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The Mu Opioid Receptor A118G Gene Polymorphism Moderates Effects of Trait Anger-Out on Acute Pain Sensitivity

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Abstract

Both trait anger-in (managing anger through suppression) and anger-out (managing anger through direct expression) are related to pain responsiveness, but only anger-out effects involve opioid mechanisms. Preliminary work suggested the effects of anger-out on post-operative analgesic requirements were moderated by the A118G single nucleotide polymorphism of the mu opioid receptor gene. This study further explored these potential genotype X phenotype interactions as they impact acute pain sensitivity. Genetic samples and measures of anger-in and anger-out were obtained in 87 subjects (from three studies) who participated in controlled laboratory acute pain tasks (ischemic, finger pressure, thermal). McGill Pain Questionnaire (MPQ) Sensory and Affective ratings for each pain task were standardized within studies, aggregated across pain tasks, and combined for analyses. Significant anger-out X A118G interactions were observed ($p < .05$). Simple effects tests for both pain measures revealed that whereas anger-out was nonsignificantly hyperalgesic in subjects homozygous for the wild-type allele, anger-out was significantly hypoalgesic in those with the variant G allele ($p < .05$). For the MPQ-Affective measure, this interaction arose both from low pain sensitivity in high anger-out subjects with the G allele and heightened pain sensitivity in low anger-out subjects with the G allele relative to responses in homozygous wild-type subjects. No genetic moderation was observed for anger-in, although significant main effects on MPQ-Affective ratings were noted ($p < .005$). Anger-in main effects were due to overlap with negative affect, but anger-out X A118G interactions were not, suggesting unique effects of expressive anger regulation. Results support opioid-related genotype X phenotype interactions involving trait anger-out.

Keywords

Anger-In; Anger-Out; Anger Expression; Pain; Opioid; Genetic; A118G

Introduction

Two common styles of managing anger are anger-in (suppressing anger and hiding the emotion from others) and anger-out (expressing anger directly either verbally or physically; [42]). Both elevated anger-in and anger-out are associated with increased acute and chronic pain

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responsiveness [7,12,13,23,26,38]. While both anger management styles are linked to increased pain sensitivity, only anger-out appears to influence pain through opioid mechanisms.

Results of studies using multiple acute pain stimuli (finger pressure, ischemic, thermal, noxious electrical stimulation) and various measures of opioid function (opioid blockade with naloxone and naltrexone, pain-induced plasma beta-endorphin release) all point towards an association between elevated trait anger-out and diminished endogenous opioid analgesia [6,7,9,10]. For example, opioid blockade with naloxone increased self-reported pain intensity in those scoring low on a measure of anger-out but not those high on anger-out, suggesting functional endogenous opioid analgesia in the former but not the latter [7].

To the extent that effects of anger-out are opioid-mediated and that endogenous opioid analgesic function is influenced by genetic differences in opioid receptors (e.g., beta-endorphin receptor binding; [5]), genotype X phenotype interactions might be expected to occur. One reasonable genetic target is the A118G single nucleotide polymorphism (SNP) of the mu opioid receptor gene. This SNP appears to affect experimental acute pain responses and responsiveness to both exogenous and endogenous opioid agonists. The variant G allele has been associated with lower acute pain responsiveness [20,32], greater beta-endorphin receptor binding [5], and greater Ca^{2+} inhibition on opioid receptor activation [33]. Other work indicates the variant G allele does not influence exogenous opioid receptor binding [5], but is associated with reduced receptor expression [28,44] and increased exogenous opioid analgesic requirements [16,27,30,37,39]. Results of a preliminary study supported possible genetic moderation of the pain-related effects of anger-out by the A118G SNP [11]. In homozygous wild-type subjects (A/A genotype), the relationship between anger-out and post-operative opioid analgesic requirements was weakly positive ($r = 0.07$), whereas it was significantly larger in subjects with the variant G allele ($r = 0.53$). Thus, elevated anger-out appeared to be associated with diminished exogenous opioid efficacy particularly in subjects with the A118G variant allele, suggesting possible genotype X phenotype interactions.

The current study sought to further evaluate possible genetic moderation of anger-out effects on pain by the A118G SNP using controlled acute laboratory pain stimuli. Based on previous work [11], we expected the effects of anger-out on acute pain responses to be greater for individuals with the variant G allele than for individuals homozygous for the wild-type allele.

