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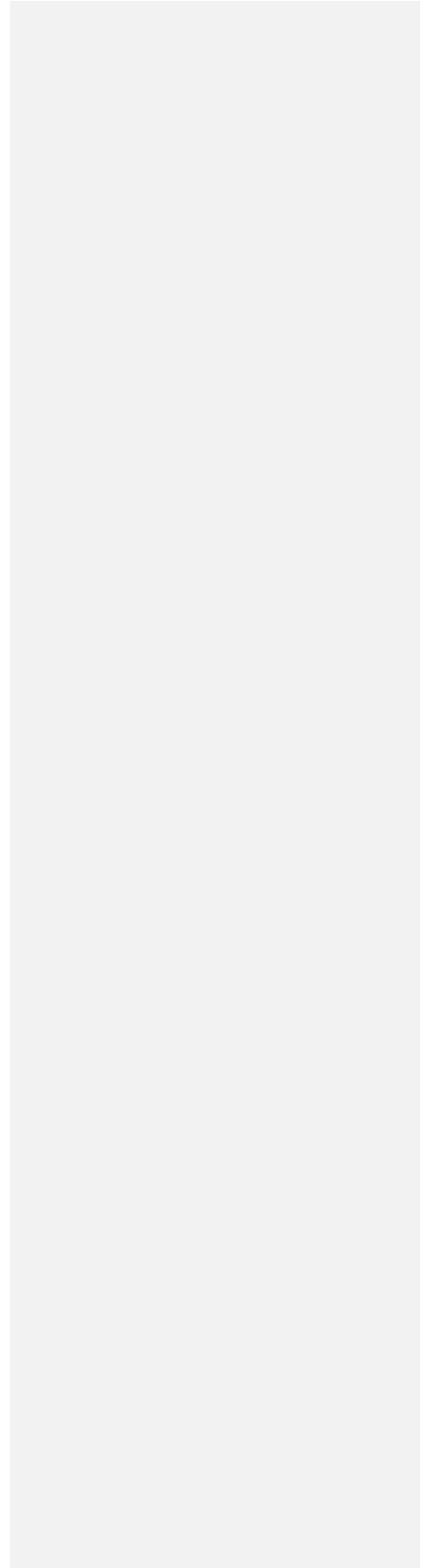
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Potassium Channel Allele Modulates Chronic Musculoskeletal Pain Experience

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Abstract

Chronic musculoskeletal pain is a complex disorder that often causes physical and psychological symptoms. While acute pain promotes healing of damaged tissue, chronic pain is caused by long-term maladaptive changes that result in hyperexcitability of pain-signaling neurons. Potassium channels have recently been implicated in pain syndromes because they largely determine the characteristics for cell activation. The gene KCNS1 encodes for a potassium channel alpha subunit, and a common single nucleotide polymorphism (SNP) in this gene was recently correlated with more severe chronic neuropathic pain. Therefore, this study explores the effects of this KCNS1 SNP on the symptomology of chronic musculoskeletal pain and response to treatment with cognitive behavioral therapy (CBT) or an educational control condition (EDU). The sample included 201 participants with chronic musculoskeletal pain who provided saliva samples from which their DNA was extracted, amplified, and sequenced to determine each participant's KCNS1 genotype. Participants answered questionnaires measuring physical pain symptoms and psychological suffering at baseline and again following three months of treatment. At baseline, those homozygous for the Val mutation were found to have reduced catastrophizing, lower total pain experience, and greater mental functioning compared to their Val/Ile and Ile/Ile counterparts. Interestingly, all participants improved to the same level of physical and psychological functioning after both treatments regardless of genotype, with greater improvements seen in CBT compared to EDU for all genotypes. Additionally, Ile/Ile individuals tended to improve more in psychological domains, whereas Val/Ile individuals improved more physically. This suggests that KCNS1 genotypic variation can alter the symptomology of chronic musculoskeletal pain and that CBT is an effective chronic pain treatment regardless of KCNS1 genotype.

1. Introduction

Chronic pain is a complex sensory experience that typically involves both physical and psychological symptoms. While acute pain is adaptive and even protective, chronic pain is the result of long-term maladaptive changes within the central and peripheral nervous systems that perpetuates the sensation of pain after the noxious stimulus has dissipated (Ji, Xu, Strichartz, & Serhan, 2011). Chronic pain includes both musculoskeletal and neuropathic pain that lasts for six months or longer, and research indicates the two may share some similar underlying mechanisms (Ji et al., 2011). Musculoskeletal pain occurs following tissue injury that elicits an inflammatory response involving a cascade of chemical signals that increase sensitivity of the injured region in order to promote healing (Li et al., 2014). These inflammatory mediators activate secondary messengers within the periphery that then signal nociceptors in the central nervous system, resulting in the perception of pain. However, it appears this inflammatory response can also stimulate long-term transcriptional and translational changes within peripheral neurons to increase the signaling of pain pathways, potentially resulting in a transition from an acute to a chronic pain state (Woolf & Costigan, 1999).

The shift from acute to chronic pain likely involves several biological mechanisms, including peripheral and central sensitization, long-term potentiation, and windup, with potential variations for chronic musculoskeletal versus chronic neuropathic pain (Ji et al., 2011). Research suggests that while chronic neuropathic pain may be caused by long-term maladaptive sensitization of nociceptors, chronic musculoskeletal pain may instead involve prolonged stimulation of nociceptors (Costigan et al., 2010; Everill & Kocsis, 1999; Nielsen & Henriksson, 2007). These processes may lower the activation thresholds for pain-signaling

neurons and cause spontaneous and/or exaggerated neuronal firing in response to noxious stimuli (Woolf & Costigan, 1999). This increased sensitivity and hyperexcitability may maintain the sensation of pain without the appropriate stimulation because the nervous system itself is now generating the painful signals via chemical mediators.

