GENETIC INFLUENCES ON THE DYNAMICS OF PAIN AND AFFECT IN FIBROMYALGIA

by

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ABSTRACT

Fibromyalgia (FM) is a chronic musculoskeletal disorder characterized by widespread pain, fatigue, and a variety of other comorbid physiological and psychological characteristics, including a deficit of positive affect. Recently, the focus of research on the pathophysiology of FM has considered the role of a number of genomic variants. In the current manuscript, case-control analyses did not support the hypothesis that FM patients would differ from other chronic pain groups in catechol-O-methyltransferase (COMT) and mu-opioid receptor (OPRM1) genotype. However, evidence is provided in support of the hypothesis that functional single nucleotide polymorphisms on the COMT and OPRM1 genes would be associated with risk and resilience, respectively, in a dual processing model of pain-related positive affective regulation in FM. Forty-six female patients with a physician-confirmed diagnosis of FM completed an electronic diary that included once-daily assessments of positive affect and soft tissue pain. Multilevel modeling yielded a significant gene X environment interaction, such that individuals with met/met genotype on COMT experienced a greater decline in positive affect as daily pain increased than did either val/met or val/val individuals. A gene X environment interaction for OPRM1 also emerged, indicating that individuals with at least one asp allele were more resilient to elevations in daily pain than those homozygous for the asn allele. In sum, the findings offer researchers ample reason to further investigate the contribution of the catecholamine and opioid systems, and their associated genomic variants, to the still poorly understood experience of FM.

To my family, who has unflinchingly given me love, support, and trust in my decisions.

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Introduction

Fibromyalgia (FM) is a chronic musculoskeletal disorder characterized by widespread pain, fatigue, and a variety of other comorbid physiological and psychological conditions (Thieme, Turk, & Flor, 2004; Wolfe, Smyth, Yunus et al., 1990). Diagnosing FM is typically accomplished through the palpation of 18 tender points, of which 11 must be reported by the patient as painful to meet criteria for diagnosis. Due to the heterogeneity of symptom presentation, however, physicians often do not rely solely on musculoskeletal examination in making a diagnosis and structuring treatment, instead deferring to a broad-based assessment of comorbid symptoms and conditions, including mood disorders, sleep disturbance, fatigue, headache, and irritable bowel disorders (Katz, Wolfe, & Michaud, 2006). Recent research has identified a deficit in positive affect (PA) among FM patients, relative to other chronic pain groups, as another distinctive feature characteristic of the disorder (Zautra, Fasman, Reich et al., 2005). In the absence of PA, FM patients may be more vulnerable to the effects of stress and pain on negative affect (NA; Zautra, Johnson, & Davis, 2005).

FM affects approximately 6 million people in America, often causing long-lasting physical discomfort, reduced quality of life, and functional impairment (Bernard, Prince, & Edsall, 2000). The economic impact of the disorder is substantial; the physical disability that can accompany FM can result in direct patient health care costs of nearly $5,000 annually and a significant loss of work hours, and has been thought to contribute to the high rate of unemployment in this population (Penrod et al., 2004). Furthermore, the high rate of physician visits within the FM population creates a significant burden on the health care system. With an aging population, reduced pensions for retirees, and

increasing difficulty in finding adequate insurance coverage, the need for effective and efficient diagnostic and treatment options for FM patients has never been greater.

In the exploration of the etiopathology of FM, researchers have begun to focus on the role of genetics. Interindividual differences in pain sensitivity are often reported to be substantially greater than intraindividual differences, and so a genetic approach provides an opportunity to target biologically latent sources of that variation (Mogil, 1999). Further, evidence for a strong familial aggregation of FM has led researchers to similarly conclude that genetic influences may account for a significant proportion of variance in the population. As such, the focus of research on FM has shifted to a variety of genomic variants (Buskila & Sarzi-Puttini, 2006; Limer, Nicholl, Thomson, & McBeth, 2008). Candidate gene association studies are popular because populations can be compared on the basis of the relative frequency of target alleles, and conclusions can be drawn about how behaviors observed between populations may relate to observed allelic differences. However, genetic associations with FM have been plagued by inconsistencies common to many candidate gene studies. For instance, some studies have reported an association of the serotonin transporter gene with FM (e.g. Cohen, Buskila, Neumann, & Ebstein, 2002), but other studies have failed to replicate those results (e.g. Gursoy, 2002). Both the nonspecificity in phenotypic associations in the candidate genes and the heterogeneity of symptom presentation in FM may be to blame for such inconsistencies.

The preponderance of candidate gene studies have chosen outcomes related to pain sensitivity, which is often an index of continuous pain self-report (i.e., the amount of pain one feels), pain threshold (i.e., the point at which a stimulus becomes painful), or pain

tolerance (i.e., the point at which a stimulus becomes intolerably painful). However, none have explored affect and affective reactivity to pain within this population. Two genes with functional polymorphisms have emerged as particularly attractive candidates for the study of the dynamic relations between pain and affect in FM: the catechol-O-methyltransferase gene (COMT/*val*^{158}*met*), with a variant conferring risk, and the μ-opioid receptor gene (OPRM1/*asn*^{40}*asp*), with a variant conferring resilience to the potentially harmful effects of chronic pain and NA for FM patients.

The objective of the current study is to gain an understanding of the genetic influences on FM through the examination of candidate genes hypothesized to impact both pain and affect. Part of the difficulty in establishing a consistent pattern of genetic effects on FM stems from the heterogeneity of the disorder. Pain sensitivity is a hallmark of the FM diagnosis, and most candidate gene studies within the FM population have isolated this symptom as the phenotype of interest (Buskila & Sarzi-Puttini, 2006). However, disturbed affective regulation in FM is evident by the high incidence of depression and anxiety in this population (Thieme, Turk, & Flor, 2004), as well as a deficit in PA relative to other chronic pain groups (Zautra, Fasman, Reich et al., 2005). The current study used a daily process design to measure individual reports of pain and affect each for 30 days, allowing for repeated measures of pain fluctuations, which strengthen both the reliability and precision of pain estimation. The exploration of genetic factors related to common symptoms of FM will enhance the current understanding of the diagnosis and treatment of the disorder.

Dynamic Model of Affect and FM

Emotion is thought to exist within a dimension known as *affective space* (Cacioppo, Gardner, & Bernston, 1999). There has been some controversy over the degree to which oppositely valenced affects are related within an affective space, and part of that controversy stems from the fundamentally distinct views of how the space is organized. Russell and Carroll (1999) gave voice to the notion that affect exists on a bipolar continuum whereby, heuristically speaking, a unit gain in one valence should be met with a unit loss in its opposite valence. One can imagine simply a straight line in which each pole represents the extreme high end of the range of PA and NA, respectively. Under this framework, as an individual's NA increases, his/her PA should decrease, thereby increasing the degree of correlation between the two affects in a negative direction.

Another conceptualization of affective space considers PA and NA as separate, bivariate dimensions, and specifically views stress as an integral variable in determining the shape of the plane that unifies the two affects (Davis, Zautra, & Smith, 2004). This integrative view, known as the Dynamic Model of Affect (Zautra, Smith, Affleck, & Tennen, 2001), holds that it is possible, if not common, to experience affective independence rather than affective correlation when stress is low or absent. In contrast, however, when stress is high, people have greater difficulty differentiating between the two affects. To visualize an affective space that allows for affective differentiation, one can imagine a two dimensional space with two perpendicular lines intersecting at a single point. One axis represents PA and the other NA. As an individual's NA increases in this

model, his/her PA may or may not change, reflecting a degree of independence between the two affects. Indeed, recent data (Ong, Bergeman, Bisconti, & Wallace, 2006) collected through daily process models to capture affective relations in naturalistic settings suggests that negative and positive affect are often independent dimensions in the absence of stress. Stress, then, creates a third dimension in the affective space and serves to contort its shape, causing the space to shrink and affect ratings to fall to opposite poles of the affective distribution.

Although substantial evidence has been offered for both the bivariate and bipolar models of affect (Reich, Zautra, & Davis, 2003), the integrative perspective of the Dynamic Model of Affect perhaps comes closest to modeling what happens in daily life when emotions are influenced by both major stressors and minor hassles. A series of studies from members of our research group help elucidate why stress is so influential to the experience and expression of emotion. Zautra, Berkhof, and Nicolson (2002) tested the hypothesis that stress would narrow the affective space between PA and NA in a sample of healthy workers in the Netherlands. Utilizing the experience sampling method, a daily process design that randomly alerted people to obtain affect and event ratings ten times per day for five consecutive days, Zautra and colleagues discovered that within-person estimates of the correlation between PA and NA were higher during moments when a stressful event was reported than during non-stress moments. Hierarchical linear modeling techniques generated within-person estimates that were obtained from repeated measurements and ruled out the possible confound of individual differences accounting for the changed relation of PA and NA during times of stress. Additionally, a

contingency analysis indicated that most individuals had a higher proportion of inverse PA-NA correlations under stressful than nonstressful moments, rebuffing earlier suggestions that changes in mean levels and variance of PA and NA under stress may confound interpretation of the change in the degree of relation between the two affects.

Why would we expect stress to impact the PA-NA relation? Stress has been shown to increase uncertainty, which in turn puts attentional demands on the information processing system (Ursin & Olff, 1993). When one's information processing abilities are taxed, affective processing becomes limited and, consequently, PA and NA become more inversely correlated (Linville, 1985). During times of acute stress, this is an adaptive solution; the body must recruit energy to escape the most pertinent threat it faces. Thus, our affective complexity diminishes in order to minimize energy expenditure, escape threat, and regain homeostatic balance. Uncertainty facilitates this process by motivating the individual to cognitively attend to the emotion most closely tied to a stressor: NA. Under conditions of uncertainty, the individual must work considerably harder to maintain PA, and so we would expect it to diminish during these times.

Reich, Johnson, Zautra, & Davis (2006) reported compelling evidence of the effect of illness uncertainty on the emotional experience in a population of FM patients. These patients, who have been shown to report considerably less control over their illness than other chronic pain groups (Smith, Christensen, Peck, & Ward, 1994), were asked to give ratings of PA, NA, perceived interpersonal stress, and illness uncertainty in a weekly diary format over a 12-week span. Across measurements, increases in perceived stress predicted increases in NA and decreases in PA. When illness uncertainty was included in

the model, it interacted with stress to predict PA, such that individuals high in uncertainty experienced a greater decrease in PA during stressful weeks than individuals low in uncertainty.

The examination of affect in the context of chronic pain provides an excellent test of the DMA, as the neural pathways for pain and affect share common processing components (Price, 2000). Additionally, chronic pain can serve as an analog to chronic stress because of the severe physical and functional limitations it presents to those affected (Davis et al., 2004). Following from the notion that pain and affect exhibit a considerable degree of neural overlap, NA is expected to be especially high during painful periods. Indeed, longitudinal research has shown that, during especially stressful weeks, rheumatoid arthritis patients rated NA higher and PA lower when pain was elevated (Potter, Zautra, & Reich, 2000). For these patients, pain is typically characterized by sudden and unexpected flares that can leave the patient in a state of functional impairment. Similarly, pain perceived as uncontrollable in FM patients is associated with a shrinkage of affective space, as patients must divert resources typically used for complex affective processing and instead employ them for other homeostatic regulatory purposes. As a result, PA should be lower and NA higher during episodes of elevated pain (Davis et al., 2004).

In line with predictions offered by the Dynamic Model of Affect, Zautra, Smith, Affleck, and Tennen (2001) found that, during painful weeks, reduced levels of PA predicted heightened levels of NA in rheumatoid arthritis patients. Importantly, these findings proved to be robust to alternate methodology in a replication involving FM

patients and daily, instead of weekly, ratings of PA, NA, and pain (Zautra et al., 2001). Recent findings, currently under review, suggest that elevations in pain were related to increases in NA and decreases in PA to a greater extent in FM patients than in a control chronic pain group of osteoarthritis (OA) patients (Finan, Zautra, & Davis, In Press).

There is a great need for research on affective regulation among FM patients for several reasons. Their pain is chronic and widespread, and induces greater uncertainty than that which is experienced by other chronic pain groups with more targeted and predictable pain (Smith et al., 1994). As a result of greater uncertainty, one can expect more stress and greater bipolarity between PA and NA during times of stress. Indeed, the literature shows that stress significantly contributes to the etiopathology of FM. Over 50% of FM patients also meet criteria for post-traumatic stress disorder, compared with 6% of the general population (Sherman, Turk, & Okifuji, 2000; Staud, 2004). Furthermore, the chronicity of pain in FM may sufficiently tax the body so that the hypothalamo-pituitary-adrenal axis response to even minor elevations in stress and pain becomes hypersensitized (Blackburn-Munro & Blackburn-Munro, 2001; Okifuji & Turk, 2002). Finally, the high comorbidity of depression and anxiety in FM suggests a common source of affective disturbance in this group.

Zautra, Fasman, Reich et al. (2005) explored stress, pain, and affect reports recorded weekly over a period of 12 weeks by FM and OA patients. Across measurements, FM patients reported less PA, but similar levels of NA, compared with OA patients. Stressful weeks were associated with even lower PA reports among FM patients, indicating a deficit in PA regulation for this group. Thus, FM patients are

operating at an affective disadvantage when they encounter periods of elevated stress and pain. Not only do they experience a deficit in PA at baseline, their PA diminishes more than would be expected when stress and pain arise. Importantly, this effect was replicated with a different sample of FM and OA patients using daily, instead of weekly, diary methodology (Finan et al., In Press).

Although pain is typically associated with a decline in PA, some evidence suggests that those FM patients capable of maintaining PA during pain episodes may be protected from the wide-ranging harm pain incurs on well-being (Zautra, Johnson, and Davis 2005). In the Zautra et al. (2005) study, FM and OA patients were again assessed in a weekly diary format for 12 weeks. For all patients, higher average levels of PA were associated with lower levels of NA when either pain or interpersonal stress was elevated. Additionally, during weeks when pain was elevated, higher PA buffered against elevations in NA. The data suggest, then, that FM patients are capable of deriving benefits from PA. As an extension, it may be interpreted that the difficulties in affective regulation that are evident in FM result, at least partly, from a deficit in PA and a failure to sustain available PA throughout periods of elevated pain, stress, and NA (Zautra et al., 2005).