Method

Design

This study examined standardized placebo condition acute pain rating data from three placebo-controlled drug studies employing pharmacological opioid (naloxone, naltrexone) or alpha-2 adrenergic (yohimbine) antagonists to assess endogenous pain control systems.

Subjects

To maximize statistical power, the current study combined samples from three similar studies in which genetic assays were performed and laboratory acute pain responsiveness was evaluated. The current sample included all participants from one completed study [17] and two ongoing studies who met entry criteria for the current study. These criteria were: 1) availability of sufficient DNA for genetic assays, 2) availability of placebo condition acute pain response data, and 3) availability of pain rating data in which no intervention other than placebo administration had occurred prior to the acute pain tasks. Additionally, all subjects met basic study medical eligibility criteria which were similar across the three studies. These criteria were: age between 18–55 years, current normotensive status (resting blood pressure < 140/90),

not pregnant, no history of cardiovascular disease, hypertension, liver or kidney disorders, or opiate dependence; no current daily use of opioid analgesics, and no current use of anti-hypertensive or antidepressant medications. Subjects were asked to refrain from use of analgesics, NSAIDs, or any medications potentially affecting blood pressure (e.g., pseudoephedrine) for 12 hours prior to study participation and to avoid caffeine 3 hours prior to study participation. Subjects from Chung et al. [17] additionally were free of depression and anxiety disorders based on results of a structured clinical interview. The final study sample size was $n=87$, including 58 subjects from a study employing alpha-2 adrenergic blockade with yohimbine [17], 19 subjects from a study employing opioid blockade with naloxone, and 10 subjects from a study employing opioid blockade with naltrexone. Of the final sample, 60.9% were healthy pain-free controls and 39.1% were individuals with chronic low back pain (median pain duration: 67.4 months; past month visual analog pain intensity: 40.3 ± 2.88 out of 100mm). Other demographics and mean psychometric scores by A118G status are presented in Table 1. The two genetic subgroups did not differ significantly on any of these variables (all p 's $>.10$).

Procedures

All procedures were approved by the institutional review board and all subjects provided written informed consent prior to study participation. Relevant study procedures are detailed below, and were similar across all three studies. Subjects began by completing a packet of demographic and psychometric questionnaires. During the laboratory portion of each study, subjects remained seated upright in a comfortable chair. After an initial 10-minute seated rest period, a series of five pre-drug resting blood pressure determinations was obtained using an oscillometric blood pressure cuff. Following this, the study drug was administered (reflecting placebo only in the current study) followed by an appropriate seated rest to allow peak drug activity to be achieved (10–60 minutes depending on the study drug). Next the study pain tasks were administered.

All subjects included in the current sample ($n = 87$) participated in a forearm ischemic pain task based on procedures described by Maurset et al. [34]. Participants engaged in two minutes of dominant arm exercise using a hand dynamometer at 50% of maximum. Following this, a blood pressure cuff was immediately inflated on the participant's upper arm to 200 mmHg, with the cuff remaining inflated until participants indicated that their pain tolerance had been reached, up to a maximum of five minutes. Immediately upon cessation of this task, acute pain ratings were obtained using the McGill Pain Questionnaire-Short Form (MPQ; [35]), which includes separate subscales assessing sensory (MPQ-Sensory) and affective (MPQ-Affective) pain qualities.

Subjects in the yohimbine and naloxone studies ($n = 77$) also participated in a one-minute pressure pain task using a Forgione-Barber finger pressure pain stimulator that applied 2000 grams of pressure to the dorsal surface of the second phalanx of the index finger of the dominant hand [21]. Immediately upon cessation of this task, pain ratings were obtained using the MPQ.

Finally, subjects in the yohimbine study ($n = 58$) participated in a thermal pain task using a Medoc Thermal Sensory Analyzer (TSA-II, Medoc, Inc., Ramat, Israel). This computer-controlled device was used to apply a controlled heat stimulus to the nondominant ventral forearm using a 30×30mm Peltier thermistor probe as reported in prior studies [e.g., 19]. A series of four pain threshold trials was conducted, followed by a series of four pain tolerance trials, with the probe applied to a slightly different target site for each trial to avoid local sensitization (procedures are detailed more extensively in [17]). For each tolerance trial, the probe started at an adaptation temperature of 40°C, with the temperature increasing at a ramp rate of 0.5°C/sec until the participant indicated maximum tolerance had been reached.