This dysfunction of the nervous system results in 76 million Americans experiencing chronic pain syndromes every year (Zheng & Peltz, 2010). This clinical population experiences reduced quality of life and costs taxpayers over \$100 billion annually (Zheng & Peltz, 2010). Despite the severity of this issue, the mechanisms underlying chronic pain remain poorly understood and the available treatments tend to be problematic with regards to long-term efficacy and abuse potential. Therefore, the present study seeks to augment current knowledge with potential predisposing factors to susceptibility and treatment of chronic musculoskeletal pain.

Current commonly available treatments for chronic pain present a rising health concern, as they mostly rely on prescription opiates and non-steroidal anti-inflammatory drugs (NSAIDs) without further intervention (King, Fraser, Boikos, Richardson, & Harper, 2014). Evidence suggests that taking commonly prescribed NSAIDs, such as ibuprofen, may actually prolong inflammation after injury by disrupting endogenous pro-resolving and/or anti-inflammatory mechanisms (Ji et al., 2011). Furthermore, prescription opiates are commonly over-prescribed and have high potential for abuse, representing significant health challenges and potentially detrimental long-term effects (King et al., 2014). For example, prescription of all opioid painkillers for chronic musculoskeletal pain doubled in the United States from 1980 to 2000, and was also associated with increasing dosage, with prescription rates of the most potent opiates actually quadrupling during this time period (King et al., 2014). Moreover, the recent

opioid epidemic seen nationwide is a particular concern in Vermont, which was recently referred to as “The New Face of Heroin” in an issue of Rolling Stone Magazine released in April, 2014. The issue may be aggravated by the prescription practices of Vermont physicians, as methadone-related deaths increased 400% from 2001 to 2006 alone and 60% of methadone prescriptions were for the treatment of chronic pain (Madden & Shapiro, 2011). It is therefore of the utmost importance to find effective, therapeutic treatments for chronic pain without exacerbating health-related challenges as commonly occurs with current pharmacologic treatments.

It has previously been reported that treatment with Cognitive Behavioral Therapy (CBT) is effective at alleviating chronic pain symptoms, and so may represent an effective alternative treatment option with reduced side effects for those with chronic musculoskeletal pain (Bair, Wu, Damush, Sutherland, & Kroenke, 2008). Subsequently, the present study seeks to explore factors that may affect the susceptibility and severity of chronic musculoskeletal pain, as well as how they relate to successful psychotherapeutic treatment of the disorder.

The huge phenotypic variation in chronic pain symptoms, coupled with the fact that not all those who sustain a tissue injury develop such disorders, suggests multiple biological mechanisms may be involved. Emerging evidence has emphasized the role of genetic predispositions to chronic pain, and suggests up to 60% of the variability in the development of chronic pain is heritable (Young, Lariviere, & Belfer, 2012). Due to the fact that neuronal misfiring is involved in chronic pain symptoms, potassium channels are likely involved as they largely determine the characteristics for neuronal cell activation (Costigan et al., 2010; Richardson & Kaczmarek, 2000). Therefore, genes that encode for potassium channels could

potentially alter the pain phenotypes seen in patients experiencing chronic pain by modulating sensory neuron excitability. Subsequently, the present study focuses on the gene KCNS1, which encodes for a potassium channel alpha subunit and is constitutively transcribed in sensory neurons (Costigan et al., 2010; Richardson & Kaczmarek, 2000).

Research suggests this subunit is nonfunctional alone, but serves to modulate the currents of potassium channels in sensory neurons when conjoined with other subunits (Costigan et al., 2010; Richardson & Kaczmarek, 2000). For example, it was determined that when combined with other subfamilies of potassium channel alpha subunits, KCNS1 inhibited the firing of action potentials during maintained stimulation in excitable cells (Richardson & Kaczmarek, 2000). Such modulatory effects on first-order sensory neurons suggest KCNS1 can influence the pain phenotypes that manifest as chronic pain syndromes (Costigan et al., 2010; George et al., 2014; Richardson & Kaczmarek, 2000; Smolin, Karry, Gal-Ben-Ari, & Ben-Shachar, 2012; Tsantoulas et al., 2012; Zheng & Peltz, 2010). This idea is further supported by the fact that KCNS1 is substantially downregulated after injury in three distinct chronic neuropathic pain models, including spared nerve, chronic constriction, and spinal nerve ligation injuries (Costigan et al., 2010; Tsantoulas et al., 2012; Zheng & Peltz, 2010). The fact that the expression of KCNS1 is reduced after nerve injury lends support to the notion that this gene may be involved in neuropathic pain signaling, potentially implicating the gene in other chronic pain disorders. In fact, recent literature suggests that variation in the KCNS1 genotype can alter an individual's sensitivity to neuropathic pain as well as their susceptibility to developing chronic neuropathic pain syndromes (Costigan et al., 2010; Young et al., 2012). Due to the potentially significant overlap between chronic pain disorders,

these findings validate investigating the effect of genotypic variation of KCNS1 on pain, suffering, and response to treatment in patients with chronic musculoskeletal pain.