Despite the recent identification of a PA deficit in FM, and several replications of those findings, there is currently a gap in the knowledge of what mechanisms may predispose FM patients to experience affective dysregulation, and how that disturbance may perpetuate and prolong the experience of chronic pain. Affective responses to stress and pain characterized by low PA and high NA are salient phenotypes that describe a

common affective profile found in FM. As such, their development over time may be determined by specific and identifiable genetic factors. Two commonly studied genes, COMT and OPRM1, have emerged as sound candidates for the study of pain, and have additionally been implicated in emotional processing independent of pain states. Several studies reviewed below indicate that a common variant in COMT is overrepresented in FM and may contribute to dysfunctional responding to affective and painful stimuli. Additionally, there is some indication that a polymorphism of OPRM1 may contribute to a lessening of pain and an enhancement of PA in FM. Building off Zautra et al's (2005) finding that PA may be a resilient resource for FM patients, it is important to seek out genetic factors that may influence that resource. To help understand the role of these genes in affective regulation for FM patients, their neurobiological functions will now be explained.

Catecholamines and the COMT Gene

Catecholaminergic Neurotransmission. A substantial body of literature suggests a role for epinephrine, norepinephrine, and dopamine in the pathophysiology of FM. All three catecholamines are intricately involved in the neural processing of affective, stressful, and painful stimuli. The catecholamines take part in an exceedingly complex network of information processing that may, depending upon environmental conditions, lead to analgesia, hyperalgesia, euphoria, or negative mood. The following section will briefly review the mechanisms through which the catecholamines have been proposed to influence the neural processing of both pain and affect.

Both epinephrine and norepinephrine are powerful stimulators of sympathetic nervous system activity, causing increased heart rate and vasodilation, among other metabolic effects in response to stress (Lundberg, 1999). They arise in the adrenal medulla and are released as hormones recruited for the hypothalamic-pituitary-adrenal (HPA) axis response to stress. In addition, both are neurotransmitters released by adrenal neurons that govern the behavioral drive toward fight or flight. With regard to pain, a role for a dysregulated sympatho-adrenal-medullary response in nociception and, more specifically, FM has been described (Petzke & Clauw, 2000).

Stress-induced analgesia is thought to occur as a result of a descending pathway whereby norepinephrine exerts inhibitory influence over the pronocieptive peptide known as substance P (Kuraishi, Hirota, Sato, 1985). FM patients have been shown to have increased levels of substance P in the cerebrospinal fluid, suggesting noradrenergic dysfunction in these patients (Russell et al., 1994). In an experimental test of pain threshold in FM patients compared with controls, Kosek & Hansson (1997) found evidence for a failure in descending noxious inhibitory control in response to ischemic pain stimulus, a process typically moderated by norepinephrine.

In addition to its influence in facilitating stress-induced analgesia, norepinephrine has been implicated in hyperalgesia and the maintenance of chronic pain. Norepinephrine delays the release of adrenocorticotropic hormone in FM patients, and this may contribute to their inability to regulate stress and pain states (Wallace et al., 2001). Additionally, norepinephrine injection produced painful states in 80% of FM patients compared to 30% of rheumatoid arthritis patients and 30% of control subjects

(Martinez-Lavin et al., 2002). It is unclear, however, whether norepinephrine is overexpressed or underexpressed in FM. Torpy, et al. (2000) found basal plasma norepinephrine levels to be elevated in FM patients. In contrast, another study found norepinephrine metabolites to be underrepresented in FM patients compared to healthy people (Russell et al., 1994). Petzke & Clauw (2000) highlight the difficulty in the interpretation of data involving plasma norepinephrine levels due to variable rates of release and reuptake in the sympathetic nerve terminals.

There is also evidence that epinephrine-mediated sympathetic dysregulation contributes to the maintenance of chronic pain states. Injection of epinephrine produces hyperalgesia to nociceptive stimulation in mouse (Khasar, Miao, Gear et al., 2002) and human (Chen & Levine, 2005; Choi & Rowbotham, 1997) models. The hyperalgesic effect of epinephrine is thought to occur through the activation of β-2 adrenergic receptors on primary afferent nociceptors sent from the periphery to the spinal cord (Khasar, Green, Miao, & Levine, 2003). Further, peripheral epinephrine has also been associated with an increase in anxiety in the context of hyperalgesia to pain stimuli (Janssen, Arntz, & Bouts, 1998). Among FM patients, a reduction in the epinephrine response to hypoglycemia is associated with greater impairment and may indicate a generally deficient autonomic nervous system response to stress (Adler & Geenen, 2005; Adler, Kinsley, Hurwitz Mossey, & Goldenberg, 1999).

Autonomic arousal has long been thought to influence emotional arousal (Schachter & Singer, 1962). During emotional arousal, norepinephrine is released in the locus coeruleus and projects to an abundance of brain regions, including the hippocampus

and amygdala (Southwick, Bremner, Rasmusson et al., 1999). The primary role of the locus coeruleus in its release of norepinephrine is to attend the organism to environmental stimuli that require an enhanced level of vigilance. Threatening situations that evoke fear result in an adaptive efflux of norepinephrine in multiple brain regions (Southwick et al., 1999). Repeated exposure to fear-eliciting stimuli, however, can have harmful consequences that are mediated by norepinephrine. Individuals with post-traumatic stress disorder (PTSD) display exaggerated levels of sympathetic arousal to trauma-related cues (Orr, 1997). This effect is supported by evidence that plasma norepinephrine is elevated among combat veterans with PTSD compared to those without (Yehuda , Siever, & Teicher, 1998). Emotion-evoked norepinephrine projections to the hippocampus may enhance the salience of emotional memory formation, which, consequently, may serve as a precursor to PTSD for those exposed to trauma (Hu, Real, Takamiya et al., 2007). As PTSD is a common psychiatric comorbidity with FM, it is possible that emotion-related norepinephrine dysregulation may contribute to the pathophysiology of the disorder.

There is evidence indicating that epinephrine may also influence emotion through its effect on physiological arousal. Mezzakappa, Katkin, & Palmer (1999) injected volunteers with either epinephrine or saline and then showed them short film clips intended to evoke fear, anger, or amusement. Subjects were continuously monitored for cardiac arousal as well as electrodermal arousal and, following the viewing of the films, were assessed for current emotional state. Fear-related emotion was rated as most intense in the epinephrine group. This, coupled with the finding that sympathetic arousal was greatest in the epinephrine group during the fear-related films, may suggest that

epinephrine, via its effect on physiological arousal to negative cues, elicits a feeling best described as a negative mood state (Marshall & Zimbardo, 1979; Mezzakappa et al., 1999). For FM patients, who may already suffer from a PA deficit, elevated epinephrine levels could create a vulnerability to heightened NA, a malady from which these patients may have little respite.

There is a need for research to address questions of the influence of norepinephrine and epinephrine in fibromyalgia, and particularly in the patient's response to stress and pain. There are data attesting to both hyperalgesic and analgesic effects of these catecholamines; however, studies that simply assess their relative presence or absence in FM patients fall short of explaining how they may contribute to the maintenance of chronic pain in this population. The examination of COMT in the daily response to stress and pain among FM patients could provide some valuable insight into the role of epinephrine and norepinephrine in this patient population.

Dopamine is another catecholamine with distinct influences on pain and affective processing. The mesolimbic dopaminergic pathway is one of the primary neural pathways for the neurotransmitter dopamine. Dopamine is synthesized in the substantia nigra and the ventral tegmentum and projects to the nucleus accumbens, as well as other areas in the limbic system, such as the anterior cingulate cortex and the anterior insula. Typically, dopamine has been implicated in the reward circuitry of substance abusers (Robinson & Berridge, 2003), such that dopaminergic neurons are thought to mediate the appraisal and interpretation of reward and uncertainty from environmental stimuli (Fiorillo, Tobler, & Schulz, 2003; Tobler, Fiorillo, & Schultz, 2005). Painful stimuli

have also been shown to evoke a dopaminergic response in the mesolimbic dopaminergic pathway. Gear, Aley, & Levine (1999) demonstrated this effect with rodents. Exposure to two different forms of noxious stimuli (i.e., injection of capaicin and paw immersion) resulted in an analgesic response among mice being electrically stimulated, such that the jaw opening reflex produced by the electrical stimulation was attenuated for mice that had received noxious stimuli compared to mice that only received electrical stimulation. Further, the antinociceptive effect of the noxious stimuli was eliminated for mice injected in the nucleus accumbens with a dopamine antagonist. Other research has shown that substance P activates dopaminergic neurons in the ventral tegmental area, producing an analgesic response to the formalin test in rodents (Altier & Stewart, 1999). Additionally, lesions to the ventral tegmental area lead to increased sensitivity to pain, while stimulation to the same area produced analgesia (Sotres-Bayon, Torres-Lopez, Lopez-Avila, del Angel, & Pellicer, 2001). Thus, the literature provides strong support for the role of dopamine in the regulation of pain states.

A tonic/phasic model of mesolimbic dopaminergic activity (Grace, 1991; Wood, 2006) has been proposed to explain the intercellular synaptic transmission of dopamine during pain. The following summarizes Wood's (2006) model. The phasic dopaminergic response occurs rapidly and transiently as a result of acute environmental stimuli, and is terminated through reuptake by the dopamine transporter. Phasic dopaminergic firing, then, is most active in response to threats to homeostasis like pain and psychological stress. Tonic dopaminergic activity is relatively constant in the extracellular space and is regulated by the COMT enzyme; COMT degrades tonic, free-floating dopamine, but not

phasic dopamine following burst firing. Tonic dopaminergic binding typically occurs at the high-affinity D2 receptors expressed on dopaminergic neurons. When tonically activated dopamine binds to dopaminergic neurons, phasic firing becomes inhibited. Thus, high tonic dopamine concentrations in the extracellular space diminish phasic activity. Chronic stress can result in a reduction in phasic activity through a variety of pathways, including hippocampal activation, which increase tonic expression in the nucleus accumbens and ultimately result in hyperalgesia.

Mesolimbic dopaminergic activity has also been shown to mediate the affective response to pain. However, the findings appear counterintuitive with respect to its known action as a neurotransmitter of reward. Scott, Heitzeg, Koeppe, Stohler, & Zubieta (2006) examined the human dopaminergic response to prolonged painful stimuli (i.e., continuous injection of saline solution into the jaw) through the use of positron emission tomography (PET). A radioactive tracer that binds with dopaminergic D2 and D3 receptors in the dorsal and ventral basal ganglia was imaged throughout the pain administration. Mesolimbic ventral D2 and D3 receptor activity correlated with increased negative affect throughout the pain stimulus, as well as with the sensory and affective ratings of the pain itself. Interestingly, dorsal caudate and putamen activation of dopamine receptors were only associated with the sensory and affective ratings of the pain, and not with the individual's general affective state. The authors concluded that mesolimbic dopaminergic neurotransmission extends beyond the appraisal of reward to include appraisal of emotionally salient stimuli of any valence. Indeed, a similar study by Pruessner, Champagne, Meaney, & Dagher (2004), reported an increase in dopaminergic

D2 and D3 activity in the ventral striatum during a psychological stress challenge. These data suggest that neural pathways for emotion may differ as a function of environmental demands like pain and stress. Although dopamine has traditionally been thought to amplify the positive affective experience of reward, its function is clearly not limited in that capacity.

Genetic Effects of COMT on Pain and Affect. The COMT gene codes for an enzyme that facilitates the degradation of the catecholamine neurotransmitters epinephrine, norepinephrine, and dopamine (Li, Warsh, & Godse, 1984). A common functional single nucleotide polymorphism (SNP) of COMT (rs 4680) contains a single nucleotide change of guanine to adenine, which results in an amino acid substitution of methionine for valine at codon 158. Individuals homozygous for the val^{158}allele of the $val^{158}met$ polymorphism have a three to four-fold increase in enzymatic activity compared to those homozygous for ^{158}met as a result of changes in thermostability of the enzyme (Spielman & Weinshilboum, 1981). The heterozygous genotype (*val/met*) produces intermediate enzymatic activity, indicating that the alleles are codominant. Lower COMT enzymatic activity results in a higher than normal level of catecholamines, while higher enzymatic activity results in a relative dearth of catecholamines. As reviewed above, alterations in the presence of these neurotransmitters may have profound effects on the systems on which they operate. Thus, the $val^{158}met$ polymorphism, through its ability to alter the circulating levels of catecholamines, may be associated with functional changes in neurological systems as well as the behaviors those systems influence.

Allelic differences in the frequency of the *val*^{158}*met* polymorphism have been reported in multiple samples. Gursoy et al. (2003) were the first to report a higher incidence in the ^{158}*met* variant in FM patients compared to controls. In their study, the met/met, val/met, and val/val genotypes were represented in 19.7%, 54.1%, and 26.2% of patients, respectively, and 16.4%, 36.1%, and 47.5% of healthy controls, respectively. The ^{158}*met* allele, alone, was not differentially represented in the FM patients over controls. Taken together, however, the met/met and val/met genotypes were overrepresented in patients compared to controls. Both Garcia-Fructoso, Lao-Villadoniga, Beyer, & Santos (2006) and Vargas-Alarcon et al. (2007) reported similar differences in allelic frequencies for FM patients compared to controls in separate samples of Spaniards. Interestingly, however, Vargas-Alarcon et al. found no COMT polymorphic differences between FM patients and controls in a population of Mexicans. These data support a previous finding indicating that the met/met and val/met genotypes conferred risk for migraine, while the val/val genotype protected against the development of migraine (Erdal et al., 2001). Similarly, Hagen, Petterson, Stovner, Skorpen, & Zwert (2006) reported an association between the *val*^{158}*met* polymorphism and headache. Migraine is highly comorbid with FM (Peres et al., 2001), and so these findings may be generalizable to the FM population.

The *val*^{158}*met* polymorphism has also been shown to contribute to increased pain sensitivity to experimental noxious stimuli in a sample of healthy women, although this effect was smaller than that observed for other highly related SNPs on COMT (Diatchenko et al., 2005). Within FM, the met/met genotype is associated with greater

illness severity, as determined by the Fibromyalgia Impact Questionnaire, which assesses function, pain level, fatigue, sleep disturbance, and psychological distress (Garcia-Fructoso et al., 2006). Thus, the genetic influence of polymorphisms of COMT may reach a wide range of phenotypic characteristics of FM that depend, at least partially, on catecholaminergic neurotransmission.

The affective domain is of particular interest in the clinical portfolio of FM patients, and, as detailed above, catecholamines play a regulatory role when affective stimuli are processed. Indeed, there is some evidence to support a role for the *val*^{158}*met* polymorphism in affect regulation (Smolka et al., 2005). Healthy volunteers were presented with pleasant and unpleasant images during functional magnetic resonance imaging (fMRI). A gene-dose effect of the ^{158}*met* variant was found on blood oxygen level-dependent response to the affective stimuli such that increased presence of the ^{158}*met* variant resulted in increased reactivity to aversive stimuli. fMRI images showed activation in the limbic system, as well as the prefrontal cortex and the visuospatial attention system. Interestingly, no genetic effects were observed for the response to pleasant stimuli. These data support evidence from Zubieta et al. (2003) that the met/met genotype was associated with a negative affective, but not positive affective, response to a sustained pain stimulus.

