–878 (1997).
14. Lei J, Benson B, Tran H, Ofori-Acquah SF & Gupta K Comparative Analysis of Pain Behaviours in Humanized Mouse Models of Sickle Cell Anemia. *PLoS One* 11, e0160608 (2016). [PubMed: 27494522]
15. Zappia KJ, Garrison SR, Hillery CA & Stucky CL Cold hypersensitivity increases with age in mice with sickle cell disease. *Pain* 155, 2476–2485 (2014). [PubMed: 24953902]
16. Darbari DS et al. Frequency of Hospitalizations for Pain and Association with Altered Brain Network Connectivity in Sickle Cell Disease. *J. Pain* 16, 1077–1086 (2015). [PubMed: 26291276]
17. Jacob E et al. Sensory and Thermal Quantitative Testing in Children With Sickle Cell Disease. *J. Pediatr. Hematol. Oncol* 37, 185–189 (2015). [PubMed: 25014619]
18. Brandow AM, Stucky CL, Hillery CA, Hoffmann RG & Panepinto JA Patients with sickle cell disease have increased sensitivity to cold and heat. *Am. J. Hematol* 88, 37–43 (2013). [PubMed: 23115062]
19. Hillery CA et al. Transient receptor potential vanilloid 1 mediates pain in mice with severe sickle cell disease. *Blood* 118, 3376–3383 (2011). [PubMed: 21708890]
20. Caterina MJ, Rosen TA, Tominaga M, Brake AJ & Julius D A capsaicin-receptor homologue with a high threshold for noxious heat. *Nature* 398, 436–441 (1999). [PubMed: 10201375]
21. Caterina MJ et al. The capsaicin receptor: A heat-activated ion channel in the pain pathway. *Nature* 389, 816–824 (1997). [PubMed: 9349813]
22. Kobayashi K et al. Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with delta/c-fibers and colocalization with trk receptors. *J. Comp. Neurol* 493, 596–606 (2005). [PubMed: 16304633]
23. Valtschanoff JG, Rustioni A, Guo A & Hwang SJ Vanilloid receptor VR1 is both presynaptic and postsynaptic in the superficial laminae of the rat dorsal horn. *J. Comp. Neurol* 436, 225–235 (2001). [PubMed: 11438926]
24. Cavanaugh DJ et al. Trpv1 Reporter Mice Reveal Highly Restricted Brain Distribution and Functional Expression in Arteriolar Smooth Muscle Cells. *J. Neurosci* 31, 5067–5077 (2011). [PubMed: 21451044]
25. Tóth A et al. Vanilloid Receptor-1 (TRPV1) Expression and Function in the Vasculature of the Rat. *J. Histochem. Cytochem* 62, 129–144 (2014). [PubMed: 24217926]
26. Saunders CI, Kunde DA, Crawford A & Geraghty DP Expression of transient receptor potential vanilloid 1 (TRPV1) and 2 (TRPV2) in human peripheral blood. *Mol. Immunol* 44, 1429–1435 (2007). [PubMed: 16777226]