Emergence of pain phenotypes associated with peripheral injuries appears to be altered by a missense single nucleotide polymorphism (SNP) at the rs734784 KCNS1 allele that results in a valine-to-isoleucine substitution (Costigan et al., 2010; George et al., 2014). This mutation is quite prevalent, with approximately 50% of the population heterozygous and 20% homozygous for this SNP, making it a clinically relevant gene to investigate (Costigan et al., 2010). When this gene was examined in patients with chronic neuropathic pain, the valine (Val) allele was associated with greater pain intensity in five different patient cohorts (Costigan et al., 2010). Subsequent research found that the KCNS1 Val mutation was associated with greater upper extremity disability and more frequent pain catastrophizing, indicative of more fear-avoidance behaviors, and suggesting KCNS1 may affect more than just the physical symptoms of pain (George et al., 2013). This indicates that KCNS1 genotype may modify the prognosis of chronic pain, making our study particularly relevant as the effect of KCNS1 has never before been examined in a chronic musculoskeletal pain population. The present study will therefore investigate how genotypic variation in KCNS1 affects the pain phenotypes in those with chronic musculoskeletal pain, as well as their response to treatment with cognitive behavioral therapy (CBT) compared to a control educational materials mailing program (EDU).

1. Methods

2.1 Participants

The study sample comprised 201 male and female adults, ages 18 to 65, of any racial or ethnic background concurrently enrolled in a chronic musculoskeletal pain study at the University of Vermont’s MindBody Medicine Clinic (Table 1). Participants were recruited from advertisements and flyers posted around the Burlington area as well as clinical referrals from other physicians. The sample included patients with diagnoses of chronic back pain, osteoarthritis, post-trauma pain, temporomandibular disorder, and fibromyalgia. Inclusion criteria included: at least 1 year of chronic musculoskeletal, non-neuropathic pain, and a subjective typical pain rating of at least 4 out of 10 (with 0 “no pain” to 10 “worst pain”) for the preceding month. Exclusion criteria included malignancy, awaiting pain-related surgery, involvement in pain-related litigation, an Axis I disorder (except for controlled mild to moderate depression and anxiety), and severe personality disorders.

Table 1. Descriptive Statistics for Demographic Factors

Variable	Mean \pm SD or Frequency	Median (Min, Max) or %
Age	48.5 \pm 13.6	50.5 (18, 81)
Gender		
Female	131	68%
Male	62	32%
Race		
White	178	92%
Black or African American	4	2%
Other	11	4%
KCNS 1 Genotype (rs734784)		
GG	34	18%
AG	73	38%
AA	86	45 %

2.2 Materials

Clinical and behavioral measures were self-administered at baseline and then again following three months of treatment with either CBT or EDU. Participants responded to

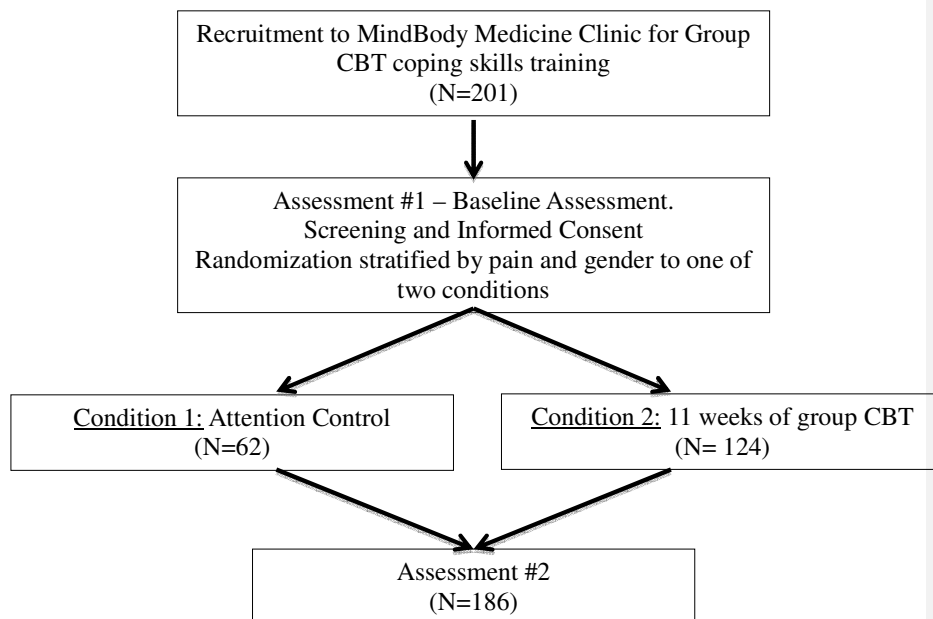
questionnaires in regards to their musculoskeletal pain. The McGill Pain Questionnaire was used to assess how patients perceived and rated their typical pain intensity on a 0 to 10 scale (with 0 “no pain” to 10 “worst pain”) (Melzack, 1975). Additionally, the Treatment Outcomes in Pain Survey (TOPS) subscales SF-36 Physical Health Composite (PHC) and Total Pain Experience (TPE) were used to evaluate several measures of physical functioning, including bodily pain, pain symptoms, functional limitations, and perceived disability (Rogers, Wittink, Ashburn, Cynn, & Carr, 2000). All of these scales are composite scores from multiple items and range from 0 to 100.

Emotional functioning was also assessed using a TOPS subscale, the SF-36 Mental Health Composite (MHC), which evaluates several domains including mental health and emotional and social functioning, with composite scores ranging from 0 to 100 (Rogers et al., 2000). Participant pain-related catastrophizing was also evaluated using either the Pain Catastrophizing Scale (PCatS) (Sullivan, Weinshenker, Mikail, & Bishop, 1995) or the Catastrophizing Subscale of the Coping Strategies Questionnaire (CSQ) (Lawson, Reesor, Keefe, & Turner, 1990) were used. A total of five overlapping questions (5 out of 6 in CSQ and 5 out of 13 in PCatS) were found in both of these questionnaires and were used in order to compare the two scales. The scale ranges from 0 to 6 for CSQ and 0 to 4 for PCatS, so scores were transformed to a scale of 0-100 and expressed as an average. Participant depression was also assessed using the Beck Depression Inventory (BDI) questionnaire (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961). Subjects with BDI scores below 13 were considered minimally depressed, 14-19 mildly depressed, 20-28 moderately depressed, and 29-63 severely depressed.