Immediately following the final thermal tolerance trial, participants completed the MPQ to describe the pain experienced during this trial.

Psychometric Measures

Psychometric measures included the Beck Depression Inventory (BDI; [3]), the trait form of the State-Trait Anxiety Inventory (STAI; [40]), the Trait Anger Scale (TRANG; [41]), and the Anger Expression Inventory [42]. The last measure includes separate subscales assessing trait anger-in (tendency to manage anger via inhibition of expression) and anger-out (tendency to manage anger through direct verbal or physical expression). This anger-out scale has demonstrated previous associations with measures of opioid function [6,7,9,10]. The BDI, STAI, and TRANG were included to evaluate whether hypothesized effects were due to anger management style per se, or rather, might be better accounted for by overlap with general negative affect.

Genetic Assays

Genetic samples were obtained via blood drawn from an indwelling venous cannula (yohimbine and naloxone studies) or via buccal sampling (naltrexone study). DNA was extracted using standard methods in the Vanderbilt University DNA Resources Core. As in our past work on this topic [11], assays of the A118G polymorphism were conducted using the *DpnII* restriction fragment length polymorphism, in a manner similar to that of Gelernter et al. [22]. Briefly, amplification using the specified primers was conducted in 25 μ l reactions using a GeneAmp 9700 PCR Thermal Cycler (Applied Biosystems, Foster City, CA). Amplification consisted of 30 cycles of 95°C for 30 seconds, 69.7°C for 30 seconds, and 72°C for 30 seconds, with a 5 min final extension at 72°C. Amplification products were verified for appropriate size (154 bp) and subjected to restriction digest with *DpnII* at 37°C for 3 hours. Digestion products were then examined by electrophoresis on 3% agarose gel. Persistence of the 154-bp band indicated homozygous 118A genotype, while cleavage to a 129-bp band indicated presence of the 118G allele. Genotyping was conducted by individuals blinded to clinical study data and hypotheses. Duplicate assays were run and indicated complete agreement as to genotype status. Blank samples were also run, which were all negative. In the final sample, 81.4% of subjects had the A/A (wild-type) genotype and 18.6% had the 118G variant allele (no subjects were homozygous for the variant allele). These genotype frequencies closely parallel results reported in prior work in predominately Caucasian samples [e.g., 11, 20,25], and support the validity of the genotyping results.

Statistical Analysis

All analyses were conducted using the SPSS for Windows Version 13 statistical package (SPSS Inc., Chicago, IL). Because MPQ-Sensory and MPQ-Affective pain ratings were obtained for each acute pain task used in all three studies, this provided an opportunity to combine these pain response data across studies. To address possible differences across studies and samples, MPQ-Sensory and MPQ-Affective pain ratings were first standardized within each pain task separately for each study sample (i.e., Z-scores were derived). Next, for each of the two pain rating measures, Z-scores were averaged across all acute pain stimuli presented in each study, thereby providing separate standardized measures of aggregate sensory and affective pain intensity across all stimuli within each study. Finally, these aggregate sensory and affective pain ratings for each study were combined into the current sample to permit examination of possible genetic moderation of the effects of anger-out on pain responses. Aggregate standardized pain ratings were employed to reduce the number of analyses performed to minimize possibility that results would be influenced by inflated type I error resulting from multiple comparisons. Moreover, aggregate pain rating measures may improve the validity of pain assessment (and thus increase statistical power) given that random measurement error

across tasks should cancel out [18]. The aggregate pain measures are hereafter referred to in text as MPQ-Sensory and MPQ-Affective pain ratings. MPQ-Sensory and Affective ratings were compared between samples from the three studies, with all differences between studies found to be nonsignificant (p 's > .43). This finding suggested that it was appropriate to analyze pain ratings in the pooled study sample.

Chi Square tests were used to determine deviation from Hardy-Weinberg equilibrium within each subject type (health control and chronic low back pain). Preliminary analyses of relationships between study measures used Pearson correlation coefficients for continuous measures and point-biserial correlations for associations involving the dichotomous genetic measure (there were no homozygous GG subjects). Primary analyses tested for moderation by the A118G SNP of the relationship between anger management style measures and acute pain responses. Given previous work suggesting opioid-related effects and genetic moderation effects for anger-out but not anger-in [6,7,9–11], it was expected that any genetic moderation effects would be limited to the anger-out variable.