The ^{158}*met* allele has also been associated with higher levels of anxiety (Enoch, Xu, Ferro, Harris, & Goldman, 2003) and depression (Ohara et al., 2006). However, recent evidence that the ^{158}*met* allele was associated with increased PA in a sample of healthy participants (Wichers, Aguilera et al., 2007) appears to contradict some of the data on the

COMT gene's role in the regulation of mood. Subjects were assessed daily through a diary format on the experience of positive events, as well as their appraisal of those events. As the number of ^{158}*met* alleles increased within the individual, so too did one's ability to experience reward from positive events. Additionally, daily PA was positive correlated with incidence of the ^{158}*met* allele. How can these data be resolved with prior evidence that the ^{158}*met* allele may predispose somebody to anxiety, depression, and heightened negative affective arousal? At this point, the data are not mature enough to adequately answer this question. One obvious difference between the Wichers et al. study and those that have linked the ^{158}*met* allele to negative affective processing (e.g. Smolka et al., 2005) is the longitudinal nature of its design. Perhaps the ^{158}*met* effect of enhanced tonic dopaminergic activity does not inhibit the experience of reward in the flow of daily life. However, this would contradict Smolka et al.'s (2005) laboratory evidence reviewed above, as well as other imaging evidence that phasic dopamine release, which is inhibited by tonic activity, is positively correlated with reports of euphoria following administration of amphetamine (Drevets et al., 2001). The problem, of course, becomes even more complex when pain is a factor in the experience of emotion, as dopamine has been shown to promote analgesia (e.g., Altier & Stewart, 1999), but also negative affective appraisal of painful stimuli (e.g., Zubieta et al., 2003). More research is clearly necessary to distinguish between the seemingly disparate threads of evidence for the association of COMT and affective processing.

Some additional issues related to the mechanism of the *val*^{158}*met* polymorphism remain unclear. First, it is not known whether the *val*^{158}*met* polymorphism, alone, can

account for phenotypic traits associated with the etiopathogenesis of FM. For example, Diatchenko et al. (2005) mapped three separate haplotypes that, together, accounted for roughly 11% of the variability in pain perception, a substantial amount for a genetic effect. Haplotypes are groupings of alleles from SNPs that fall within a common range of loci on the gene and are often in high linkage disequilibrium with each other. The authors determined that the three haplotypes, each of which included an allele from the *val*^{158}*met* polymorphism, differed in the amount of pain sensitivity attributable to genetic influence. Both the haplotype which conferred the highest pain sensitivity and that which conferred the least pain sensitivity contained the G allele, which codes for the *val*^{158} variant, while the intermediate haplotype contained the A allele, which codes for the *met*^{158} variant. The implication of this finding is that the variant that has been previously associated with FM and pain severity does not confer the greatest vulnerability to pain when analyzed as part of a group of alleles from other closely related SNPs, suggesting that interactions of SNPs, rather than SNP variations alone, may account for between-person effects of COMT on pain sensitivity (Diatchenko et al., 2005). Thus, any exploration of the *val*^{158}*met* polymorphism independent of other SNPs in its genomic region should lead to cautious interpretations of the findings until replications can be reported.

Second, it is not known exactly how the changes in enzymatic activity produced by the *val*^{158}*met* polymorphism translate into vulnerabilities to the development and maintenance of FM. It is undisputed that the polymorphism changes the rate at which catecholamines are degraded, but it is less clear how those changes impact function. As

noted above, norepinephrine has been shown to, under different circumstances, contribute to analgesia, hyperalgesia, and increased emotional arousal. Thus, the enzymatic torpidity conferred by the ^{158}*met* variant, through its effect on norepinephrine may also result in varying functional outcomes. Additionally, increased incidence of all three catecholamines simultaneously may result in outcomes different from that which would be expected from changes in metabolic activity on any one catecholamine alone. Association studies can generally only provide one small piece of this ever-expanding puzzle, so there is still quite a long road ahead to understanding the behavioral and affective implications of the COMT gene.

Despite its broad and sometimes contradictory phenotypic associations, COMT remains an attractive candidate gene for the study of the dynamics of pain and affect in FM. *Specifically, there is evidence to support a role for the* ^{158}met *allele as a "risk" allele in the regulation of PA in the face of chronic pain. The* ^{158}met *allele has been consistently associated with FM and pain hypersensitivity in healthy individuals (e.g., Garcia-Fructoso et al., 2006; Vargas-Alarcon et al., 2005), and has also been associated with higher sensory and affective ratings of lab-induced pain (Zubieta et al., 2003).*

Given the evidence reviewed above, a next step is to explore the ^{158}*met* allele in the context of affect reports as they occur throughout daily life with chronic pain. Daily reports of stress, pain and affect would extend beyond the laboratory work that has already been done and provide a unique window into the influence of COMT within the FM condition. Another necessary research step is to establish the allelic frequency of COMT in FM relative to other chronic pain groups. The ^{158}*met* allele is found more

frequently in FM patients compared to healthy controls, but it is not known if FM patients differ from other chronic pain groups in this regard. Such a test would aim to answer the question of how specific the effects of COMT are on the FM population.

Opioids and the OPRM1 Gene

Opioidergic Neurotransmission. The opioid system is large and complex, involving a variety of peptides and receptors that become endogenously activated during painful episodes and can be exogenously stimulated through the administration of opiates. For the purposes of the current review, I will provide a brief introduction to the molecular mechanisms of opioids and their receptors, and then highlight some evidence in support of the importance of one particular receptor class, the μ-opioid receptor (MOR), in the neurotransmission of pain and affect.

Three primary peptide groups account for most of the antinociceptive activity of the opioid system: dynorphin, enkephalin, and β-endorphin (Przewlocki & Przewlocka, 2001). All three peptides act as neurotransmitters and bind to any of the three primary opioid receptor sites: μ, κ, and δ. β-endorphins have a strong binding affinity at the MOR and exert a peripheral analgesic action in response to hyperalgesia (Przewlocki & Przewlocka, 2001). Exogenous morphine also selectively binds to the MOR, in addition to the κ-receptor, and has the effect of inhibiting spinally ascending transmission from primary nociceptive afferents (Holden, Jeong, & Forrest, 2005). The MOR facilitates endogenous opioid release, as well as an exogenous morphine-induced antinociceptive response to inflammatory pain, neuropathic pain, and chronic widespread pain, although

morphine's action at the MOR tends to be least effective in combating neuropathic pain (Ballantyne & Mao, 2003; Holden et al., 2005; Przewlocki & Przewlocka, 2001).

Endogenous opioid binding at the MOR may also modulate the emotional response to emotionally-salient environmental stimuli. There is a high density of MOR receptors in the limbic areas, which include the amygdala and hippocampus among other temporal lobe structures (Liberzon et al., 2002). This suggests that the MOR may be particularly active during the situations in which the limbic system is most active: the processing of emotional stimuli. Furthermore, there is evidence that amygdalar MOR activity regulates the anxiolytic effect of benzodiazepams (Kang, Wilson, & Wilson, 2000). In a PET scan study of brain activity during emotion processing, Liberzon et al. (2002) showed that higher baseline MOR binding potential resulted in diminished cerebral blood flow during the presentation of aversive emotional stimuli, suggesting that MOR binding buffers against the negative affective response to aversive stimuli. Additionally, MOR agonists have been shown to enhance pleasantness ratings in the face of pain, possibly via activation of the anterior cingulate cortex (Casey et al., 2000), which plays a crucial role in modulating both the sensory and affective dimensions of pain (Price, 2000).

Although the opioid system has been suggested to participate in the pathogenesis of FM, it is as yet unclear whether it is an overabundance of certain opioid peptides, a relative lack of peptides, a dysfunction in the binding action at the level of the receptor, or some combination of mechanisms that contributes to the heightened pain sensitivity found in FM. Additionally, it is unclear which peptide and/or receptor should be targeted

in the study of opioids in FM. For example, Dynorphin A, an opioid peptide operating at the κ-receptor, has been hypothesized to be involved in the pathogenesis of FM, as it has been known to cause allodynia in rodents when administered exogenously (Russell, 1998). This hypothesis has been supported by evidence that Dynorphin A is elevated in the cerebrospinal fluid of FM patients (Vaeroy, Nyberg, & Terenius, 1991). Another group reported that elevated levels of Met-enk-Arg-Phe (MEAP), an opioid polypeptide with strong binding affinity at the MOR, are associated with reduced pain threshold in FM patients (Baraniuk, Whalen, Cunningham, & Clauw, 2004). In contrast, β-endorphin, which exacts an analgesic effect at the MOR, is found to be diminished in FM patients compared to controls (Pannerai et al., 2002).

Genetic Effects of OPRM1 on Pain and Affect. The gene for MOR function, OPRM1, has been touted as a highly focused candidate for the study of genetic influences on pain (Uhl et al., 1999). As mentioned before, β-endorphin exacts its endogenous analgesic effect primarily at the MOR, while morphine depends exclusively on the MOR for its analgesic function. In addition, disrupted MOR function may disturb the function of other opioid receptors, further compromising the opioidergic response to painful stimuli (Sora, Funada, & Uhl, 1997). A functional polymorphism located at position 118 results in the substitution of guanine for adenine. This nucleotide substitution causes a change in amino acid of asparagine to aspartate at position 40 on exon 1 of the mu receptor protein. Functionally, the *asn*^{40}*asp* polymorphism in OPRM1 results in a three-fold increase in binding affinity for β-endorphin at the MOR, presenting a rationale for its association with pain processing (Bond, LaForge, Tian, et al., 1998; Fillingim, Kaplan,

Staud et al., 2005). Unlike the *val*^{158}*met* polymporphism, in which both alleles are distributed relatively evenly throughout the population, the ^{40}*asp* allele is found in only 20-30% of the population, with homozygous asp/asp genotypes representing a substantially smaller fraction of the distribution than heterozygous genotypes (Bond et al., 1998; Fillingim et al., 2005).

Despite the wide-ranging implications of MOR activity in pain and affective processing, OPRM1 has only recently been explored in these contexts. Two studies have reported null findings with regard to the gene's effect on pain sensitivity (Comptom, Geschwind, & Alarcon, 2003; Kim, Mittal, Iadarola, & Dionne, 2006). One study has reported significant genetic variability in self-reported pain threshold. Fillingim et al. (2005) administered thermal, mechanical, and ischemic pain stimuli on healthy men and women. PA and NA were additionally measured using the PANAS (Watson, Clark, & Tellegen, 1988). Individuals with at least one copy of the rare ^{40}*asp* allele had a higher pressure (mechanical) pain threshold than those homozygous for *asn*^{40}. No main effects for genotype on pain emerged for the other painful stimuli. However, a sex by genotype interaction on thermal pain threshold suggested that men with the ^{40}*asp* variant may have higher thresholds than women that have the variant. A trend was also reported for a sex by genotype interaction on PA, such that men with the rare allele tended to report less PA than men with the common allele. No differences were found between genotype in women, or between sexes.

Lotsch, Stuck, & Hummel (2006) found that the *asn*^{40}*asp* polymorphism modulates cortical activation in response to nociceptive stimuli without influencing the

cortical response to nonnociceptive stimuli. Specifically, individuals carrying the ^{40}asp allele displayed cortical potentials in response to a nasal nociceptive carbon dioxide gas impulse that amounted to only half of the amplitude of individuals without the rare allele.

The data suggest that the $asn^{40}asp$ polymorphism on OPRM1 is a promising target for an association study involving a clinical pain population like FM patients. However, knowledge is still extremely limited as to the value of the $asn^{40}asp$ polymorphism in predicting pain.

There is some evidence in support of $asn^{40}asp$-mediated reward processing among alcoholics (Oroszi & Goldman, 2004), but, to the author's knowledge, no studies have linked the variant to affective components of pain. Any attempt to explore the utility of the $asn^{40}asp$ polymorphism in the affective processing of pain, then, will remain exploratory.

Although any number of opioid peptides could be justifiably examined in the context of FM, the functions of β-endorphin, which is upregulated at the MOR by the ^{40}asp allele, are relevant to the current study for two reasons. First, the peptide is important in mediating the central nervous system response to nociception, resulting in analgesia when binding occurs at the MOR (Bond et al., 1998). Second, β-endorphin mediates hypothalamo-pituitary-adrenal (HPA) axis function in response to stress. In opioid dependent people, blockade of β-endorphin by the opioid antagaonist naloxone results in elevated serum cortisol levels (Culpepper-Morgan & Kreek, 1997). FM patients characteristically experience an erosion of positive affective resources when stress and pain are elevated. Through genomic regulation of these negative and

homeostatically taxing states, FM patients with the ^{40}*asp* allele could be expected to sustain PA despite the perceived threat of stress and pain. Thus, it is possible that *the asn^{40}asp polymorphism of OPRM1 may promote resilient positive affective responding to elevations in pain and NA within the FM population.*

β-endorphin binding at the MOR could represent a target for pharmacotherapy, and knowledge of an FM patient's MOR receptor activity could inform clinical treatment options. With regard to pain, FM patients may benefit from targeted pharmacotherapeutics aimed at enhancing MOR affinity for the β-endorphin. From a behavioral standpoint, FM patients' stress-management skills present a clinical concern, as these patients often report hypersensitivity to the effects of stress on mood and pain levels (Okifuji & Turk, 2002). From this perspective, enhanced β-endorphin binding at the MOR would be expected to benefit FM patients by helping regulate glucocorticoid levels in response to stress.

Both COMT and OPRM1 satisfy criteria recently offered by Belfer and colleagues (2004) for optimizing the choice of candidate genes in the study of pain processes in humans. First, there is strong evidence, outlined above, supporting a role for each candidate gene in both pain and affective processing. Second, both genes have alleles with a relatively high population frequency, thereby increasing their chances of having an impact on behavioral phenotypes. The ^{158}*met* allele in COMT has a population frequency of greater than 40% (Egan et al., 2001) while the ^{40}*asp* allele on OPRM1 has been shown to have a frequency of between 20 and 30% (Bond et al., 1998; Fillingim et al., 2005). Finally, as outlined above, both proposed polymorphisms have well-

documented functional implications at both the cellular and behavioral levels. Taken together, the literature suggests that COMT and OPRM1 are attractive candidate genes relevant to the perception of pain and the regulation of PA in the face of chronic pain and stress. It is unknown, however, whether either gene contributes to individual differences in the experience of naturally occurring pain and the affective regulation that occurs in its midst. The current study will provide the first test of hypotheses directly related to daily pain and affect regulation in the FM population.