2.3 Procedure

The University of Vermont’s institutional review board for human subjects approved this study, and all subjects provided informed consent prior to participating. Subjects underwent clinical evaluation and cognitive testing to determine their eligibility. Participants were then randomized into either CBT intervention or a control educational condition. The study design is summarized in Figure 1.

Figure 1. Study Design



The CBT intervention is referred to as Coping Skills Training and was developed specifically for treatment of chronic pain. Typically, between 7-10 patients participate in each group CBT session, and participants randomized to this condition attend weekly 90 minute group sessions for 11 weeks and receive on-going feedback and encouragement.

Details of Group Cognitive Behavioral Therapy - CBT

The purpose of this CBT intervention is to 1) reduce maladaptive coping skills, such as catastrophizing, and 2) increase the ability of the patient to use adaptive strategies to cope with their pain, including attention diversion and changes in activities. To apply CBT to a pain management group program, a simplified gate control model of pain is used, emphasizing to patients that pain is a complex and multifaceted experience involving thoughts, feelings, and behaviors (Melzack & Wall, 1965). The coping strategies used here emphasize skills patients can practice so as to better control their pain.

A. Changing Cognitions. Cognitive-restructuring is used to emphasize to patients the relationship between their thoughts, feelings, and behavior. Such techniques help participants to identify irrational and maladaptive thought patterns so that they can replace them with more rational thoughts to better cope with their pain. Patients were also encouraged to engage in calming self-statements to deal with severe pain.

B. Attention Diversion Methods. Patients are trained in three attention diversion methods, including relaxation, imagery, and distraction. Relaxation training involves using muscle tension signals as a cue to relax. Mini-practices, or brief relaxation methods, are used to teach patients how to apply relaxation techniques during daily activities. Patients are also taught to focus on pleasant imagery to aid their relaxation (Rosenstiel & Keefe, 1983). Finally, distraction techniques emphasize focusing on physical and/or auditory stimuli.

C. Controlling Pain by Changing Activity Patterns. Activity-rest cycling and pleasant activity scheduling are used to help patients reduce their pain and more effectively increase their activity level (Houpt, Keefe, & Snipes, 1984; Keefe, 1982). Activity-rest cycling involves patients identifying activities that cause them to overexert themselves and

learning to break them up with periods of rest. Patients also identify pleasant activities they enjoy and set and record weekly activity goals.

Details of Attentional Control Condition

Participants randomized to the control group receive 11 weekly mailings of educational materials with information on the nature of pain. This includes pain physiology and methods of maintaining a healthy lifestyle, emphasizing the importance of physical activity and of weight control. However, the mailings do not specifically address the coping strategies taught during CBT, nor do they provide feedback. For ethical reasons patients randomized to the control group may enroll in CBT after completing the educational program free of charge to ensure all participants are given adequate clinical treatment regardless of randomization. This program has been described previously (Naylor, Helzer, Naud, & Keefe, 2002; Naylor, Keefe, Brigidi, Naud, & Helzer, 2008).

Genetic Analysis

Participants provided saliva samples from which their DNA was extracted following typical guanidine hydrochloride extraction protocol with DNA STAT-60TM, amplified according to standard polymerase chain reaction (PCR) protocol, and sequenced in order to determine each participant's genotype at the rs734784 KCNS1 allele. The extraction involves homogenization of the DNA followed by phase separation, precipitate formation, and removal of contaminants. The yield and purity of all samples are then tested using a NanoDropTM spectrometer, which measures nucleic acid concentrations. PCR amplification serves to replicate the DNA so that it can be sequenced, and involves separating the DNA strands with

heat and adding specific primers that bind to opposite strands, allowing for synthesis and replication of the desired allele. Finally, the Qiagen PyroMark Q24 pyrosequencer used for sequencing is highly susceptible to detecting SNPs and is therefore appropriate for this study.

After three months of intervention with either CBT or educational materials, questionnaires were compared to evaluate patient levels of physical pain, psychological suffering, and ability to cope with these pain symptoms. These data were used in order to identify any significant differences within and between intervention groups according to variations in KCNS1 genotype.

2.4 Statistical Analysis

This analysis used data from patients involved in a chronic musculoskeletal pain study who had completed at least one baseline assessment. A regression analysis to control for age and sex as covariates was conducted to characterize the baseline sample, and their interactions with KCNS1 genotype were not found to be significant. After removing any participants for missing data, the population means for each of the six clinical measures were determined. Any scores greater than three standard deviations from the mean were determined to be outliers and excluded from the analysis. Independent F-statistics were calculated to determine variance differences between measures of interest and corresponding T-tests were run to analyze within and between group differences in six clinical measures for each of the three genotypes and the two intervention groups. Given the exploratory nature of this study the significance level ($\alpha=0.05$) was retained.