Tests of possible genetic moderation were conducted (separately for anger-in and anger-out) using a series of hierarchical multiple linear regressions with acute pain ratings (MPQ-Sensory and MPQ-Affective) as the dependent measures. Given the lack of association between the A118G SNP and subject type in preliminary analyses [Chi Square (1) = 0.006, p >.93] and the expected main effects influence of subject type on pain outcomes irrespective of genetic factors (i.e., healthy pain-free subjects frequently display lower acute pain responsiveness than those with chronic pain; [e.g., 7,24,29]), subject type was entered in the first step of each regression as a control variable to address this potential confound. Gender was not associated significantly with A118G status (Φ i= 0.08, p >.46) and inclusion of gender as a control variable in step 1 did not alter the pattern of primary findings. A118G status also did not differ significantly as a function of race/ethnicity (Φ i= 0.26, p >.21), and as with gender, inclusion of race/ethnicity as a control variable in step 1 left the pattern of significance in primary findings unchanged. To simplify the model, only participant type is included as a control variable in the analyses presented.

To illustrate primary analyses for anger-out, subject type was entered in Step 1, with main effect terms for anger-out scores and A118G SNP status (dummy coded) entered in Step 2, followed by a multiplicative interaction term (A118G $\times$ anger-out scores) that was entered in Step 3. A significant interaction would support a genetic moderation model. The source of significant interactions was determined using simple effects analysis; that is, by examining the relationship between, in this case, anger-out scores and pain ratings separately for the wild-type and 118G variant allele groups. A scatterplot of MPQ-Sensory pain ratings by anger-out scores is presented to display the pattern of raw data by genotype. To depict significant genetic interactions graphically in an optimal way, the regression equations computed for each genetic subgroup (wild-type AA versus G-allele) were solved for hypothetical low and high anger management style values (-1 SD and $+1$ SD from the mean score of the anger-out or anger-in scales) as described by Aiken and West [1]. MPQ-Sensory or MPQ-Affective pain ratings were then predicted for these representative low and high anger management style values and were plotted by genotype.

The measures of anxiety (STAI), depression (BDI), and trait anger (TRANG) that were obtained permitted analyses to address potential influences of general negative affect on the pattern of findings. Specifically, measures of anger-in and anger-out are associated positively with other measures of general negative affect [4,7,14,43]. It is therefore possible that effects of anger management style, and genetic moderation of those effects, on acute pain responses might not reflect unique effects of anger management style, but rather the effects of shared variance with general negative affect. To address this possibility, significant primary analyses

were rerun, entering these three negative affect measures in Step 1. If entry of these negative affect measures greatly reduces the effects of anger management style, then this suggests that the latter effects might be better explained by overlap with negative affect.

All analyses used the maximum number of cases available, and a two-tailed probability value of $p < .05$ was used as the criterion for statistical significance.

Results

Preliminary Analyses

The mu opioid receptor genotype distribution was in Hardy-Weinberg equilibrium in both the healthy controls (Chi Square = 0.125, $p = .72$) and the chronic back pain subjects (Chi Square = 0.17, $p = .68$). Table 2 summarizes the associations between A118G SNP status, MPQ-Sensory and Affective pain ratings, and the various psychometric measures. A118G status did not correlate significantly with any of the acute pain or psychometric measures, indicating that it did not mediate any effects of anger management style on pain responses (as per Baron & Kenny [2]). Anger-in and anger-out scores were significantly, but only modestly, correlated. The anger-in scale was correlated significantly with MPQ-Affective pain ratings, but only marginally with MPQ-Sensory ratings. Anger-out scores were not correlated significantly with either acute pain measure. Of the general negative affect measures, only the STAI was correlated significantly with acute pain responses (MPQ-Affective ratings). Of note regarding potential confounding by general negative affect, anger-in scores were highly correlated with all three negative affect measures. The anger-out scale was uncorrelated with BDI scores, but was significantly correlated with STAI scores and to an even greater extent with TRANG scores.