27. Bodó E et al. Vanilloid receptor-1 (VR1) is widely expressed on various epithelial and mesenchymal cell types of human skin [4]. *Journal of Investigative Dermatology* 123, 410–413 (2004). [PubMed: 15245445]
28. Ross RA Anandamide and vanilloid TRPV1 receptors. *British Journal of Pharmacology* 140, 790–801 (2003). [PubMed: 14517174]
29. Jordt SE, Tominaga M & Julius D Acid potentiation of the capsaicin receptor determined by a key extracellular site. *Proc. Natl. Acad. Sci. U. S. A* 97, 8134–9 (2000). [PubMed: 10859346]
30. Brandow AM et al. Children and adolescents with sickle cell disease have worse cold and mechanical hypersensitivity during acute painful events. *Pain* (2018).
31. Kohli DR et al. Pain-related behaviors and neurochemical alterations in mice expressing sickle hemoglobin: modulation by cannabinoids. *Blood* 116, 456–65 (2010). [PubMed: 20304807]
32. Cain DM, Vang D, Simone DA, Hebbel RP & Gupta K Mouse models for studying pain in sickle cell disease: effects of strain, age, and acuteness. *Br. J. Haematol* 156, 535–544 (2012). [PubMed: 22171826]
33. He Y et al. CaMKII α underlies spontaneous and evoked pain behaviors in Berkeley sickle cell transgenic mice. *Pain* 157, 2798–2806 (2016). [PubMed: 27842048]
34. He Y et al. PKC δ -targeted intervention relieves chronic pain in a murine sickle cell disease model. *J. Clin. Invest* 126, 3053–7 (2016). [PubMed: 27348590]
35. Zappia KJ et al. Characterization of a mouse model of sickle cell trait: parallels to human trait and a novel finding of cutaneous sensitization. *Br. J. Haematol* 179, 657–666 (2017). [PubMed: 29027199]
36. TAYLOR SC, SHACKS SJ, MITCHELL RA & BANKS A Serum Interleukin-6 Levels in the Steady State of Sickle Cell Disease. *J. Interf. Cytokine Res* 15, 1061–1064 (1995).
37. Sarray S et al. Serum IL-6, IL-10, and TNF α levels in pediatric sickle cell disease patients during vasoocclusive crisis and steady state condition. *Cytokine* 72, 43–47 (2015). [PubMed: 25569375]
38. Obara I, Géranton SM & Hunt SP Axonal protein synthesis: a potential target for pain relief? *Curr. Opin. Pharmacol* 12, 42–48 (2012). [PubMed: 22033338]
39. Schmidt M, Dubin AE, Petrus MJ, Earley TJ & Patapoutian A Nociceptive Signals Induce Trafficking of TRPA1 to the Plasma Membrane. *Neuron* 64, 498–509 (2009). [PubMed: 19945392]
40. Camprubi-Robles M, Planells-Cases R & Ferrer-Montiel A Differential contribution of SNARE-dependent exocytosis to inflammatory potentiation of TRPV1 in nociceptors. *FASEB J* 23, 3722–3733 (2009). [PubMed: 19584302]
41. Julius D TRP Channels and Pain. *Annu. Rev. Cell Dev. Biol* 29, 355–384 (2013). [PubMed: 24099085]
42. Jung J et al. Phosphorylation of Vanilloid Receptor 1 by Ca²⁺/Calmodulin-dependent Kinase II Regulates Its Vanilloid Binding. *J. Biol. Chem* 279, 7048–7054 (2004). [PubMed: 14630912]
43. Plant TD et al. Endothelin potentiates TRPV1 via ETA receptor-mediated activation of protein kinase C. *Mol. Pain* 3, 35 (2007). [PubMed: 18001466]
44. Rybicki AC & Benjamin LJ Increased levels of endothelin-1 in plasma of sickle cell anemia patients. *Blood* 92, 2594–6 (1998). [PubMed: 9746804]
45. Graido-Gonzalez E et al. Plasma endothelin-1, cytokine, and prostaglandin E2 levels in sickle cell disease and acute vaso-occlusive sickle crisis. *Blood* 92, 2551–2555 (1998). [PubMed: 9746797]
46. Qari MH, Dier U & Mousa S. a. Biomarkers of inflammation, growth factor, and coagulation activation in patients with sickle cell disease. *Clin. Appl. Thromb. Hemost* 18, 195–200 (2012). [PubMed: 21949038]
47. Vincent L et al. Mast cell activation contributes to sickle cell pathobiology and pain in mice. *Blood* 122, 1853–1862 (2013). [PubMed: 23775718]
48. Ryu S, Liu B, Yao J, Fu Q & Qin F Uncoupling Proton Activation of Vanilloid Receptor TRPV1. *J. Neurosci* 27, 12797–12807 (2007). [PubMed: 18032651]
49. Ueda Y, Nagel RL & Bookchin RM An increased Bohr effect in sickle cell anemia. *Blood* 53, 472–480 (1979). [PubMed: 32940]