2. Results

3.1 Baseline Differences in Clinical Measures by Genotype

A total of 201 participants completed the study and all results are summarized in Table 2. Of the six clinical measures examined, three were found to have significant interactions dependent on KCNS1 genotype, and seem to suggest that the valine risk allele has opposite effects in patients with chronic musculoskeletal pain compared to those with chronic neuropathic pain. Participants' McGill Typical Pain levels were significantly lower in homogeneous Val/Val individuals than for those with Ile/Ile, but this difference did not maintain significance when comparing Val/Val to heterogeneous Ile/Val patients. This suggests that just one copy of the mutated gene is not sufficient to alter an individual's musculoskeletal pain experience. Similarly, Val/Val homogeneous individuals scored significantly higher than Ile/Ile patients. Finally, the valine allele was associated with much lower pain catastrophizing scores when comparing homogeneous Val/Val to Ile/Ile and to heterogeneous Ile/Val subjects. These effects were additive, with even just one copy of the valine allele significantly reducing an individual's frequency of catastrophizing. The three additional clinical measures did not show any statistically significant differences regardless of genotype. All of these findings have been reported previously (French, Bishop, Lieberman, Atkinson, May, & Naylor, 2014 Submitted to Pain)

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3.2 Treatment Response by Genotype

A total of 193 participants also completed a second time point and were included in the post-treatment analysis. All results are summarized in Tables 3 and 4 and in Figures 2 and 3. All individuals improved to the same level of physical and mental functioning after either

treatment and regardless of genotype across all six clinical measures, suggesting both treatments were successful in alleviating pain-related symptoms. However, it is important to note that because Val/Val participants already had greater levels of physical and psychological functioning at baseline, they required a lower degree of improvement compared to Val/Ile and Ile/Ile individuals to attain the same level of functioning after treatment.

Both Val/Ile and Ile/Ile participants showed statistically significant improvements across all six clinical measures, whereas Val/Val participants did not have statistically significant improvements after treatment in their mental or physical composite scores. Furthermore, both Val/Ile and Ile/Ile individuals improved to the same level of functioning as Val/Val individuals. This means that both treatments, either psychotherapy with CBT or mailings of educational materials, are effective in reducing symptoms related to chronic musculoskeletal pain regardless of KCNS1 genotype, although CBT tended to result in greater improvements in functioning

Additionally, heterozygous Val/Ile and homozygous Ile/Ile individuals responded differentially to each intervention, with CBT treatment resulting in greater improvements than the EDU condition. Individuals with Val/Ile KCNS1 genotype improved significantly more from CBT than from EDU in Total Pain Experience, PCS, and Catastrophizing scores. This is in contrast to Ile/Ile individuals, who improved significantly more from CBT than from EDU in MCS, BDI, and Catastrophizing scores. It is interesting that Ile/Ile participants had the greatest improvements regarding their psychological symptoms whereas Val/Ile individuals had larger improvements in their physical functioning, and the effects of these genotypes on chronic musculoskeletal pain should be investigated further to elucidate the exact nature of this relationship.

Within the CBT intervention, Val/Val participants had significantly greater improvements in depression levels when compared to heterozygous Val/Ile individuals. Conversely, within the EDU intervention Val/Ile participants had significantly greater improvements in both BDI and MCS compared to Ile/Ile individuals. Also, it appears as if participants with Ile/Ile and Val/Ile KCNS1 genotypes may actually be more susceptible to responding to treatment than those homozygous for the Val mutation. It is interesting that there were no significant differences between treatments for Val/Val participants across any of the clinical measures, suggesting these individuals actually improved less from either treatment than those with Val/Ile and Ile/Ile KCNS1 genotypes. Although this may seem counterintuitive, it could be explained given that Val/Val individuals started out with higher physical and mental functioning, and therefore had less room for improvement.

Table 2. Baseline Differences in Clinical Factors by Genotype

Genotype [mean (n)]	BDI	McGill Typical	SF-36 PCS	SF-36 MCS	TOPS TPE	Catastro-phizing
Val/Val	16.0 (n=41)	5.9 (n=42)	34.7 (n=42)	42.3 (n=42)	54.1 (n=42)	48.6 (n=41)
p-value vs. Val/Ile	0.56	0.78	0.67	0.14	0.47	0.03
p-value vs. Ile/Ile	0.37	0.02	0.63	0.04	0.30	0.01
Val/Ile	16.7 (n=73)	6.0 (n=76)	34.3 (n=76)	39.0 (n=76)	55.7 (n=76)	52.7 (n=76)
Ile/Ile	18.5 (n=82)	6.6 (n=83)	35.3 (n=83)	37.7 (n=83)	57.5 (n=83)	53.7 (n=83)

Table 3. Treatment Response Differences in Clinical Measures by Genotype

Genotype [mean (n)]	BDI	McGill Typical	SF-36 PCS	SF-36 MCS	TOPS TPE	Catastro-phizing
Val/Val	9.7 (n=28)	5.3 (n=31)	32.9 (n=26)	46.2 (n=31)	44.4 (n=28)	6.0 (n=32)
p-value vs. Val/Ile	0.18	0.25	0.22	0.48	0.22	0.39
p-value vs. Ile/Ile	0.30	0.44	0.10	0.21	0.10	0.43
Val/Ile	11.1 (n=69)	5.0 (n=74)	32.8 (n=62)	46.1 (n=66)	46.9 (n=61)	5.7 (n=71)
Ile/Ile	10.5 (n=76)	5.2 (n=88)	33.7 (n=73)	44.1 (n=76)	48.6 (n=70)	6.1 (n=87)