Anger-Out

To test for possible genetic moderation of the effects of anger-out on acute pain responses by the A118G SNP, hierarchical regressions as described above were conducted (see Table 3). For MPQ-Sensory pain ratings, the anger-out X A118G interaction was significant, uniquely accounting for a 5% increase in MPQ-Sensory variance. A scatterplot of MPQ-Sensory pain ratings by anger-out score is presented as a function of genotype to summarize the pattern of raw data (Figure 1). The source of this significant interaction was determined in simple effects analyses by examining the slopes between anger-out scores and MPQ-Sensory ratings separately in the wild-type and variant G allele groups. In subjects with the wild-type mu opioid receptor gene, the beta coefficient between anger-out scores and MPQ-Sensory ratings was positive and nonsignificant ($\beta = 0.12$, $p > .10$), whereas in subjects with the variant G allele, anger-out scores showed a significant negative association with pain ratings ($\beta = -0.85$, $p < .001$). To depict the source of the significant interaction above most clearly, the regression equations computed for wild-type and A118G variant allele groups were solved for hypothetical anger-out values (-1 SD and $+1$ SD from the mean anger-out value). These values are displayed in Figure 2. The pattern of values suggests that whereas greater anger-out appeared modestly hyperalgesic in subjects homozygous for the wild-type A allele, low anger-out subjects with the variant G allele displayed much higher pain sensitivity than any of the other groups.

Similar genetic moderation tests were conducted on MPQ-Affective pain ratings (Table 3). The anger-out X A118G group interaction was again significant, and accounted for a 8% increase in variance accounted for in MPQ-Affective intensity. Simple effects analyses indicated that in subjects with the wild-type mu opioid receptor gene, the beta coefficient between anger-out and MPQ-Affective ratings was positive and nonsignificant ($\beta = 0.15$, $p > .10$), whereas in subjects with the variant G allele, anger-out scores displayed a significant

negative association with pain ratings ($b = -0.59$, $p < .05$). This interaction is depicted graphically in Figure 3. As for MPQ-Sensory ratings, the pattern of values suggests that greater anger-out was mildly hyperalgesic in subjects homozygous for the wild-type A allele. However, among those with the G allele variant, low anger-out subjects reported greater affective pain intensity and high anger-out subjects reported lower affective pain intensity than subjects with the wild-type allele regardless of anger-out status.

Anger-In

A series of regressions like those described above were conducted to test for a genetic moderation model for anger-in (Table 4). For MPQ-Sensory and MPQ-Affective pain ratings, the anger-in X A118G interactions were nonsignificant. Thus, effects of anger-in on affective pain intensity (see above) did not depend on genotype.

Role of General Negative Affect

The analyses for anger-out scores were re-run with general negative affect variables entered before entry of the anger-out X A118G term to determine whether observed effects were due uniquely to anger-out, or rather, due to shared variance with negative affect. For anger-out X A118G groups effects on MPQ-Sensory pain ratings, controlling for BDI, STAI, and TRANG scores in the model did not alter the pattern of results. The anger-out X A118G interaction still accounted for a 5% increase in incremental variance accounted for ($p < .05$). Similar analyses of MPQ-Affective pain ratings also indicated that the anger-out X A118G interaction remained significant even after controlling for negative affect, still accounting for more than 7% of the variance in pain ratings ($p < .02$). Both of these findings suggest that overlap with negative affect, although present, could not account for the genetically-moderated effects of anger-out on acute pain responses. Rather, these effects appear to be due to the unique effects of a greater tendency to manage anger via expressive strategies.

A possible role for negative affect in the main effects of anger-in on MPQ-Affective ratings was also examined. In contrast to findings for anger-out, control of general negative affect eliminated the significant main effect for anger-in scores, reducing the variance accounted for by this variable from 11% to less than 4% ($p > .22$). This finding indicates that the influence of trait anger-in on acute pain responses may better be accounted for by shared variance with general negative affect.

Discussion

This study evaluated interactions between phenotype (anger management style) and genotype (A118G SNP) vis-à-vis acute pain responses. Prior work suggests that anger-out, but not anger-in, may impact on pain via endogenous opioid mechanisms [6,7,9,10]. Moreover, a growing literature indicates the A118G SNP of the mu opioid receptor gene affects opioid receptor function and pain responses [5,20,28,30–33,44]. Based on the influence of both anger-out and the A118G SNP on opioid analgesic systems, one previous study tested for phenotype X genotype interactions between anger-out and the A118G SNP [11]. Results of that study suggested little effect of anger-out on post-operative exogenous opioid requirements for those with the wild-type gene, yet among those with the variant G allele, greater anger-out was associated with significantly greater exogenous opioid requirements. The current study sought to further explore possible genetic moderation of anger-out effects on pain using controlled laboratory acute pain stimuli.