Summary and Hypotheses

The current study seeks to associate two candidate genes, COMT and OPRM1, with the process of affective regulation during pain. At issue is how variants on those genes will influence pain and affect as it is reported in the flow of daily life. The current study has several advantages over previous attempts to associate genes with FM. First, the control group in the current study consists of patients with OA who should experience pain that is relatively comparable to that of the FM patients. The use such a control group extends beyond previous attempts to establish allelic frequency rates in FM, in which FM patients were compared to healthy controls, by establishing allelic frequency comparisons between two chronic pain groups. Second, the current study will make use of daily diaries to obtain within-person estimates of change in pain levels from day to day. By examining such an association with PA, the current study may shed light on how previous associations of COMT with negative affective reactivity and pain sensitivity may contribute to the pathogenesis of FM. Finally, the current study will uniquely contribute to the literature an exploration of a possible association of OPRM1 with

phenotypic characteristics related to resilience within FM. Studies to date that have examined genetic associations with phenotypic traits related to FM have almost exclusively reported on risk to elevations in pain and NA. It is well-established that the OPRM1 gene influences pain sensitivity by increasing pain threshold. The current study will explore whether such an association exists within the FM population and whether it has implications for positive affect regulation.

The specific aims and hypotheses are as follows:

1. Determine if the frequencies of the ^{158}*met* allele in the catechol-O-methyltransferase (COMT) gene and the ^{40}*asp* allele in the μ-opioid receptor (MOR) gene (OPRM1) differ by rheumatic diagnosis. Past research has shown the ^{158}*met* allele to confer a vulnerability to pain sensitivity, and FM patients have been shown to carry the allele at a greater frequency than healthy controls. However, to the applicant's knowledge no published study has compared FM patients with another chronic pain group with regard to the representation of this allele.

Hypothesis 1a. The ^{158}*met* allele will have a greater frequency in FM patients and patients with a dual diagnosis of OA and FM compared to patients with OA only. Additionally, the ^{40}*asp* allele in OPRM1 has been shown to be more prevalent in people with a low as opposed to high pain sensitivity. FM patients have been shown to have a lower threshold for pain than other chronic pain sufferers. Thus, it is expected that this allele will be underrepresented in the FM population relative to individuals with OA.

Hypothesis 1b. FM and OA/FM patients will have a relative absence of the ^{40}*asp* allele compared to OA patients.

2. Examine whether the *val*^{158}*met* polymorphism influences affect reports differentially for FM patients with and without the "risk" ^{158}*met* allele. Past research has indicated that FM patients exhibit a deficit in positive affect (PA) compared to other chronic pain patients (Zautra et al., 2005a), and that FM patients evidence a loss of PA when pain is elevated relative to other groups. However an endogenous source of this deficit has not been identified. The minor ^{158}*met* allele on the COMT gene has been shown to have a role in the processing of emotional stimuli (Zubieta et al., 2003), but its influence on affective processing in the FM population has not been examined. A model for Hypothesis 2 is presented in Figure 1.

Hypothesis 2. The ^{158}*met* allele will exert an effect on PA, across 30 days of daily reporting, and a gene X environment effect will be observed, such that the ^{158}*met* allele will have an effect on PA on days in which pain is elevated.

3. Examine whether the *asn*^{40}*asp* polymorphism on OPRM1 influences affect reports differentially for FM patients with and without the "resilient" *asp* allele. Past research has supported a role for the the minor ^{40}*asp* allele of OPRM1 in resistance to painful stimuli (Fillingim et al., 2005). Further, the MOR has been implicated in the processing of aversive emotional stimuli (Liberzon et al., 2002). A model for Hypothesis 3 is presented in Figure 2.

Hypothesis 3. The ^{40}*asp* allele will exert an effect on PA, across 30 days of daily reporting, and a gene X environment effect will be observed, such that the ^{40}*asp* allele will have an effect on PA on days in which pain is elevated.

In the current study, the ^{158}*met* allele on COMT is proposed as a "risk" allele, with the potential to differentiate FM patients in PA reports and positive affective reactivity to daily elevations in pain and NA. To the author's knowledge, the current study will be the first to establish comparable frequency rates of the ^{158}*met* allele in COMT between FM and OA. The inclusion of OA as a comparison group in this analysis will be crucial to the identification of unique risk among FM patients compared to patients with chronic pain of known organic origin. Within FM, the examination of COMT as a risk factor for a PA deficit will add to the body of evidence established by Zautra, Davis and colleagues indicating that FM patients have difficulty sustaining positive affect through pain episodes. The current study would be the first to explore a possible endogenous source of this affective disturbance in FM. The ^{40}*asp* allele in OPRM1 is proposed as a "resilience" allele, with the potential to identify a group of FM patients who are capable of sustaining PA throughout daily elevations in pain and NA. The identification of genotypes associated with risk and resilience to affective regulation in FM will advance theory related to the etiopathology of FM. Additionally, it will aid researchers interested in the development of targeted pharmacotherapeutics involving the catecholaminergic and opioid systems. Finally, the current research will inform clinicians interested in more efficient means of diagnosing FM and establishing effective behavioral and pain management programs.

Method

Overview

The data that were analyzed for the current project were collected as part of a larger study (R01 AR46034) designed to identify factors related to adaptation to pain and stress in FM. The participants from that study completed 30 days of electronic diaries and submitted samples containing swabbed buccal cells, from which DNA has been genotyped. The current study sought to analyze the role of those polymorphisms in the context of daily reports of pain and affect.

Participants

Participants were 214 women between the ages of 38 and 72 with a physician-confirmed diagnosis of either OA (N=86) or FM (N=46), or a dual diagnosis of OA/FM (N=82). Participants were recruited in the Phoenix, AZ metropolitan area from physician's offices, advertisements, senior citizen groups, and mailings to members of the Arthritis Foundation. Included in the study were participants who had no diagnosed autoimmune disorders, a pain rating above 20 on a 0-100 scale, and/or who were not involved in litigation regarding their condition. All participants reported their diagnosis to research staff and subsequently signed a HIPAA release form. Research staff then contacted each participant's physician, who sent a written confirmation of the participant's stated diagnosis and disconfirmed diagnosis of other autoimmune disorders. All participants, regardless of physician diagnosis, underwent a tender point exam conducted by trained research personnel supervised by licensed rheumatologists in a method consistent with medical standards. A member of the research staff used a

dolorimeter to palpate 18 musculoskeletal regions identified by the A merican College of Rheumatology as tender points that can aid in FM diagnosis (Wolfe et al., 1990). The results of the tender point exam were used primarily to identify outliers whose reported pain may have differed from expectations based on physician diagnosis. However, the results of our tender point exam were not used as inclusionary criteria.

Procedure

After being screened into the study, participants were visited by a clinician to reconfirm FM diagnosis. Next, participants were trained in our laboratory by a research assistant to use a laptop computer to complete daily diaries each night for 30 days. Participants were encouraged to call our laboratory staff immediately if a problem occurred with the laptop. A built-in date-checking software program prevented data entry on days other than the correct day. In the event of laptop malfunction, a research assistant traveled to the participant's home to replace the malfunctioning laptop with a working one. After completing the 30-day diary, participants were visited by a clinician, and DNA was collected from buccal cells via a cheek swab method following published procedures (Walker et al., 1999). Subsequently, participants were debriefed and compensated for their efforts.

Genotyping

Genomic DNA was purified from buccal cheek swab samples by the University of Connecticut Health Center GCRC Core Lab. DNA samples were placed in 96-well plates and genotyped using PCR based TaqMan 5'-nuclease allelic discrimination assay methods in the GCRC Core Lab. Ten percent of genotypes were randomly repeated to

monitor reproducibility. Additionally, the core lab repeated samples from each plate in a known fashion as well included water blanks and DNA samples with known genotypes to monitor quality control. Assays for both markers were already in use in the GCRC Core Lab. The primer and probe combinations for these assays are: COMT val158met polymorphism (rs4680) primers (CCCAGCGGATGGTGGAT and AACGGGTCAGGCATGCA), and dual labeled probes (Vic-TCCTTCAcGCCAGCGA-MGB and Fam- TCCTTCAtGCCAGCGA-MGB); OPRM1 Asn40Asp polymorphism (rs1799971) primers (CCCAGCCCCGGTTCCT and TGATGGCCGTGATCATGGA), and probes (Vic-AGATGGCGACCTGTCC-MGB Fam-AGATGGCAACCTGTCC-MGB).

Measures

Positive and Negative Affect. PA and NA were measured in the daily diary using the Positive and Negative Affect Schedule (PANAS; Watson, Clark, & Tellegen, 1988). Participants rated 10 standard mood adjectives each for PA and NA using a 5-point scale from 1 (very slightly or not at all) to 5 (extremely). Scores for the two scales were obtained by computing means and between-person reliabilities were computed by aggregating each participant's items across all days. Cronbach's alpha was .95 for PA and .86 for NA.

Interpersonal Stress. Both positive and negative interpersonal life events were assessed by administering an abridged version of the Inventory of Small Life Events (ISLE; Zautra, Guarnaccia, & Dohrenwend, 1986). Events were grouped in four domains of the ISLE: a) friends or acquaintances, b) spouse or partner, c) family members, and d)

employment and co-workers. Participants were first asked to report whether or not each event occurred during the day of reporting. After responding to each domain of items, participants were asked, "Overall, how stressful were your relations with your (domain) today?". Responses were made on a four-point Likert scale where 1 = Not at all, 2 = A little, 3 = Moderately, and 4 = Extremely. Stressfulness ratings from each of the four domains were then aggregated to create a single daily index of perceived stress. This method has been reported elsewhere with weekly diaries (Zautra, Hoffman, Potter et al., 1997).

Soft Tissue Pain. Participants were shown a body diagram with areas marked out in zones covering the major quadrants of the body (Affleck et al., 1996) and instructed to rate their soft tissue pain in 15 total areas including parts of their neck, shoulders, chest, upper and lower arms, upper and lower legs, back, and buttocks on a scale of zero-to-three where zero was "No pain" and a three meant "Severe pain." Sum sores were computed from the 15 items to create an overall score of soft tissue pain. Cronbach's alpha was .93.

Data Analysis

The frequencies of the alleles that code for the *val*^{158}*met* substitution in COMT have been reported as follows in the FM population: 12% AA (*val*/*val*), 51% AG (*val*/*met*), 37% GG (*met*/*met*). To date, allelic frequency estimates are not available for the OA population. However, those estimates were hypothesized to be similar to those observed in the general population: 22% AA (*val*/*val*), 59% AG (*val*/*met*), 19% GG (*met*/*met*). Due to the functionally intermediate action of the heterozygous *val/met*

genotype, gene effects were expected to be largest for individuals with homozygous genotypes. However, COMT gene effects were explored both by using the raw frequency of the *met* allele as a predictor, as well as using the trichotomous genotype as a predictor. The *asn*^{40}*asp* substitution on OPRM1 was expected in fewer than 25% of FM participants based on previous reports that about 25% of the general population carries the ^{40}*asp* allele (Fillingim et al., 2005). No known allelic frequency estimates were available within the FM or OA populations. Given the rarity of the homozygous *asp/asp* genotype (2-3% in the general population; Ray & Hutchison, 2004), individuals were analyzed based on the presence or absence of at least one copy of the ^{40}*asp* allele.

There were three waves of analyses for the current study. First, the frequency of the "risk" ^{158}*met* allele in COMT and the "resilience" ^{40}*asp* allele in OPRM1 was compared between diagnostic groups. Three groups were compared: FM-only patients, OA-only patients, and patients with a dual diagnosis of OA/FM. It was hypothesized that FM patients will carry the *met* allele with a greater frequency than OA patients, and that OA patients will carry the ^{40}*asp* allele with greater frequency than FM patients. Comparison of FM-only with OA-only diagnostic groups provides a test of the relative difference in the frequency of the ^{158}*met* and ^{40}*asp* alleles, but does not distinguish between a higher frequency of the ^{158}*met* allele in FM, for example, from the relative absence of that allele in OA. The third group provides more definitive evidence that it is the presence of the risk allele that is associated with FM symptom presentation, and not the absence of the allele that characterizes OA symptoms, provided the allele is observed with greater frequency in both the FM-only and OA/FM groups compared with the OA-

only group. Diagnostic group differences in *val*^{158}*met* genotype were explored, with differences expected to be greatest for the homozygous genotypes. Chi-square tests were performed using SPSS 15.0 statistical software to test these hypotheses.

The second wave of analyses tested the association of COMT and OPRM1 genotypes with PA reported in the daily diaries by FM patients. Multiple regression was used to determine if, within the FM population, main effects of genotype on PA are observed. NA and pain were averaged across days and used as covariates, and other factors such as age and ethnicity were probed for use as covariates. To enhance the power of detecting individual differences between genotype, OA/FM patients were grouped with FM patients for these analyses, while OA-only individuals were excluded from the initial analysis.

The third wave of analyses tested if there was an interaction of genotype and daily pain on daily PA. Specifically, these analyses tested if, on days in which pain is elevated: 1) FM patients with the "risk" ^{158}*met* allele in COMT would experience a loss of PA relative to patients without the "risk" allele; 2) FM patients with the "resilience" ^{40}*asp* allele in OPRM1 would experience more PA than individuals without the "resilience" allele. For COMT, it was also expected that PA would be lower with higher levels of ^{158}*met* loading. To test this, trichotomous genotype differences for COMT were examined.

Repeated daily measurements resulted in a hierarchical nested data structure, with up to 30 observations nested within people. Given such a data structure, multilevel modeling was the appropriate data analytic tool. SAS PROC MIXED (Littell et al.,

1996) was used to conduct all multilevel analyses. Change in daily pain relative to each individual's mean pain level across diary days was used as an interaction term with genotype. NA was probed as a covariate in these analyses.

Predictor variables in the current study were centered under a procedure referred to as group-mean centering (Nezlek, 2001) or centering within cluster (Enders & Tofighi, 2007). For each observation, the participant's mean was subtracted from the daily score, yielding an index of within-person *daily change*. Deviation scores are denoted in this manuscript by the Greek letter Δ. The independent variables selected as predictors were modeled as deviation scores because this procedure allows for the interpretation of the intercept based on the individual's mean of the independent variable of interest. I chose to group-mean center all predictors because the resulting values allow for a "pure estimate of the Level 1 relationship between X and Y," without the influence of variance at Level 2 (Enders & Tofighi, 2007, pg. 127). In contrast, the independent variables that were selected as covariates in our analyses were all modeled as raw scores because they control for both between- and within-person differences. As an example, if PA is the outcome, pain is the predictor, and NA is controlled for, our centering decisions allow for an interpretation of the intercept as the average value for PA when individuals are at their mean pain and are experiencing no NA.