50. Premkumar LS & Raisinghani M Nociceptors in cardiovascular functions: Complex interplay as a result of cyclooxygenase inhibition. *Mol. Pain* 2, 26 (2006). [PubMed: 16916451]
51. Scotland RS et al. Vanilloid receptor TRPV1, sensory C-fibers, and vascular autoregulation: A novel mechanism involved in myogenic constriction. *Circ. Res* 95, 1027–1034 (2004). [PubMed: 15499026]
52. Ristoiu V et al. Hypoxia-induced sensitization of transient receptor potential vanilloid 1 involves activation of hypoxia-inducible factor-1 alpha and PKC. *Pain* 152, 936–45 (2011). [PubMed: 21376466]
53. Ross JL, Queme LF, Lamb JE, Green KJ & Jankowski MP Sex differences in primary muscle afferent sensitization following ischemia and reperfusion injury. *Biol. Sex Differ* 9, 2 (2018). [PubMed: 29298725]
54. Xing J, Lu J & Li J Contribution of nerve growth factor to augmented TRPV1 responses of muscle sensory neurons by femoral artery occlusion. *Am. J. Physiol. Heart Circ. Physiol* 296, H1380–7 (2009). [PubMed: 19286963]
55. Jhun EH et al. Transient receptor potential polymorphism and haplotype associate with crisis pain in sickle cell disease. *Pharmacogenomics* 19, 401–411 (2018). [PubMed: 29620434]
56. Mosaku S et al. Avascular necrosis significantly impairs quality of life in sickle cell disease. *J. Clin. Sci* 12, 41 (2015).
57. Ghilardi JR Selective Blockade of the Capsaicin Receptor TRPV1 Attenuates Bone Cancer Pain. *J. Neurosci* 25, 3126–3131 (2005). [PubMed: 15788769]
58. Blinder MA, Russel S & Barnes M Prevalence of symptomatic avascular necrosis and the operative treatment in adult patients with sickle cell disease. *Blood* 124, 1379 (2014). [PubMed: 25170108]
59. VanderJagt DJ, Bonnett C, Okolo SN & Glew RH Assessment of the bone status of Nigerian children and adolescents with sickle cell disease using calcaneal ultrasound and serum markers of bone metabolism. *Calcif. Tissue Int* 71, 133–140 (2002). [PubMed: 12200647]
60. Sarrai M, Duroseau H, D'Augustine J, Moktan S & Bellevue R Bone mass density in adults with sickle cell disease. *Br. J. Haematol* 136, 666–672 (2007). [PubMed: 17223909]
61. Xiao L et al. Loss of bone in sickle cell trait and sickle cell disease female mice is associated with reduced igf-1 in bone and serum. *Endocrinology* 157, 3036–3046 (2016). [PubMed: 27171384]
62. Yuan FL et al. The roles of acidosis in osteoclast biology. *Frontiers in Physiology* 7, 222 (2016). [PubMed: 27445831]
63. Jankowski MP, Rau KK, Ekmann KM, Anderson CE & Koerber HR Comprehensive phenotyping of group III and IV muscle afferents in mouse. *J. Neurophysiol* 109, (2013).
64. Light AR et al. Dorsal Root Ganglion Neurons Innervating Skeletal Muscle Respond to Physiological Combinations of Protons, ATP, and Lactate Mediated by ASIC, P2X, and TRPV1. *J. Neurophysiol* 100, (2008).
65. Tajeja N, Racovan M, Valent J & Zonder J Myonecrosis in sickle cell anemia-Overlooked and underdiagnosed. *Case Rep. Med* 2010, 659031 (2010). [PubMed: 20300585]
66. Ravelojaona M et al. Evidence for a profound remodeling of skeletal muscle and its microvasculature in sickle cell anemia. *Am. J. Pathol* 185, 1448–56 (2015). [PubMed: 25773175]
67. Valeriano-Marcet J & Kerr LD Myonecrosis and Myofibrosis as Complications of Sickle Cell Anemia. *Ann. Intern. Med* 115, 99 (1991). [PubMed: 2058869]
68. van Beers EJ et al. Exercise tolerance, lung function abnormalities, anemia, and cardiothoracic ratio in sickle cell patients. *Am. J. Hematol* 89, 819–824 (2014). [PubMed: 24799296]
69. Wang L et al. Cognitive and behavior deficits in sickle cell mice are associated with profound neuropathologic changes in hippocampus and cerebellum. *Neurobiol. Dis* 85, 60–72 (2016). [PubMed: 26462816]
70. Wang L et al. The role of nitrite in muscle function, susceptibility to contraction injury, and fatigability in sickle cell mice. *Nitric Oxide - Biol. Chem* 80, 70–81 (2018).
71. Rong W et al. Jejunal afferent nerve sensitivity in wild-type and TRPV1 knockout mice. *J. Physiol* 560, 867–881 (2004). [PubMed: 15331673]