Prior work indicates that the A118G SNP is associated simultaneously with reduced acute pain responsiveness [20,32] and increased exogenous opioid analgesic requirements [e.g., 27,30]. Results of the current study suggest the former effect is more pronounced in those highest in

anger-out (for MPQ-Affective ratings). Our prior related work suggests the latter effect is also more pronounced in those higher in anger-out [11]. In this sense, both studies addressing genetic moderation of anger-out's effects are consistent with prior literature, further suggesting that previously reported pain-related effects of the A118G SNP are greatest in individuals highest in trait anger-out. Despite these consistencies, it is also important to examine differences in findings between these two genetic moderation studies.

As in Bruehl et al. [11], the current study found modest, nonsignificant positive associations between anger-out and pain ratings among homozygous wild-type subjects. The source of genotype X phenotype interactions in past work was primarily greater exogenous opioid analgesic requirements among high anger-out subjects with the G allele. In contrast, significant interactions in the current study resulted from elevated acute pain responsiveness in *low* anger-out subjects with the variant G allele, with high anger-out subjects possessing the G allele displaying *lower* acute pain sensitivity than their wild-type counterparts (see Figure 3). The different nature of these interactions across studies might be due to the different outcome measures used (clinical analgesic requirements versus pain intensity ratings). However, it may be useful to examine the current findings in context of the literature regarding pain-related effects of the A118G SNP.

Using Figure 3 as an example, it appears that high anger-outs, whom prior work suggests have inadequate endogenous opioid analgesia [7,9,10], do in fact display effective endogenous analgesia (presumably opioid-mediated) if they possess the variant 118G allele. Such speculation is consistent with work indicating that the variant allele is associated with greater beta-endorphin receptor binding [5], increased Ca²⁺ channel inhibition for a given level of opioid agonist activation [33], and diminished acute pain responsiveness [20,32].

In contrast, the subgroup of low anger-out subjects who possess the variant G allele appear to exhibit poor endogenous opioid analgesia, as reflected in substantially greater pain sensitivity relative to the three remaining study subgroups. Although seemingly at odds with prior work demonstrating that individuals low in anger-out *as a group* display more effective endogenous opioid analgesia [7,9,10], these discrepant findings may be reconciled by the fact that approximately 18% of subjects in past samples likely possessed the variant G allele, and thus may have had relatively limited influence on overall group findings. The mechanisms by which the 118G allele is associated with more effective endogenous analgesia in some (high anger-outs) but less effective endogenous analgesia in others (low anger-outs) remain to be determined. Regardless, these results underscore the value of considering genotype X phenotype interactions as they impact on pain responses.

Results for trait anger-in failed to demonstrate any evidence for genetic moderation, consistent with prior work [11]. Although genetic moderation effects were not indicated, greater anger-in was associated with higher affective pain intensity (main effect). The pain-related effects of anger-in were due to the fact that those exhibiting an anger suppressive style tend to be more affectively distressed (depressed, anxious, and angry), which in turn negatively influences pain responsiveness. This finding supports prior work suggesting that statistical overlap between anger-in and general negative affect may account for many of the reported health-related effects of anger-in [9,15,36]. In contrast, while anger-out was significantly correlated with negative affect in this study, this overlap did not account for its genetically-moderated influence on pain responsiveness. This finding of unique effects of an anger expressive style is supported by several previous studies [7,9,12,14].

Several potential study limitations must be considered. While the sample size was reasonably large, statistical power issues might nonetheless have contributed to nonsignificant findings. Because the only nonsignificant results were for anger-in, which was not expected to be