Both Level 1 (within-person) and Level 2 (between-person) variables, as well as cross-level interactions (Level 1 x Level 2), were modeled as predictors. As an example, I will highlight the basic equations used in the present study, involving daily PA as the criterion:

$$\text{Level 1: } y_{ij}\,(\text{Daily PA}) = \beta_{0j} + \beta_{1j}\,(\Delta\text{Pain}) + \beta_{2j}\,(\text{NA}) + r_{ij} \quad (1)$$

There are *i* observations of PA for *j* individuals. β_{0j} yields an estimate of the average level of PA at the individual's mean level of pain, when they are experiencing no NA. β_{1j} is the coefficient for the daily influence of pain on PA and β_{2j} is the coefficient for the relationship between NA and PA, serving in this model as a covariate. r_{ij} is the within-person error component. At Level 2, individual differences in the average level of PA are probed, along with cross-level interactions. The Level 2 intercept is specified as follows:

$$\text{Level 2: } \beta_{0j} = \gamma_{00} + \gamma_{01}\,(\text{Average Pain}) + \gamma_{02}\,(\text{Genotype}) \quad (2)$$

where the equation for β_{0j} predicts each person's Level 1 intercept from the grand mean, the mean level of pain, and the individual's genotype. The Level 2 slopes are specified as follows:

$$\text{Level 2: } \beta_{1} = \gamma_{10} + \gamma_{11}\,(\text{Genotype}) \quad (3)$$

The second Level 2 equation models a cross-level interaction, whereby between-person differences in genotype (Level 2) moderate the relationship of within-person changes in pain (Level 1) and the outcome, daily PA. In all models, a first-order autoregressive variance-covariance matrix was chosen to model the within-person variance on the dependent variables.

Here we are only concerned with fixed effects, and so the Level 2 equations lack a random error component. Thus, for the analyses presented in the current study, only the intercept was modeled as random. The decision to model only fixed effects was motivated by the expectation, supported by the literature, that the gene and gene X

environment effect sizes would be relatively small. In such cases, it is considered statistically justifiable to model only fixed effects (Nezlek, 2001).

Weekly and daily process designs have been crucial to advancing theory regarding affect relations during stress and pain (Tennen & Affleck, 2002). Early criticism of approaches that incorporated a bivariate model of affective processing suggested that unmodeled individual differences could account for some of the findings that indicated separate dimensions for PA and NA. Longitudinal naturalistic studies have tempered some of that criticism for several reasons. First, the calculation of within-person slopes allows for an examination of changes in the individual over time, irrespective of sample peers. Second, as will be discussed below, repeated measurements over time provide a significant advantage in power compared to cross-sectional designs. Finally, measurements of stress, pain, and affect can be obtained exceptionally close to when events that influence such states actually occur.

Power

The phenotypes proposed in the current study likely arise from complex mechanisms of genes, environmental stimuli, and some interaction of the two. As a consequence, determining sample size is typically a crucial methodological step in ensuring that there will be enough statistical power to detect an effect attributable to a single polymorphism. Many factors affect power in a genetic association study, and these have been summarized by Evans (2008). The current study sought to examine two different types of genetic association. First, differences in allelic frequency between diagnostic groups were probed. Second, gene and gene X environment effects on

phenotypic traits were examined. The following will address which factors are most likely to affect power in the proposed analyses, will present an estimate of the number or cases necessary to achieve 80% power with an alpha of .05 (Cohen, 1992).

In analyzing allelic differences between FM and OA patients, disease prevalence is pertinent. Power increases as a function of disease prevalence, and so more common diseases come with greater power to detect an association. FM is a relatively common condition, affecting 2-4% of the population, with that number increasing among women. The sample in the current study allows for comparisons between OA and FM patients, as well as between OA patients and those with OA/FM. The OA-only controls in the current study were pre-screened to ensure that no control participant has been diagnosed with FM. This pre-screening measure will also increase the power to detect allelic differences between groups. In a case-control design such as this, power is greatest with equal numbers of cases and controls. The analysis comparing OA/FM to OA patients satisfies this criterion, as there are nearly equal numbers of cases (N=82) and controls (N=86). Evans (2008) asserts that in situations when cases and controls are not equal, power is greater if there are a greater number of controls than cases. In the analysis comparing FM patients to OA patients, there are nearly twice as many controls (N=86) as cases (N=46).

Linkage disequilibrium (LD) refers to the degree to which a selected variant is related to a different allele at a different locus on the same gene. Power to detect an effect is greater when LD is high. As the measure for linkage disequilibrium, *D'*, approaches 1, one can have greater confidence that an effect can be detected from the

selected variant, as it may be representative of the effects of other marker alleles in its genomic region. Additionally, when *D'* approaches 1, it becomes more likely that the marker allele has a population frequency equal to the selected variant. For COMT, the *val*^{158}*met* polymorphism has been reported to be in high LD with an allelic marker in close proximity on the gene (*D'*=.96; Diatchenko et al., 2005). For OPRM1, the *asn*^{40}*asp* polymorphism has also been reported to be in high LD with an allelic marker in close proximity (*D'*=.91; Tan, Tan, Karupathivan, & Yap, 2003). Thus, LD is a factor that should enhance the power to detect both *val*^{158}*met* and *asn*^{40}*asp* allelic differences between diagnoses.

Using Purcell, Cherny, and Sham's (2003) genetic power calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/), power was calculated at 80% for an alpha level of .05 with the following parameters: population disease prevalence, population frequency of the high risk allele, population frequency of a marker allele, *D'*, control-case ratio, and the relative risk of the genotype. The relative risk of the genotype is determined based upon the mode of inheritance—that is, whether the target allele effect is dominant, recessive or additive. A dominant model assesses how many times more likely an individual either homozygous or heterozygous for the risk allele is to contract the disease than an individual homozygous for the non-risk allele. For the *asn*^{40}*asp* polymorphism, a dominant model was used since most studies have analyzed the presence of the ^{40}*asp* compared to its absence. Also, it is important to note that, although the examination of the *asn*^{40}*asp* polymorphism will hypothesize that fewer FM patients will carry the allele than OA patients, the calculation of the parameters for mode of

inheritance will not change. For the *val*^{158}*met* polymorphism, an additive model was used because the alleles are codominant. That is, the presence of one allele does not mask the effect of the other, and so heterozygous individuals are expected to express an intermediate phenotype. In terms of allelic differences, it was expected that the met/met genotype would be most highly represented in FM patients, followed by the val/met genotype. The val/val genotype, in contrast, was expected to be most highly represented in OA patients.

Calculation of the parameters reviewed above through the genetic power calculator (for a review of the mathematics involved, see Evans, 2008) indicated that, for 80% power, 61 FM cases are recommended to detect a *val*^{158}*met* allelic difference between FMs and OAs, while 81 OA/FM cases are recommended to detect a *val*^{158}*met* allelic difference between OA/FMs and OAs. For the *asn*^{40}*asp* polymorphism, 115 OA cases are recommended to detect an allelic difference beween FMs and OAs, while 78 OA cases are recommended to detect an allelic difference between OA/FMs and OAs.[1]

Other factors that cannot be included in a case-control calculation may, nonetheless, affect the power to detect the effects proposed in Hypotheses 2 and 3 in the current study. Two factors should significantly enhance power. First, the repeated-measure design of the study will increase the stability of the measurements, as they are assessed over 30 consecutive days, and allow for within-person estimates of changes over time in the variables of interest. As a result, power to detect a genetic effect on phenotypic traits other than diagnosis should substantially increase. Second, the primary

[1] For this latter power calculation, the number of OA cases is listed because that is the group in which the allele is proposed to have greater representation. Thus, the number of cases required for power in this calculation should be compared to the number of actual OA cases (N=86).

outcome measure, PA, is a continuous measure that contains more information than a categorical variable and, thus, should increase the power to detect an effect (Evans, 2008).

Population stratification is widely considered a significant threat to internal validity in association studies (Cardon & Bell, 2001) and was addressed in the proposed analyses. Population stratification occurs when a sample consists of individuals from several different subgroups in which mating over time has been non-random, potentially having caused intergroup differences in allelic representation. Ethnocentric mating is the cardinal example of a behavior that can lead to population stratification. Vargas-Alarcon et al. (2007) reported ethnic differences within the FM population in the frequency of ^{158}*met*, and other ethnic differences have been reported elsewhere (Mannisto & Kaakkola, 1999). Allelic frequencies reported for the ^{40}*asp* allele in OPRM1 have varied from 10-32% in different ethnic groups (Laforge, Yuferov, & Kreek, 2000). A preliminary analysis revealed that the patient sample is relatively ethnically homogenous, with 92%, 88%, and 93% of OA, FM, and OA/FM patients, respectively, identifying as Caucasian. Due to the general ethnic homogeneity of the sample, Hypothesis 1, in which allelic differences between diagnoses were sought, may not be vulnerable to the effects of population stratification. Ethnic differences were probed on the primary outcome variable (e.g., PA), and all analyses for Hypotheses 2 and 3 will be re-run excluding non-Caucasian participants to test the generalizability of the results for Caucasian-only participants. It is unknown if there are significant differences across ethnic groups within FM with respect to PA regulation. Therefore, it was not clear whether population

stratification would ultimately pose a threat to Hypotheses 2 and 3 in the current study (Hutchison, Stallings, McGeary, & Bryan, 2004).

Another potential concern is whether there was power enough to detect the gene X environment interactions hypothesized. Few gene X environment interactions have been consistently replicated (for an exception, see Caspi et al., 2003; Kendler, Kuhn, Vittum, Prescott, & Riley, 2005), and a variety of possibilities may account for this problem, including population stratification, model misspecification, inconsistency in measurement, and failure to properly identify a salient phenotype with endogenous correlates. Additionally, it has been argued that a 'pathology of scale,' in which continuous measures are artificially dichotomized for the purposes of analysis, significantly increases the risk of finding spurious gene X environment interactions (Eaves, 2006). To account for this potential threat to validity, the primary outcome measure, PA, was analyzed as a continuous measure on all gene X environment analyses.

Results

Demographics

First, I examined demographic differences by diagnosis, the means and standard deviations of which are presented in Table 1. OAs were older than both other groups (FM: $p<.001$; OA/FM: $p<.05$), while OA/FMs were older than FMs ($p<.05$). FMs reported a higher average income range than the other groups (FM: \$40-50,000; OA: \$30-40,000; OA/FM: \$25-30,000), but the diagnostic groups were not statistically different in percent reporting at or above the sample median of \$30-40,000, $\chi^2=4.23$,

p=.12. Diagnosis did not differentiate people in terms of education level, F(2, 202)=1.56, p=.21, with all groups reporting "some college" on average, or ethnicity, with all groups mostly comprised of Caucasians, F(2, 205)=.051, p=.95. Diagnostic groups did significantly differ in body mass index [BMI: weight in kilograms/((height in meters)$^{2)}$)] (FM: M=27.88, SD=5.17; OA: M=30.12, SD=8.75; OA/FM: M=31.84, SD=7.63), F(2, 178)=3.34, p<.05, with FM patients having a lower BMI than either OA or OA/FM groups. All demographic variables were also tested as predictors of PA and pain among FM patients. However, no significant associations were found.

Diagnostic Differences in Pain and Affect

Next, I explored diagnostic differences in pain and affect. Means and standard errors of PA, NA, and pain, by diagnostic group are also listed in Table 2. Across diary days, both FM and OA/FM participant groups reported more soft tissue pain on average than the OA group, F(2, 210)=33.66, p<.0001. FMs and OA/FMs did not significantly differ in average soft tissue pain reports. Thus, there were diagnostic differences indicating greater pain overall if FM was present. Additionally, both the FM and OA/FM groups evidenced greater variance in soft tissue pain scores than OAs, F(2, 211)=11.91, p<.001. Again, the FM and OA/FM groups did not significantly differ in that regard. Correlations between study variables for the full sample can be found in Table 3.

Consistent with past findings regarding reports of PA among FM patients, there was a significant main effect for diagnosis on daily PA, F(2, 210)=10.94, p<.01, indicating that OA/FMs and FMs reported significantly less PA across diary days than

OAs (see Table 1 for means and standard errors). FMs and OA/FMs did not significantly differ in PA levels. Further, controlling for soft tissue pain did not change the significance or direction of the main effect of diagnosis on PA. Diagnostic groups did not significantly differ in PA variance, $F(2, 211)=1.46$, $p=.24$. Overall, the findings point to an affective disturbance characterized primarily by a deficit in PA for individuals carrying an FM diagnosis compared to those with OA-only.

Allelic Frequency

For the entire sample, COMT genotypes were present in frequency rates similar to published findings. The *met/met* genotype was present in 18.2% of the sample (N=39), compared with 25.7% for *val/val* (N=55) and 56.1% for the intermediate *val/met* genotype (N=120). Table 4 presents allelic frequencies for each measured genotype within participant diagnosis. Contrary to my hypothesis, *met/met* genotype frequencies did not significantly differ between diagnoses, $\chi^2(4) =3.35$, $p=.50$. However, within both FM and OA/FM groups, the *met/met* genotype was present for 21.7% and 21.9%, respectively, of participants (FM: N=10; OA/FM: N=18), compared with only 12.8% of OA participants (N=11). Combining the FM and OA/FM participants into a single group did not significantly change the results, $\chi^2(2)=3.07$, $p=.21$.

As expected, the homozygous *asp/asp* genotype on OPRM1 was rare in the current sample (Entire sample: N=6; FM-only: N=1). As a result, *asp/asp* and *asp/asn* genotypes were combined such that the presence of the *asp* allele was contrasted with the *asn/asn* homozygous genotype. Across the entire sample, the *asp* allele was present in 28.0% of participants, while the *asn/asn* genotype was present in 72.0%. Significant

diagnostic differences were not found in OPRM1 allelic distribution, $\chi^2(2)=.59$, $p=.74$. The percentages for both OPRM1 genotypes between diagnoses can be found in Table 4. Among FM participants, 23.9% (N=11) carried the asp allele, compared with 28.0% of OA/FM participants (N=23) and 30.2% of OA participants (N=26). As these are the first allelic frequencies of the *asn*^{40}*asp* polymorphism on OPRM1 reported for a clinical sample of FM patients, comparisons to other similar clinical samples are impossible. However, it is apparent that the frequency rates found in the current study, regardless of diagnosis, are consistent with general population estimates (Fillingim et al., 2005).