Figure 2. Response to Treatment with EDU

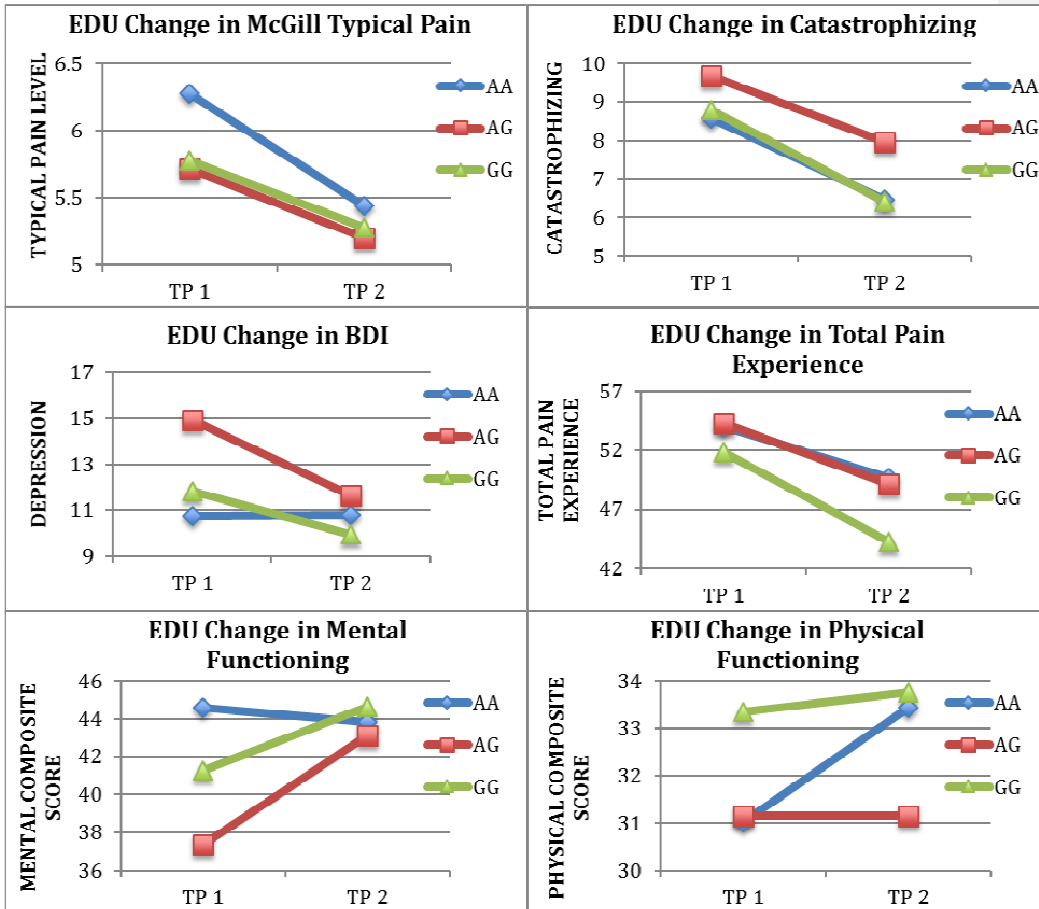


Figure 2. Change in Clinical Measures after Treatment with EDU. All participant data for each genotype was calculated at baseline (TP 1) and after treatment (TP 2). Independent sample T-tests were used to compare data between genotypes within each clinical measure at TP 1 and TP 2. Independent sample T-tests were run comparing clinical data scores within each genotype to determine which group did or did not improve significantly. GG = Val/Val; AG = Val/Ile; AA = Ile/Ile