72. Dang K Differential Responses of Bladder Lumbosacral and Thoracolumbar Dorsal Root Ganglion Neurons to Purinergic Agonists, Protons, and Capsaicin. *J. Neurosci* 25, 3973–3984 (2005). [PubMed: 15829649]
73. Christianson JA, McIlwrath SL, Koerber HR & Davis BM Transient receptor potential vanilloid 1-immunopositive neurons in the mouse are more prevalent within colon afferents compared to skin and muscle afferents. *Neuroscience* 140, 247–257 (2006). [PubMed: 16564640]
74. Schwartz ES et al. Synergistic role of TRPV1 and TRPA1 in pancreatic pain and inflammation. *Gastroenterology* 140, 1283–1291 (2011). [PubMed: 21185837]
75. Malin S et al. TRPV1 and TRPA1 Function and Modulation Are Target Tissue Dependent. *J. Neurosci* 31, 10516–10528 (2011). [PubMed: 21775597]
76. Ahmed S, Shahid RK & Russo LA Unusual causes of abdominal pain: Sick cell anemia. *Best Practice and Research: Clinical Gastroenterology* 19, 297–310 (2005). [PubMed: 15833695]
77. Zappia KJ et al. Characterization of a mouse model of sickle cell trait: parallels to human trait and a novel finding of cutaneous sensitization. *Br. J. Haematol* 179, 657–666 (2017). [PubMed: 29027199]
78. Garrison SR, Kramer AA, Gerges NZ, Hillery CA & Stucky CL Sick cell mice exhibit mechanical allodynia and enhanced responsiveness in light touch cutaneous mechanoreceptors. *Mol. Pain* 8, 62 (2012). [PubMed: 22963123]
79. Jones S et al. Windy weather and low humidity are associated with an increased number of hospital admissions for acute pain and sickle cell disease in an urban environment with a maritime temperate climate. *Br. J. Haematol* 131, 530–533 (2005). [PubMed: 16281945]
80. Caterina MJ et al. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 288, 306–13 (2000). [PubMed: 10764638]
81. Watanabe M, Ueda T, Shibata Y, Kumamoto N & Ugawa S The role of TRPV1 channels in carrageenan-induced mechanical hyperalgesia in mice. *Neuroreport* 26, 173–178 (2015). [PubMed: 25590988]
82. Szabo A et al. Role of Transient Receptor Potential Vanilloid 1 Receptors in Adjuvant-Induced Chronic Arthritis: In Vivo Study Using Gene-Deficient Mice. *J. Pharmacol. Exp. Ther* 314, 111–119 (2005). [PubMed: 15831443]
83. Walder RY et al. TRPV1 is important for mechanical and heat sensitivity in uninjured animals and development of heat hypersensitivity after muscle inflammation. *Pain* 153, 1664–72 (2012). [PubMed: 22694790]
84. Eskander MA et al. Persistent Nociception Triggered by Nerve Growth Factor (NGF) Is Mediated by TRPV1 and Oxidative Mechanisms. *J. Neurosci* 35, 8593–603 (2015). [PubMed: 26041925]
85. Mickle AD, Shepherd AJ, Loo L & Mohapatra DP Induction of thermal and mechanical hypersensitivity by parathyroid hormone-related peptide through upregulation of TRPV1 function and trafficking. *Pain* 156, 1620–1636 (2015). [PubMed: 25970319]
86. Shepherd AJ, Mickle AD, Kadunganattil S, Hu H & Mohapatra DP Parathyroid Hormone-Related Peptide Elicits Peripheral TRPV1-dependent Mechanical Hypersensitivity. *Front. Cell. Neurosci* 12, 38 (2018). [PubMed: 29497363]
87. Brandow AM, Farley RA & Panepinto JA Neuropathic pain in patients with sickle cell disease. *Pediatr. Blood Cancer* 61, 512–7 (2014). [PubMed: 24167104]
88. Mittal AM, Lamarre YY & Gupta K Observer Based Objective Pain Quantification in Sick Mice Using Grimace Scoring and Body Parameters. *Blood* 124, 4907 LP-4907 (2014).
89. Brederson J-D, Kym PR & Szallasi A Targeting TRP channels for pain relief. *Eur. J. Pharmacol* 716, 61–76 (2013). [PubMed: 23500195]
90. Wu H et al. Hypoxia-mediated impaired erythrocyte Lands' Cycle is pathogenic for sickle cell disease. *Sci. Rep* 6, 29637 (2016). [PubMed: 27436223]
91. Han J, Saraf SL, Lash JP & Gordeuk VR Use of anti-inflammatory analgesics in sickle-cell disease. *Journal of Clinical Pharmacy and Therapeutics* 42, 656–660 (2017). [PubMed: 28695614]
92. Perlin E et al. Enhancement of pain control with ketorolac tromethamine in patients with sickle cell vaso-occlusive crisis. *Am. J. Hematol* 46, 43–7 (1994). [PubMed: 7514356]
93. McKemy DD, Neuhauser WM & Julius D Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 416, 52–58 (2002). [PubMed: 11882888]