Gene and Gene X Environment Effects on PA

The next analytic step was to determine if there were any gene and/or gene X environment effects on PA, as reported across the span of diary days, within the FM sample. All analyses modeled PA as the outcome and NA as a covariate. For FM-only patients, COMT genotype was not associated with PA across diary days, $F(2, 42)=.05$, $p=.95$ (for means and standard deviations, see Table 5). This result did not change significantly when FM-only and OA/FM patients were analyzed together, $F(2, 124)=.82$, $p=.45$. However, a significant gene X environment interaction was evident for FM-only patients, $F(2, 1217)=4.49$, $p<.05$ (for complete multilevel statistics, see Table 6), such that individuals with *met/met* genotype experienced a greater decline in PA as daily soft tissue pain increased than did either *val/met* or *val/val* individuals. The latter two genotypic groups were not significantly different from each other, suggesting that the effect observed was recessive and, thus, was present only for *met/met* homozygotes. Figure 3 graphically depicts the COMT X daily pain interaction after dichotomizing pain

into top and bottom thirds of responding within the sample. Additional analyses revealed useful information pertaining to the chosen phenotype. First, with regard to the outcome, COMT genotype was not related to NA across diary days, $F(2, 42)=.52$, $p=.60$, and did not interact with daily pain in the prediction of NA, $F(2, 1217)=1.90$, $p=.15$. Second, with regard to the predictor, COMT did not interact with daily interpersonal stress in the prediction of PA, $F(2, 1192)=1.01$, $p=.36$. Taken together, the findings suggest that the chosen phenotype, daily PA reactivity to *pain*, most closely approximated the endogenous influence of the $val^{158}met$ polymorphism on COMT in FM patients.

OPRM1 genotype evidenced a trend toward a significant prediction of PA across diary days for FM-only patients, $F(1, 43)=3.62$, $p=.06$. FM-only patients with the ^{40}asp allele (M=2.78, SD=.80) reported higher PA across diary days than those homozygous for asn^{40} (M=2.26, SD=.74). Means and standard deviations of PA by genotype and diagnosis can be found in Table 5. The hypothesis that an OPRM1 X daily pain interaction would be observed was supported, $F(1, 1218)=6.06$, $p<.05$ (for complete multilevel statistics, see Table 7). This effect is graphically displayed in Figure 4. As the graph indicates, FM-only patients with the ^{40}asp allele experienced a greater decline in PA as daily soft tissue pain increased than did those carrying two asn^{40} alleles. Still, as Figure 4 indicates, ^{40}asp carriers reported substantially greater PA than their counterparts on high pain days. NA did not significantly moderate that interaction, $F(1, 1215)=.39$, $p=.53$. However, further analyses revealed a significant OPRM1 X daily pain interaction on NA, $F(1, 1218)=5.22$, $p<.05$, such that FM-only ^{40}asp carriers reported a greater increase in NA as pain increased than those without the ^{40}asp allele. As with COMT,

OPRM1 did not interact with daily interpersonal stress to predict PA, $F(1, 1193)=.10$, $p=.76$.

Each genotype was entered as a predictor of both soft tissue pain variance and PA variance. Met/met individuals evidenced significantly greater soft tissue pain variance than val/val individuals, $F(2, 43)=3.40$, $p<.05$, but did not differ from the heterozygous genotype. FM patients did not significantly vary by COMT genotype in PA variance across diary days, and did not significantly vary by OPRM1 genotype in either pain or PA variance.

Two population stratification control measures were taken with all gene and gene X environment effects. First, when ethnicity was included as a covariate, the form and significance of the effects reported for both COMT and OPRM1 remained consistent. Second, when all primary COMT and OPRM1 analyses were re-run with a Caucasian-only sample, the N decreased from 43 to 35. The result was that the significance of each effect reported above dropped below the *a priori* alpha threshold ($p<.05$), but the form and direction of all effects remained consistent. In sum, there is little evidence to suggest that population stratification was a threat to the hypotheses tested in the current study.

Influence of Tender Point Endorsement

When assessed by trained members of our research staff, some participants endorsed tender point pain discordant with their physician-confirmed diagnosis. For a diagnosis of FM, pain should be present in 11 of 18 tender points. Ten FM-only and 11 OA/FM patients reported pain in fewer than 11 out of 18 tender points, while 27 OA-only participants reported pain in 11 or more tender points. One FM and three OA/FM

participants lacked tender point exam data. Ultimately, the physician-confirmed diagnosis is the gold-standard and was used to classify diagnosis for the analyses presented above. However, because the present study is concerned with FM-specific risk and resilience to daily pain, this finding motivated several tests for the possible confound of individual differences in tender point endorsement. First, correlations between tender point endorsement and genotype were examined. Tender point endorsement was not significantly related to either COMT, r=.13, p=.07, or OPRM1, r=-.08, p=.26, genotype. The case-control chi square analyses of allelic frequency were re-run to include only those physician-diagnosed FM and OA/FM participants who endorsed pain in 11 or more tender points, and only those physician-diagnosed OA participants who endorsed pain in fewer than 11 tender points. The results using the modified sample according to tender point endorsement were consistent with those reported above using the sample determined by physician diagnosis. That is, diagnostic groups still did not significantly differ in the allelic representation of either the COMT or OPRM1 genes after individuals with tender point endorsements discordant with their physician diagnosis were removed from the analyses. In analyses involving continuous phenotypic traits of FM patients, two controls were implemented. First, all analyses were run separately including only those FM patients who reported pain in 11 or more tender points. Results of these separate analyses did not significantly differ from results run with the full sample. Second, the number of tender points endorsed was included as a covariate in analyses involving the full sample of FM patients. No effects were significantly altered through the inclusion of raw tender point endorsement as a

covariate. Thus, it is unlikely that individual differences in tender point endorsement affected any of the results reported in the current study.

Discussion

The purpose of the present study was to test the relation of two hand-selected candidate genes to positive affective regulation during pain. To meet this aim, the study took two approaches. The first approach sought to determine if alleles on COMT and OPRM1 that were hypothesized to confer risk and resilience, respectively, to the maintenance of positive affect through elevations in daily pain were differentially represented by rheumatic diagnosis. The motivation of this aim was driven by the fact that both of the chosen genes have previously been associated with pain processes and affective processes, and one of the genes, COMT, has been identified as disproportionately represented in FM patients compared to healthy controls. My goal was to further specify the relation of those genes to the dual processes of widespread pain and positive affective regulation that distinguish FM patients from patients with more targeted chronic pain. In pursuit of this aim, allelic frequencies were compared between three diagnoses: FM, OA, and individuals with a dual diagnosis of OA and FM.

Contrary to the hypothesis, the *met/met* allele of the *val*^{158}*met* single nucleotide polymorphism on the COMT gene was not significantly overrepresented in the FM group compared to the OA group. However, examination of the raw percentage representation within each diagnosis warrants future testing of this hypothesis with a larger sample. The *met/met* allele was similarly represented in both the FM and OA/FM groups and represented a nearly 60% greater proportion of COMT genotypes in those groups compared to OA participants. However, several abnormalities in the current data deserve mentioning. Although the 18.2% overall met/met representation in the current sample

was consistent with previously published estimates of healthy controls, the representation within the OA group, at 12.8%, was somewhat lower than expected (≈19%), and the representation for patients with an FM diagnosis, at roughly 22% between the two FM groups, was markedly lower than published estimates (≈37%). Given the limited sample size in the current study, explanations for these discrepancies will require further testing with a larger sample. From the current data, competing hypotheses about allelic differences can be generated. Either the current estimates are disproportionately low due to the low sample size in the current study, or the previously published estimates are disproportionately high due to the low sample sizes in those studies (N=110 in Garcia-Fructoso et al., 2005; N=29 in Vargas-Alarcon et al., 2007). Another possible explanation for the discrepancies in allelic frequency observed here could be the threat of population stratification in previous samples. In the Garcia-Fructoso et al. and Vargas-Alarcon et al. studies, Spanish and Mexican populations comprised the entire samples. The current study, in contrast, was comprised of mostly Caucasians which, theoretically, should represent a more heterogeneous background.

It was also hypothesized that allelic differences would emerge between diagnostic groups in the representation of the *asp* allele on the OPRM1 gene. However, the results indicated that the *asp* allele was relatively evenly distributed across diagnostic groups. Thus, there is a lack of evidence to suggest that the *asp* allele is underrepresented in FM patients compared to other chronic pain groups. This finding suggests that the OPRM1 gene may not be involved in the development of FM. That is, those who lack an *asp*

allele do not appear to be at any significant risk of developing FM relative to the rest of the population.

The second major focus of the current study was to examine the relation of the candidate genes to the process of positive affective reactivity to daily pain, among the FM-only participants, through a daily diary format. Indeed, an interesting pattern of results emerged. With regard to COMT, there was no evidence for a main effect of genotype on positive affect, across diary days. In the development of hypotheses for the current study, it was clear that COMT has been implicated in affective processing, but the specificity of that association was in question. For example, Smolka et al. (2005) found that the ^{158}*met* allele was associated with increased reactivity to aversive, but not pleasurable, stimuli. Zubieta et al. (2003) found the ^{158}*met* allele to be associated with the negative affective, but not the positive affective, component of pain on the McGill Pain Questionnaire. In contrast, Wichers et al. (2007) found that the ^{158}*met* allele was associated with a greater capacity to experience reward. The present study sought to determine if the ^{158}*met* allele was related to PA across diary days for FM patients. In essence, this served as a test of the association of the ^{158}*met* allele with trait positivity in this patient group. The results of the present investigation clearly indicate an absence of such an association, suggesting that the relation of COMT to FM as previously displayed through case-control studies of allelic frequency does not primarily occur via an affective circuit. However, as hypothesized, COMT moderated the relation of daily pain to daily PA such that elevations in pain were associated with lower PA to a greater degree among met/met individuals than their counterparts. A gene-dose effect was not observed, as the

heterozygous group closely resembled the homozygous val/val group. Thus, allelic codominance was not supported for the PA reactivity phenotype in FM patients. This finding suggests that the lower enzymatic activity on catecholamines conferred by the met/met genotype affects FM patients' ability to maintain PA in the face of pain flares. Importantly, this finding stands in contrast to Zubieta et al.'s (2003) finding that COMT was not associated with the positive affective components of pain. The current study differs in sample constituency (FM patients vs. normative population), methodology (daily diary vs. laboratory pain manipulation), and measurement (PANAS vs. McGill Pain Questionnaire), and so comparing results must be qualified by those major differences. Still, the contrast offers a chance to highlight the concomitant PA deficit with widespread pain in FM. It seems that the chronic pain process for FM patients is almost inextricably intertwined with a deficit in PA regulation, and COMT may bear some responsibility for that effect. The upshot is that the association of COMT and pain is variable and context-dependent.

As reviewed above, the COMT moderation of the relation of daily pain and daily PA could stem from the *val*^{158}*met* SNP's effect on adrenergic, noradrenergic, or dopaminergic activity, or some combination thereof. One goal of behavioral genetics is to reduce the gap between the gene and distal, macro-level behaviors, the cause of which can be polygenic, environmental, or both. This is accomplished through the identification of brain systems, and specific processes within them that can serve as more proximal representatives of the gene's work—the endophenotype (Gottesman & Gould, 2003). The findings presented in the current study should serve as a starting point for the

identification of a putative endophenotype for the deficit in positive affective regulation for FM patients. Of the three catecholamine systems, the dopaminergic system offers the most promising direction for future study in that regard. The tonic/phasic model of mesolimbic dopaminergic neurotransmission, in particular, is an attractive choice because it is discreet and observable, directly regulated by COMT, functions in the absence of disease, and has been reported to affect both the perception of pain and the experience of reward. A next-step could be to target the tonic/phasic firing of dopamine in subjects, perhaps non-human, who are homozygous for the val^{158} allele (i.e., high enzymatic activity) and determine if disruption of the phasic response to pain results in similar affective deficits.

In contrast to COMT, both gene and gene X environment effects on daily PA were found for OPRM1. FM patients with at least one ^{40}asp allele reported significantly more PA across diary days than those homozygous for asn^{40}. On days when pain was elevated, however, ^{40}asp individuals report a greater decrease in PA than those homozygous for asn^{40}. This interaction could have multiple interpretations. One possibility could be that ^{40}asp individuals experience more PA, in general, than their counterparts, but face greater instability their positive affective reactivity to pain. An alternate interpretation could be that asn^{40} individuals are displaying a floor effect and, if not for their maximally low PA, we would only be seeing an OPRM1 main effect on PA. The likelihood of this possibility is bolstered by the comparison of PA levels in past studies using a similar sample. For example, Zautra et al. (2005) reported an average PA level of 2.78 (SD=.58) for the FM patients in that study. In comparison, the FM patients

homozygous for asn^{40} in the current study reported a mean PA level across diary days of 2.26 (SD=.74). Thus, the average PA level experienced by homozygous asn^{40} carriers was quite low, even by FM standards. This suggests that there may have been little room for those individuals to decline in PA on days of elevated pain. Either way, the findings suggest that the ^{40}asp allele is a source of resilience for FM patients. Even on high pain days, they are able to maintain substantially higher PA levels than those of their genotypic counterparts.

Diagnostic and genotypic differences emerged in the variance of pain reports. Both FM and OA/FM patients were more variable across diary days in their soft tissue pain than were OA patients. This finding falls in line with the common understanding of widespread pain in FM: It is often closely related to environmental context, such as the presence of external social stressors, and is subject to wide and unpredictable fluctuations from day to day. These variance scores, then, may reflect a pain process that could be described as reactionary. Within the FM group, met/met individuals evidenced significantly greater variance in their pain scores than those with the val/val genotype. How does this between-genotype difference relate to the finding that met/met individuals experienced less PA on days in which pain was elevated? It is possible that the greater variability in pain experiences for the met/met group led them to react more strongly in their positive affective response to pain elevations. The met/met group did not significantly differ from other COMT groups in PA variance, suggesting that their more highly variable pain scores could have influenced their relatively stable PA variance. Perhaps the parallel between the diagnostic and genotypic differences in pain variance

suggests that met/met individuals, within the FM group, could be more prone to externally-influenced pain than those with a *val*^{158} allele.

The current study also yielded some unexpected results with regard to tender point endorsement among participants from each diagnostic group. Contrary to diagnostic criteria, about 17% of FM and OA/FM patients endorsed pain in fewer than the minimum 11 tender points needed to meet conditions for diagnosis. In turn, nearly 31% of OA-only patients endorsed pain in 11 or more tender points, suggesting that an FM diagnosis might be considered for this group. Still, despite controlling for the discrepant tender point endorsements in the FM-only population, the primary gene and gene X environment findings remained. This result supports recent assertions (Finan et al., In Press) that the diagnosis of FM may require a broader assessment than that put forth by the American College of Rheumatology (Wolfe et al., 1991). That the effects of COMT and OPRM1 were observed with FM patients with and without the requisite number of tender points suggests that factors other than widespread pain serve to group these individuals. Although the current study was specifically designed to examine FM-only participants, further information could be gained from conducting similar gene and gene X environment analyses with the OA and OA/FM participants who met American College of Rheumatology tender point criteria. Such an investigation would help determine the extent to which genes differentially affect behavior in primary FM, in which FM is the only diagnosed rheumatic disorder, versus secondary FM, in which FM is concomitant with another rheumatic disorder.