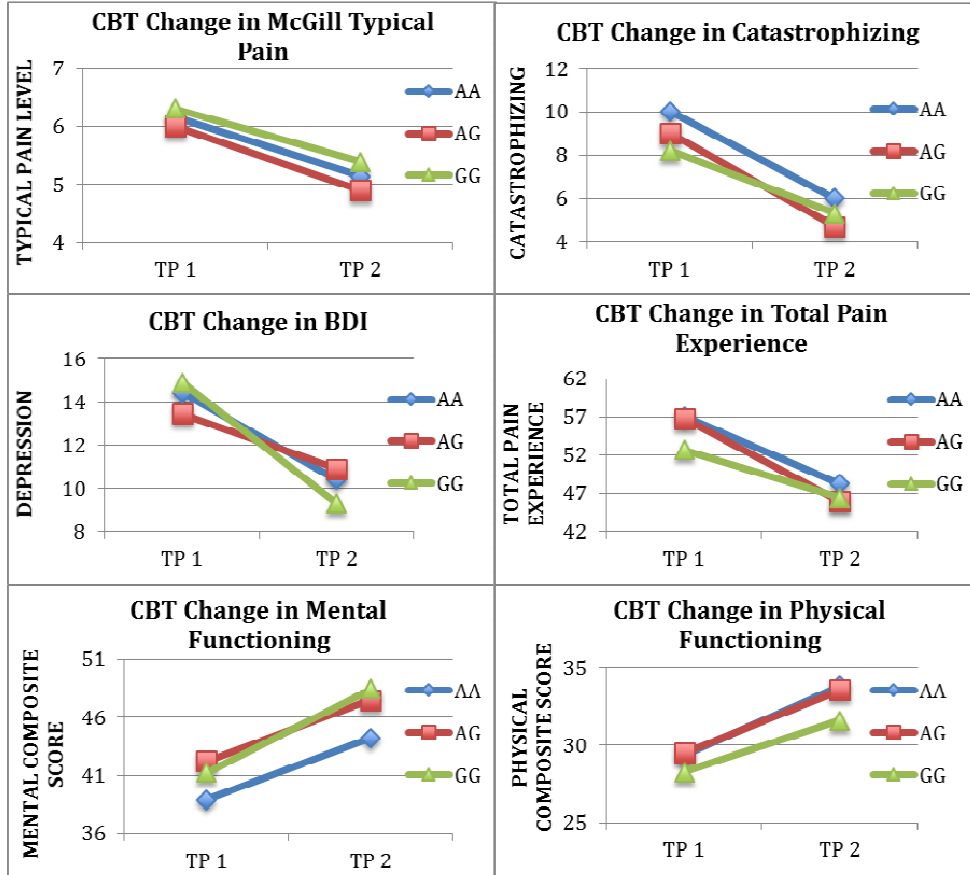


Figure 3. Response to Treatment with CBT

Figure 3. Change in Clinical Measures after Treatment with CBT. All participant data for each genotype was calculated at baseline (TP 1) and after treatment (TP 2). Independent sample T-tests were used to compare data between genotypes within each clinical measure at TP 1 and TP 2. Independent sample T-tests were run comparing clinical data scores within each genotype to determine which group did or did not improve significantly. GG = Val/Val; AG = Val/Ile; AA = Ile/Ile

Table 4. Change in Clinical Measures within Genotype Across Interventions

Genotype (CBT vs. EDU)	TPE	PCS	MCS	CATA	McGILL	BDI
Ile/Ile						
CBT	-8.9	4.5	5.3	-4.1	-1.0	-4.1
EDU	-4.4 (p=0.07)	2.4 (p=0.11)	-0.8 (p= 0.02)	-2.1 (p= 0.04)	-0.8 (p=0.36)	0.05 (p= 0.01)
Val/Ile						
CBT	-10.9	4.1	5.3	-4.3	-1.1	-2.6
EDU	-5.1 (p< 0.01)	-0.02 (p< 0.01)	5.8 (p=0.43)	-1.7 (p= 0.01)	-5.2 (p=0.09)	-3.3 (p=0.29)
Val/Val						
CBT	-6.3	3.4	7.2	-2.4	-0.9	-5.6
EDU	-8.8 (p=0.29)	0.42 (p=0.15)	3.4 (p=0.19)	-2.9 (p=0.40)	-0.5 (p=0.24)	-1.9 (p=0.07)

Table 4. Average change in clinical measure scores for each genotype within intervention groups were determined and independent sample T-tests were run to compare the change in clinical scores for each genotype across interventions.

3. Discussion

The present study investigated KCNS1 effects on the symptomology of chronic musculoskeletal pain and response to cognitive therapy. KCNS1 is a gene that encodes for a potassium channel alpha subunit that modulates potassium currents within sensory neurons, and thus is likely involved in pain signaling (Richardson & Kaczmarek, 2000). Our data suggest that the common SNP in the KCNS1 gene is associated with perception of pain and coping in patients with chronic musculoskeletal pain, but with converse effects to those demonstrated in chronic neuropathic pain (Costigan et al., 2010). Specifically, we demonstrated that while the valine-to-isoleucine mutation increases one's risk for experiencing greater chronic neuropathic pain, it actually seems to protect against chronic

musculoskeletal pain symptoms, and is associated with less physical pain symptoms and psychological suffering before treatment in these individuals. However, homogeneous Val/Val KCNS1 genotype was not associated with a greater response to treatment as was hypothesized. Rather, Val/Ile and Ile/Ile individuals showed the greatest treatment response, initially presenting with the greatest amounts of pain and suffering but reaching the same level of functioning as their Val/Val counterparts after treatment. Although this may speak more to the fact that Val/Val individuals had higher levels of functioning at baseline, and required a lower degree of improvement to reach the same functioning as other patients.

This study demonstrated that both CBT and EDU are effective at reducing pain-related physical and psychological symptoms in individuals with chronic musculoskeletal pain across all KCNS1 genotypes. After either treatment, all participants reached similar levels of physical and mental functioning regardless of KCNS1 genotypic variation. This is important to note, as it suggests psychotherapeutic approaches are a viable treatment option for those experiencing chronic musculoskeletal pain syndromes, and may reduce potential for long-term health consequences compared to prescription medications. It is of particular interest that Val/Ile and Ile/Ile participants showed different degrees of response to treatment associated with their KCNS1 genotypes. Specifically, heterozygous individuals appear to experience greater improvements related to their physical functioning, whereas homozygous Ile/Ile participants showed the greatest treatment response in regards to their psychological suffering. This may suggest wild-type heterozygous Val/Ile KCNS1 genotype, which has one copy of each allele, may optimize functioning overall. However, the present study is limited to speculation on this matter and future studies should investigate the effect of Val/Ile and Ile/Ile KCNS1 genotypes more fully.

These findings are important because while acute pain is adaptive and protective, chronic pain is analogous to a disease of the nervous system that results in significant dysfunction (Woolf, 2010; Young et al., 2012). As such, chronic pain disorders represent a huge public health concern with significant unmet need for safe and effective treatments. Given the potentially detrimental effects on quality of life for those experiencing chronic pain, gaining a better understanding of the underlying mechanisms is essential to provide more efficacious treatment. Although there remains much to learn, recent literature has illuminated key findings that may help to explain the shift from acute pain-related inflammation to chronic musculoskeletal pain.

In acute pain states, inflammatory responses serve to promote sensitivity of the damaged region so as to allow it to heal. However, chronic pain seems to hijack this system to elicit a perpetuating cycle of pain signaling. This prolonged response can cause long-term changes in dorsal horn neurons, which send pain signals to the brain, resulting in unresolved chronic pain (Woolf & Costigan, 1999). Specifically, following injury there is an initial inflammatory phase of rehabilitation that results in hypersensitivity of injured cells so as to promote their recovery (Woolf, 2010; Young et al., 2012). This hypersensitivity results in spontaneous action potentials and reorganization of synaptic connections in the spinal cord, which may lead to central sensitization that manifests as pain without a specific noxious stimulus (Woolf, 2010). The synaptic connections that communicate these pain signals can become long-term pathways, thereby shifting the experience from acute to chronic pain.