94. Voets T et al. The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. *Nature* 430, 748–754 (2004). [PubMed: 15306801]
95. Andersson DA, Nash M & Bevan S Modulation of the cold-activated channel TRPM8 by lysophospholipids and polyunsaturated fatty acids. *J. Neurosci* 27, 3347–55 (2007). [PubMed: 17376995]
96. Resar LMS & Oski FA Cold water exposure and vaso-occlusive crises in sickle cell anemia. *J. Pediatr* 118, 407–409 (1991). [PubMed: 1999780]
97. Smith WR et al. Climatic and geographic temporal patterns of pain in the Multicenter Study of Hydroxyurea. *Pain* 146, 91–98 (2009). [PubMed: 19683393]
98. Zimmermann K et al. Transient receptor potential cation channel, subfamily C, member 5 (TRPC5) is a cold-transducer in the peripheral nervous system. *Proc. Natl. Acad. Sci* 108, 18114–18119 (2011). [PubMed: 22025699]
99. McKemy DD The molecular and cellular basis of cold sensation. *ACS Chem. Neurosci* 4, 238–47 (2013). [PubMed: 23421674]
100. Story GM et al. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell* 112, 819–829 (2003). [PubMed: 12654248]
101. Zappia KJ, O'Hara CL, Moehring F, Kwan KY & Stucky CL Sensory neuron-specific deletion of TRPA1 results in mechanical cutaneous sensory deficits. *eneuro* 4, ENEURO.0069–16.2017 (2017).
102. Chen J et al. Species differences and molecular determinant of TRPA1 cold sensitivity. *Nat. Commun* 4, 2501 (2013). [PubMed: 24071625]
103. Vandewauw I et al. A TRP channel trio mediates acute noxious heat sensing. *Nature* 555, 662–666 (2018). [PubMed: 29539642]
104. Taylor-Clark TE et al. Prostaglandin-induced activation of nociceptive neurons via direct interaction with transient receptor potential A1 (TRPA1). *Mol. Pharmacol* 73, 274–81 (2008). [PubMed: 18000030]
105. Wood KC & Granger DN Sickle cell disease: Role of reactive oxygen and nitrogen metabolites. *Clinical and Experimental Pharmacology and Physiology* 34, 926–932 (2007). [PubMed: 17645642]
106. Kaul DK & Hebbel RP Hypoxia/reoxygenation causes inflammatory response in transgenic sickle mice but not in normal mice. *J. Clin. Invest* 106, 411–420 (2000). [PubMed: 10930444]
107. Andersson DA, Gentry C, Moss S & Bevan S Transient Receptor Potential A1 Is a Sensory Receptor for Multiple Products of Oxidative Stress. *J. Neurosci* 28, 2485–2494 (2008). [PubMed: 18322093]
108. McNamara CR et al. TRPA1 mediates formalin-induced pain. *Proc. Natl. Acad. Sci. U. S. A* 104, 13525–30 (2007). [PubMed: 17686976]
109. de la Roche J et al. The molecular basis for species-specific activation of human TRPA1 protein by protons involves poorly conserved residues within transmembrane domains 5 and 6. *J. Biol. Chem* 288, 20280–92 (2013). [PubMed: 23709225]
110. Watanabe H et al. Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature* 424, 434–438 (2003). [PubMed: 12879072]
111. Ho TC, Horn NA, Huynh T, Kelava L & Lansman JB Evidence TRPV4 contributes to mechanosensitive ion channels in mouse skeletal muscle fibers. *Channels* 6, 246–254 (2012). [PubMed: 22785252]

Highlights

- SCD patients and animal models exhibit mechanical, cold, and heat hypersensitivity
- TRPV1 contributes to chronic mechanical hypersensitivity in rodent models of SCD
- No preclinical studies on TRPA1 or TRPM8 confirm channel involvement in SCD pain
- Future studies should assess TRPV1 involvement in acute crisis and deep tissue pain

Figure 1: SCD pain pathology.

SCD patients experience widespread superficial and deep pain. Pain arising from the surface of the body (i.e. somatic pain) is mediated by increased activity of peripheral nerves. Both patients with SCD and animal models of SCD exhibit cold and mechanical allodynia and hyperalgesia. These sensations are driven by increased activity of C and A δ fibers that express members of the TRP channel family and terminate within the skin. Deep sites of SCD pain include muscle, bone and joints as well as visceral organs. During a vaso-occlusive crisis, sickled red blood cells (RBCs) adhere to and damage the endothelial cells

lining blood vessels and other circulating cell types. Complete blockage of small blood vessels causes endothelial cell/RBC lysis and initiates immune cell infiltration which results in a pro-inflammatory, acidic microenvironment.

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