The findings presented in the current study have several implications for the diagnosis and treatment of FM. The discovery of genetic association with behavior opens up the possibility of genetic screening to aid in treatment planning. FM patients are notoriously difficult to treat, with treatment options ranging from opiate medication, antidepressant medication, psychotherapy, and a variety of alternative therapies including acupuncture and exercise (Arnold, 2006). With genetic screening, practitioners would be able to tailor therapeutic regimens to FM patients most likely to succeed with a particular course of therapy. For example, FM patients with the met/met genotype could be considered "at-risk" for ineffective positive affective regulation and treatment planning could pay particular attention to the scheduling of positive events. For the minority of FM patients who carry the ^{40}asp allele, positive affective regulation may be resilient to the chronic insult of widespread pain, so practitioners could focus treatment resources on other aspects of the syndrome that may be in greater need of support than positive affective regulation. In any case, more research must be conducted before such treatment options are warranted. The current findings must first be replicated in a different sample with more subjects. If the findings can be replicated, clinical trials would be warranted to determine if FM patients of certain genotypes respond more favorably to one treatment approach versus another. A study is currently underway in which FM patients will undergo a multi-week course of several psychotherapeutic and psychoeducational treatments, of which one is a mindfulness-based intervention that targets positive affective regulation, one is a cognitive-behavioral intervention that targets pain, and another is an education-only control. Just such a randomized controlled trial would be an

ideal venue for testing the efficacy of genotypic grouping in the prediction of treatment outcomes.

One issue raised by the present investigation is the question of whether there is a trajectory for recovery from FM and, if so, what, if any, role genetic factors may play in recovery. The literature is sparse with empirical information pertaining to recovery from FM, and this may stem from the widespread controversy in the medical field over whether FM is really a disease. The thinking holds: If there is no objective pathology to identify the disorder as a disease at baseline, then how can one 'recover' from something that does not exist (For a counter-argument, see: Mengshoel & Heggen, 2004)? Still, longitudinal studies of the FM symptom trajectory have estimated FM recovery, as defined by failure to meet tender point criteria set forth by the American College of Rheumatology, to occur in 28-47% of the population at follow-ups ranging from 2-5.5 years from baseline (Forseth, Forre, & Gran, 1999; Granges, Zilko, & Littlejohn, 1994). But, recovery from FM symptoms is likely more complex than the endorsement of fewer tender points, as evidenced by the variation in tender point endorsement in currently diagnosed patients from the present investigation. Indeed, most patients in the Forseth et al. study still reported widespread pain at follow-up, suggesting that the impact of FM was still present despite the presence of fewer tender points. What insight can behavioral genetics add to this debate? The finding that carriers of the asp allele on OPRM1 are resilient in their positive affective regulation in the face of adversity raises the question of whether these individuals may have a greater potential for 'recovery' from FM, should such an outcome be possible. It would be important to determine if these individuals

look similar in their positive affective regulation to patients from other chronic pain groups. Hypothetically, if that were to be the case, treatment for these individuals could focus less on addressing affective issues and more on pain management. Researchers could focus on the measurement of multiple trajectories for recovery, which could include, for example, a trajectory for pain symptoms, one for affective symptoms, and one for the affective reactivity to pain. Behavioral genetic information, like that provided by the OPRM1 analyses in the present investigation, could aid in the interpretation of trajectories of recovery.

The current findings, if replicated, could aid in the development of pharmaceuticals targeted to address both the pain and affective problems that characterize FM. For instance, there is some suggestion that OPRM1 may be implicated in the pharmacological mechanisms of the antidepressant citalopram (Garriock & Hamilton, 2008). With regard to the typical narcotic treatment regimen for managing FM pain, physicians may be interested in knowing which OPRM1 variant is present in a patient before prescribing dosage. ^{40}asp individuals may need a lower opiate dosage to produce sufficient reductions in pain and increases in positive affect, given their already high binding affinity for β-endorphin, as well as the evidence presented in the current manuscript suggesting resilient affective regulation in this group. Further, a recent study found that cancer patients with the ^{40}asp allele needed a 93% lower morphine dose to achieve pain relief than asn^{40} patients (Reyes-Gibby, Shete, Rakvag, et al., 2007). Interestingly, that study additionally found that cancer patients homozygous for the ^{158}met allele on COMT needed 63% less morphine to achieve pain relief than those homozygous

for *val*^{158}. Although the ^{158}*met* allele has typically been associated with greater pain sensitivity, it has also been associated with greater mu-opioid receptor binding potential (Zubieta et al., 2003), which may explain its association with increased morphine efficacy. The findings in the present study support past evidence for pharmacological covariation of COMT and μ-opioid system functioning, and should encourage pharmacological researchers to take a multi-system approach in advancing the science on pharmaceutical treatment options for FM patients. For this patient group, an ideal pharmaceutical agent would both reduce pain and help stabilize positive affect. A systematic trial of opiate efficacy for FM patients should explore both pain relief and affective change as outcomes that may vary between COMT and OPRM1 genotypes.

The current study proposed that OPRM1 would be a genetic source of resilience for FM women in that it would be associated with higher PA levels throughout pain elevations. The evidence supported that hypothesis, but it is necessary to qualify the language used in my conceptualization of resilience. Here, resilience was the maintenance of PA levels throughout 30 days of reporting that approximated those of OA patients reported in this and other samples (Zautra et al., 2005), with only minor dips in PA on days in which pain was elevated. This form of resilience is similar to that described by Bonnano (2004), in which bereaved widows were termed resilient if they were able to live through the months following the loss of their spouse without experiencing depression symptoms. We now know that FM patients characteristically have great difficulty gleaning the positive from daily experiences, especially those that are social in nature. So, our minority group of FM patients with the ^{40}*asp* allele displayed

a pattern of positive affective regulation that looked more like that of OA patients in terms of their ability to experience reward over time. This is not to say, of course, that OPRM1 genotype was a direct cause of their resilient responding. Indeed, for reasons I will enumerate below, it is now almost universally understood that no single gene can contribute enough variance to a complex behavioral pattern, such as positive affective regulation, to justify labeling that gene as a causal agent. However, from a theoretical viewpoint, the current findings support the notion that neurobiological processes, at least partly determined by the code imparted by a genetic variant, can influence not just the risk of developing an illness, but the potential for one to be resilient through adversity.

Limitations

As alluded to above, one major limitation of the current study was the single gene approach inherent to its design. There has been much fervor in the field over the last decade about the candidate gene approach, as it was perhaps the first major technological advance in behavioral genetic research from the more inferential family/twin design. The benefit of the candidate gene approach is that it leads research closer to the specific source of biological diversity that accounts for physical and behavioral abnormalities that natural selection should be working against. However, as quickly as excitement has grown over the increasingly inexpensive methods to collect and genotype DNA that led to the proliferation of empirical studies reporting genetic associations with behavior, so, too, has that enthusiasm been tempered by the glut of small effect sizes, non-replications, and null findings. The single genetic variant approach, as used in the current study, provides a compelling entrée into the vast possibilities of behavioral genetic research, but

is limited in what it can actually tell us about the biological sources of risk and resilience to the positive affective disturbance in FM. Other currently available approaches could certainly expand the knowledge gained from the current study. For example, the haplotype approach, used in identifying COMT-moderated pain sensitivity by Diatchenko et al. (2005), allows for the grouping of multiple variants proximally located with respect to a target SNP to be blocked together and analyzed based on their cumulative contribution to a behavioral phenotype. Diatchenko et al.'s work, for example, shows that it may not be the ^{158}met allele, alone, that accounts for pain sensitivity, but rather the interaction of that allele with alleles from SNPs in high linkage disequilibrium with $val^{158}met$.

Another major limitation of the current study was the lack of control over medication usage. Participants were enrolled whether or not they were currently taking opiates to help manage their pain, and their daily dosage throughout the diary reporting period was not monitored by research staff. Naturally, this presents a confound to the effects of both OPRM1 and COMT, which are intricately related to opioid system functionality. This is a hazard inherent to the daily process design with many FM patients, as disrupting ongoing medical treatment for study purposes for such a lengthy period would present perhaps too great a participant burden. Future efforts could attempt to recruit an FM sample that is not currently taking opiate medication and compare the results to a sample that is currently prescribed opiates. Although it would be difficult to rule out opiate intrusion or non-adherence, respectively, with those samples in a daily diary study, methods do exist that would enable the researcher to test for the presence of

opiates throughout the course of the study. Such an undertaking, although burdensome, would provide greater clarity about the potentially confounding influence of opiate medication on COMT and OPRM1 effects in the FM population.

The current study was also limited in the respect that the gene and gene X environment effects were only examined in the FM population. An extension of the study should include an analysis of gene X diagnosis and gene X diagnosis X environment effects on positive affective regulation. The absence of such information limits the generalizability of the current findings, especially in light of recent data that should serve to reinvigorate the debate on primary versus secondary concomitant FM (Finan et al., In Press). Although such analyses were possible in the current study, I chose to omit them due to concerns about power. Thus, the small sample size limited the ability to examine genetic effects on positive affective regulation between rheumatic diagnoses.

Finally, the current study included only female participants. Although FM primarily affects women, gender differences are widely reported in candidate gene studies in general, and a male comparison group may have provided important information about genetic differences in dynamics of pain and affect. Future studies should include a male cohort to determine if the findings presented here are moderated by gender.

Summary and Conclusions

Evidence has been provided in support of the hypotheses that variants on COMT and OPRM1 would be associated with the dual processes of pain and affect in FM. The

findings are unique in several respects. First, they make use of repeated measurements of pain and affect collected across 30 days of electronic diary entries. This methodology allowed for the examination of genetic associations with a stable PA phenotype, observed over time, as well as positive affective reactivity to naturally occurring perturbations in pain. Naturalistic data such as those presented in the current study are crucial to our understanding of FM because pain for this patient population is often closely related to life stress and interpersonal processes that are, at best, imperfectly simulated in the laboratory. By identifying two separate, but complimentary, brain systems that have been shown to contribute to pain and affective processing, the current study sought to determine if candidate genes known to regulate those systems were associated with a dual processing model of pain-related positive affective regulation in FM. To that end, hypotheses that the *val*^{158}*met* polymorphism on COMT and the *asn*^{40}*asp* polymorphism on OPRM1 would serve as markers for FM risk and resilience, respectively, were supported. Given the complexity of the behavioral phenotype chosen for investigation, the conclusions presented do not attempt to suggest genetic determinism for the development of FM or any of the characteristics associated with it. Instead, the findings offer researchers ample reason to further investigate the contribution of the catecholamine and opioid systems, and their associated genomic variants, to the still poorly understood experience of FM. In so doing, the field of behavioral genetics may provide basic scientists, behavioral scientists, and practitioners alike the knowledge necessary to advance the current state of conceptualization and treatment for FM.

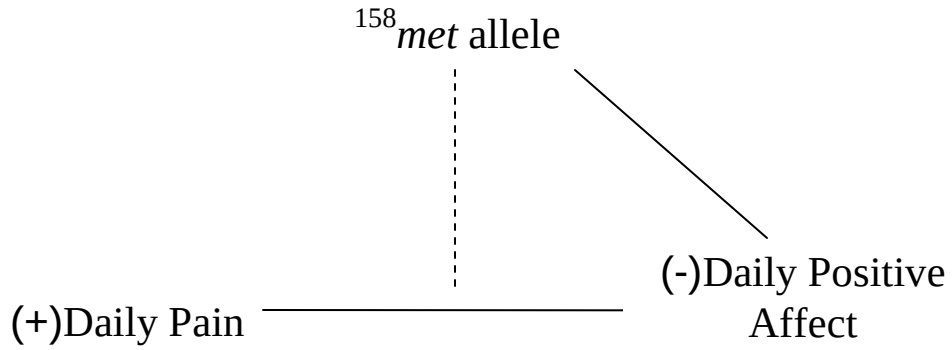

Figure 1. Hypothesized model for COMT-moderated risk for positive affect deficit in FM. Lines represent hypothesized risk of a deficit in positive affect for FM carriers of the ^{158}*met* allele relative to FM carriers of the *val*^{158} allele, as well as moderation of the association of elevated daily pain with diminished positive affect.

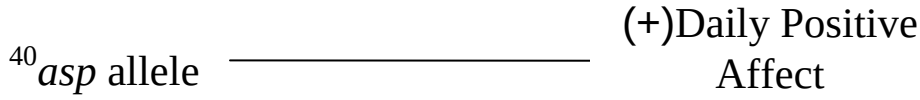

Figure 2a. Hypothesized model for OPRM1 association with daily positive affect. Line represents a hypothesized association of higher positive affect for FM carriers of the ^{40}*asp* allele relative to FM carriers of the *asn*^{40} allele.

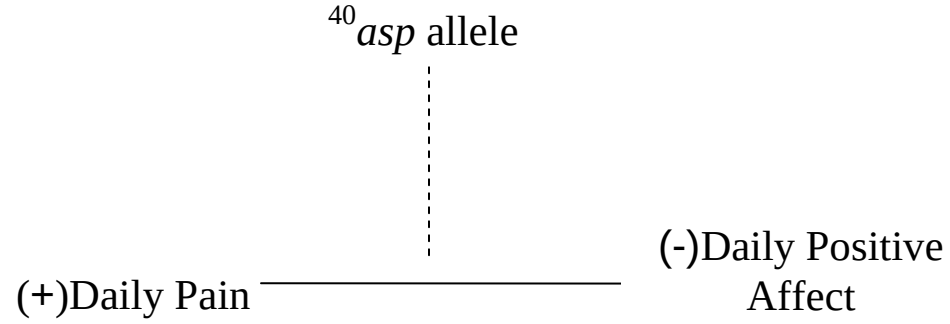

Figure 2b. Hypothesized model for OPRM1-moderated resilience to elevations in pain in FM. Lines represent a hypothesized interaction effect for FM carriers of the ^{40}*asp* allele relative to FM carriers of the *asn*^{40} allele on the association of elevated daily pain with diminished positive affect.