Initially, inflammation is characterized by inflammatory cells binding to receptors on peripheral nociceptor terminals (Ji et al., 2011). These chemical mediators induce hypersensitivity of the damaged region and increase the firing rates of peripheral nociceptors,

a phenomenon known as peripheral sensitization (Ji et al., 2011). Pain signals are then sent via two fiber types: large, heavily myelinated A-delta fibers that constitutively transcribe KCNS1 and produce the immediate sensation of pain, and small, unmyelinated C-fibers that do not express KCNS1 and produce the slow, second phase of pain (Tsantoulas et al., 2012; Woolf & Costigan, 1999). Both of these fibers project to second order dorsal horn neurons in the spinal cord, where they release the excitatory neurotransmitter glutamate and other neuromodulators (Li et al., 2014; Woolf & Costigan, 1999). Glutamate binds to AMPA and NMDA receptors on the dorsal horn to stimulate the release of calcium, which in turn activates protein kinases that phosphorylate AMPA and NMDA, causing up-regulation of these receptors and hyperexcitability of the membrane (Li et al., 2014). This is referred to as central sensitization, and serves to prolong the sensation of pain (Ji et al., 2011). If there is sustained activation of these pathways, excess glutamate release can cause excitotoxicity in the dorsal horn, resulting in the death of inhibitory neurons and causing potentiated responses of dorsal horn neurons, a phenomenon called windup (Woolf & Costigan, 1999).

Previous literature has shown that C-fiber activation can rapidly induce both central sensitization and windup to perpetuate the perception of pain (Woolf & Costigan, 1999). Pain signaling is further complicated by the fact that C-fiber activation can rapidly change the sensitivity of large, myelinated A-beta fibers that typically only transduce sensation rather than pain, causing A-beta fibers to produce painful sensations from non-painful stimuli (Woolf & Costigan, 1999). A-beta fibers can also sprout new axonal growths into regions of the spinal cord previously innervated only by pain-signaling C-fibers, resulting in the perception of pain from non-painful stimuli (Tsantoulas et al., 2012). The overall hyperexcitability of the pain signaling system results in substantial plasticity, including

changes in the channel protein transcription and gene expression that can become long-term changes in chronic pain syndromes (Woolf & Costigan, 1999).

All of these interactions are likely involved in chronic pain syndromes, although the exact nature of how chronic musculoskeletal pain differs from chronic neuropathic pain remains to be determined. The fact that the KCNS1 mutation works oppositely in chronic musculoskeletal pain compared to chronic neuropathic pain suggests that they occur through separate modalities that are modulated differently by the same potassium channel subunit. Specifically, one could postulate that if neuropathic pain is caused by long-term maladaptive sensitization of pain receptors, or nociceptors, musculoskeletal pain may conversely involve prolonged stimulation of nociceptors (Costigan et al., 2010; Woolf & Costigan, 1999). If this is the case, it may help explain why KCNS1 genotypic variation exerts different effects on chronic musculoskeletal pain than it does for chronic neuropathic pain. While Val/Val individuals tend to experience greater severity of pain and disability in chronic neuropathic pain, the same cannot be said for Val/Val individuals with chronic musculoskeletal pain. However, further research is necessary to examine the exact nature of this dynamic.

The potassium channel subunit KCNS1 encodes for serves to inhibit repeated firing of large, myelinated sensory neurons by maintaining the cell's resting membrane potential near the equilibrium potential for potassium (Everill & Kocsis, 1999). Previous literature suggests that injury-related hyperexcitability may be due to a reduction in potassium currents, which serve to repolarize the cell out of its refractory period so that it can fire another action potential (Everill & Kocsis, 1999). If KCNS1 is downregulated in chronic musculoskeletal pain disorders, as it is for chronic neuropathic pain, downregulation of this potassium channel allele may result in disinhibition because KCNS1 helps limit excessive neuronal firing.

However, it is currently unknown whether such downregulation also occurs in chronic musculoskeletal pain models and the exact effect of KCNS1 modulation depends on what other subunits are present in the channel and what other channels are present in the membrane (Richardson & Kaczmarek, 2000; Tsantoulas et al., 2012). Future research should shed some light on the nature of such mechanisms and explain the different effects seen in chronic neuropathic versus chronic musculoskeletal pain syndromes.

Clearly, chronic musculoskeletal pain is a complex disorder that involves multiple interacting factors. Although variation in KCNS1 genotype may modulate pain perception and/or experience, it is far from the only gene involved and likely has many complex interactions with other channel subunits, channels, and chemical mediators that produce specific effects in certain individuals. This is one of the main limitations of the present study, as examining a single gene encoding for a potassium channel alpha subunit is a definite oversimplification of the complexity involved in chronic pain syndromes. Future research should examine the effect of KCNS1 when coexpressed with other potassium channel subunits to determine the exact nature of its modulation of potassium currents. Additionally, this study failed to consider epigenetic factors that are likely involved in developing chronic pain disorders. It would be beneficial for future research to examine such interactions and shed light on how various lifestyle choices and environmental stimuli modulate pathology in chronic pain syndromes. These findings warrant future research on the role of potassium channels in the vast range of phenotypic variation associated with chronic pain disorders and how to most effectively treat them.

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