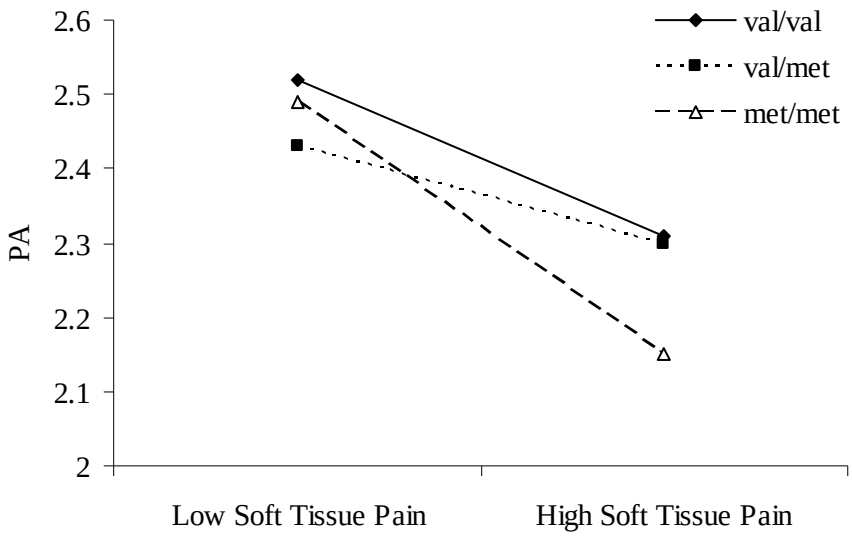

Figure 3. COMT X pain interaction. Slopes reflect PA reactivity to high versus low pain days for FM patients with either *val/val*, *val/met*, or *met/met* genotypes. X-axis parameters were generated by dichotomizing centered pain scores into the top and bottom third of responses across participants.

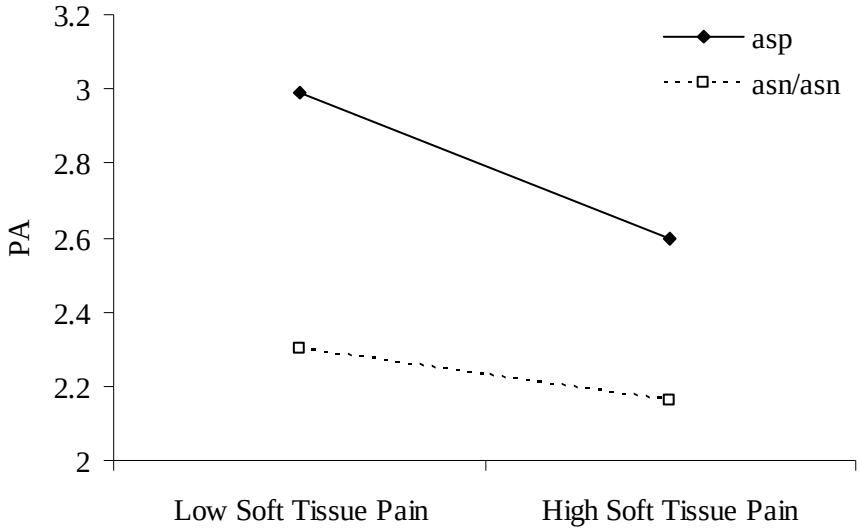

Figure 4. OPRM1 X pain interaction. Slopes reflect PA reactivity to high and low pain days for FM patients who are either carriers or not carriers of the *asp* allele. X-axis parameters were generated by dichotomizing centered pain scores into the top and bottom third of responses across participants.

Table 1

Means and Standard Deviations on Demographic Variables by Diagnosis

	Fibromyalgia N=46		Osteoarthritis/Fibromyalgia N=82		Osteoarthritis N=86	
Demographic Variables	M/%	SD	M/%	SD	M/%	SD
Age	52.98[a]	7.82	56.93[b]	8.13	60.01[c]	8.26
Ethnicity (% Caucasian)	93% [a]		94% [a]		94% [a]	
Income ($30-40,000)	76% [a]		44% [a]		67% [a]	

Note. Values in each row sharing the same superscript are not significantly different from each other. Significant differences were observed at the $p<.05$ level. Means (M), percentages, and standard deviations (SD) of demographic variables were obtained by running descriptives in SPSS. Percentage representation by Caucasians per diagnostic group is reported. Percent within each diagnostic group reporting income at or above the sample median of $30-40,000 is reported.

Table 2

Means and Standard Errors on Independent Variables by Diagnosis

	Fibromyalgia N=46		Osteoarthritis/Fibromyalgia N=82		Osteoarthritis N=86	
Daily	M	SE	M	SE	M	SE
Psychological Characteristics						
Positive Affect	2.41[a]	.11	2.23[a]	.08	2.73[b]	.08
Negative Affect	1.38[ab]	.04	1.45[b]	.04	1.30[ac]	.37
Soft Tissue Pain	1.00[a]	.08	1.08[a]	.05	.40[b]	.05
Soft Tissue Pain Variance	.13[a]	.17	.13[a]	.11	.05[b]	.07
Interpersonal Stress	1.53[a]	.05	1.55[a]	.04	1.37[b]	.04

Note. Values in each row sharing the same superscript are not significantly different from each other. Significant differences were observed at the $p<.05$ level. Means (M) and standard errors (SE) for the daily measures were obtained from the LS Means statement in PROC MIXED (Littell, 1996). Positive and negative affect descriptives

were obtained by averaging across participants' once-daily ratings, across all completed diary days, on the Positive and Negative Affect Schedule (Watson, Clark, & Tellegen, 1988). Soft tissue pain descriptives were obtained by averaging participants' once-daily ratings of pain, across all completed diary days, computed from 15 soft tissue "zones" on the body. Interpersonal stress descriptives were obtained by averaging participants' once-daily ratings of stressfulness across four domains of interpersonal interaction: spouse, family, friends, and work. Participants were instructed to give all affect, pain, and stress ratings before going to bed at night.

Table 3

Correlations Among Study Variables

	Positive Affect	Negative Affect	Soft Tissue Pain	Interpersonal Stress
Positive Affect	1.00	-.27**	-.20**	-.19**
Negative Affect	-	1.00	.30**	.56**
Soft Tissue Pain	-	-	1.00	.33**
Interpersonal Stress	-	-	-	1.00

Note. Variables included in the table above were averaged for each participant across diary days and then correlated.

**p<.01

Table 4

Allelic Frequencies for COMT and OPRM1 by Diagnosis

	Fibromyalgia N=46	Osteoarthritis/Fibromyalgia N=82	Osteoarthritis N=86
Catechol-O-Methyltransferase	% Representation	% Representation	% Representation
val/val	26.1	22.0	29.1
val/met	52.2	56.1	58.1
met/met	21.7	21.9	12.8
μ-Opioid Receptor			
Asp	23.9	28.0	30.2
asn/asn	76.1	72.0	69.8

Note. Values in each row are the percentage representation of each measured genotype, separated in columns by participant diagnosis. No significant differences between diagnoses in allelic representation were found.

Table 5

Means and Standard Deviations of Positive Affect by Genotype and Diagnosis

	Fibromyalgia N=46		Osteoarthritis/Fibromyalgia N=82		Osteoarthritis N=86	
Catechol-O-Methyltransferase	M	SD	M	SD	M	SD
val/val	2.43[a]	.73	1.98[a]	.58	2.89[a]	.98
val/met	2.37[a]	.83	2.23[ab]	.67	2.71[a]	.84
met/met	2.37[a]	.98	2.44[bc]	.84	2.69[a]	.56
μ-Opioid Receptor						
Asp	2.78[a]	.80	2.23[a]	.64	2.87[a]	.98
asn/asn	2.26[b]	.74	2.20[a]	.64	2.71[a]	.80

Note. Means and standard deviations are provided for PA, aggregated across diary days. Columns reflect values for each diagnosis, with each row representing a measured genotype. Comparisons were made between genotype, within each diagnosis. Values in each mean column (separately, for each diagnosis) that do not share a common superscript are significantly different from each other, $p<.05$.

Table 6

COMT Moderation of Daily Pain Effect on Daily Positive Affect

Random Effects					
Covariance Parameter	Subject	β	*SE*	*Z*	*p*
Estimates					
Intercept	ID	.06	.13	4.40	<.001
Residual	ID	.32	.02	20.23	<.001

Fixed Effects					
Predictor Variables	β	*SE*	*df*	*t*	*p*
Level 1					
Δ in Daily Pain (ΔPain)	-.57	.09	1217	-6.07	<.001
Level 2					
COMT (val/val vs. met/met)	.08	.35	42	.24	.81
COMT (val/met vs. met/met)	-.001	.31	42	-.01	.99
Covariate					
Negative Affect	-.29	.04	1217	-7.56	<.001
Level 1 X Level 2					
ΔPain X COMT (val/val vs. met/met)	.37	.16	1217	2.34	<.05
ΔPain X COMT (val/met vs. met/met)	.31	.11	1217	2.78	<.01

Note. The results of the multilevel analysis of the cross-level interaction of COMT genotype and within-person centered daily pain (ΔPain) on daily positive affect among fibromyalgia patients are presented. The effects presented for each Level 2 and cross-level effect are separated according to contrast with the target group, met/met individuals. met/met= individuals homozygous for the met allele; val/met = individuals with a heterozygous genotype; val/val = individuals homozygous for the val allele.

Table 7

OPRM1 Moderation of Daily Pain Effect on Daily Positive Affect

Random Effects					
Covariance Parameter	Subject	β	*SE*	*Z*	*p*
Estimates					
Intercept	ID	.53	.12	4.43	<.001
Residual	ID	.32	.02	20.27	<.001

Fixed Effects					
Predictor Variables	β	*SE*	*df*	*t*	*p*
Level 1					
Δ in Daily Pain (ΔPain)	-.28	.05	1218	-5.18	<.001
Level 2					
OPRM1 (asp vs. asn/asn)	.50	.26	43	1.93	.06
Covariate					
Negative Affect	-.29	.04	1218	-7.39	<.001
Level 1 X Level 2					
ΔPain X OPRM1 (asp vs. asn/asn)	-.32	.13	1218	-2.46	<.05

Note. The results of the multilevel analysis of the cross-level interaction of OPRM1 genotype and within-person centered daily pain (ΔPain) on daily positive affect among fibromyalgia patients are presented.

asp= individuals either homozygous for the asp allele or heterozygous for the asp and asn alleles; asn/asn = individuals homozygous for the asp allele

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APPENDIX A

THE POSITIVE AND NEGATIVE AFFECT SCHEDULE

These words describe different feelings and different emotions.

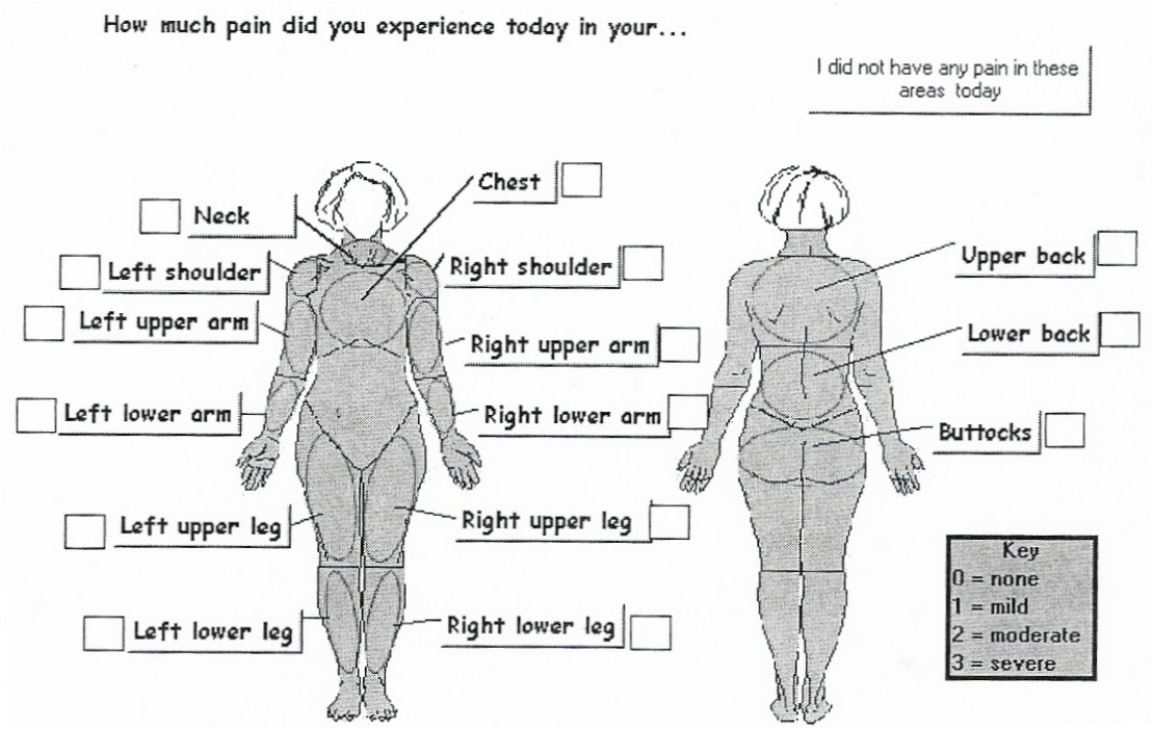
How much have you felt this way today?

1 = very slightly/not at all 2 = a little 3 = moderately 4 = quite a bit 5 = extremely

Positive Affect	Negative Affect
Interested	Distressed
Alert	Upset
Attentive	Guilty
Excited	Ashamed
Enthusiastic	Hostile
Inspired	Irritable
Proud	Nervous
Determined	Jittery
Strong	Scared
Active	Afraid

APPENDIX B

SOFT TISSUE PAIN SILHOUETTE


How much pain did you experience today in your...
I did not have any pain in these areas today
Neck
Chest
Left shoulder
Right shoulder
Left upper arm
Right upper arm
Left lower arm
Right lower arm
Left upper leg
Right upper leg
Left lower leg
Right lower leg
Upper back
Lower back
Buttocks
Key
0 = none
1 = mild
2 = moderate
3 = severe

APPENDIX C

IRB APPROVAL FOR CURRENT STUDY

To: Alex Zautra
PSYCHOLOGY
From: Anna Schwartz, Chair
Bioscience Full Board
Date: 03/18/2008
Committee Action: Approval
IRB Action Date 03/18/2008
Approval Date 01/15/2008
IRB Protocol # 0712002436
Study Title Genetic Factors in Chronic Musculoskeletal Pain
Expiration Date 01/14/2009
The above-referenced protocol has been APPROVED following Full Board Review by the Institutional Review Board. This approval does not replace any departmental or other approvals that may be required. It is the Principal Investigator's responsibility to obtain review and continued approval before the expiration date noted above. Please allow sufficient time for continued approval. Research activity of any sort may not continue beyond the expiration date without committee approval. Failure to receive approval for continuation before the expiration date will result in the automatic suspension of the approval of this protocol on the expiration date. Information collected following suspension is unapproved research and cannot be reported or published as research data. If you do not wish continued approval, please notify the Committee of the study termination. Adverse Reactions: If any untoward incidents or severe reactions should develop as a result of this study, you are required to notify the Bioscience Full Board immediately. If necessary a member of the Committee will be assigned to look into the matter. If the problem is serious, approval may be withdrawn pending IRB review. Amendments: If you wish to change any aspect of this study, such as the procedures, the consent forms, or the investigators, please communicate your requested changes to the Bioscience Full Board. The new procedure is not to be initiated until the IRB approval has been given.