associated with genetic moderation effects, this issue appears to be of limited importance. Also, while genotype/phenotype interactions for anger-out were statistically significant, the variance accounted for by these interactions was relatively small. The clinical relevance of these interactions remains to be tested. Another potential issue is that while anger-in was associated positively with pain ratings as anticipated, anger-out as a main effect was not. Even among the relatively larger subset of subjects with the wild-type gene, the anger-out/pain association was positive but did not approach statistical significance. This finding may be less surprising when one considers a recent review which revealed that although anger-out is positively correlated with acute and chronic pain measures in many published studies, in a subset of studies there is no significant correlation [8]. The current findings highlight the possibility that in some cases absence of anger-out main effects might be due to opposing trends that negate each other. That is, anger-out may be hyperalgesic in some subgroups and analgesic in others, depending on the status of a moderating variable such as genotype. Another possible study limitation is the use of combined data from three studies. Although procedures were similar across these studies, they were not identical, and it is possible this factor might have affected the pain ratings obtained. This issue was handled statistically by deriving standardized pain ratings within each study separately for each pain stimulus before combining the data. Use of aggregate pain ratings, rather than being a weakness, could instead be a strength, as it should nullify effects of random error and increase the signal to noise ratio [18]. The fact that order of placebo/drug administration in the individual studies was not controlled for in the current analyses is another potential weakness. However, examination of data from the study contributing the largest number of subjects to this combined sample suggested order effects were minimal. A final limitation is that the study sample included both healthy pain-free controls and chronic low back pain subjects. Sample composition could have impacted on the results despite statistical control of subject type. However, we consider subject type unlikely to be a confound given that chronic pain status was not associated with the A118G SNP in this study, and that prior work did not suggest that anger-out/opioid relationships were influenced by chronic pain status [7,10]. Despite this argument, it is important to note that the lack of association between the A118G SNP and chronic pain status in this study contrasts with a report by Janicki et al. [25] that chronic pain patients were less likely to have the variant 118G allele. Very different sample selection criteria (i.e., regarding chronic pain diagnoses and intensity, use of opioid analgesics, and whether patients were involved in specialty treatment) may have contributed to the differing findings across studies regarding A118G status and chronic pain.

In summary, this study showed that trait anger-out influences on responses to laboratory acute pain stimuli may depend on whether the A118G SNP of the mu opioid receptor gene is present. Anger-out was associated with nonsignificant hyperalgesic effects in subjects with the wild-type gene, but with significant hypoalgesic effects in subjects with the variant G allele. This work further underscores the importance of considering opioid factors in studies regarding the pain-related effects of expressive anger regulation. Specifically, prior work suggests that endogenous opioid system differences contribute to the pain-related effects of anger-out, and the current study raises the possibility that the impact of anger-out on pain responses may be influenced by genetically-determined opioid receptor differences. Anger-in did not show genetic moderation effects, but did demonstrate pain-related main effects.

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Figure 1. Scatterplot of MPQ-Sensory pain ratings by anger-out score as a function of A118G SNP status.

Figure 2.

Effects of anger-out on MPQ-Sensory standardized pain ratings in subjects with the wild-type (A/A) and variant G allele. Anger-out values plotted are hypothetical values representing one standard deviation (SD) below and above the sample mean. Greater positive MPQ-Sensory scores indicate greater pain intensity. AOS = Anger-Out Scale.

Figure 3.
Effects of anger-out on MPQ-Affective standardized pain ratings in subjects with the wild-type (A/A) and variant G allele.

Table 1

Sample description by A118G SNP status.

Variable	A118G Status	
	A/A Wild-Type	A/G Variant
Gender (Female %)	60.0	50.0
Race/Ethnicity (%):		
White Non-Hispanic	82.9	75.0
African-American	10.0	6.3
Hispanic	2.9	0.0
Asian	1.4	12.5
Other	2.9	6.3
Subject Type (% Healthy)	61.4	62.5
Age (years)	35.5 ± 1.15	34.3 ± 2.52
Anger-In	19.1 ± 0.65	20.6 ± 1.35
Anger-Out	19.3 ± 0.48	20.2 ± 0.66
BDI	2.8 ± 0.44	2.9 ± 1.06
STAI	29.4 ± 0.93	31.2 ± 2.21
TRANG	14.6 ± 0.42	13.8 ± 0.70

Note: Summary statistics are presented as percentages or mean ± SE. All comparisons across genotypes are nonsignificant ($p's > .10$). BDI = Beck Depression Inventory; STAI = Trait form of the State Trait Anxiety Inventory; TRANG = Trait Anger Scale.

Table 2
Intercorrelations among A118G SNP status, acute pain responses, and psychometric measures.

Variable	MPQ-Sensory	MPQ-Affective	Anger-In	Anger-Out	BDI	STAI	TRANG
A118G SNP							
MPQ-Sensory	0.06	0.00	0.11	0.10	0.00	0.09	-0.07
MPQ-Affective	--	0.62 [†]	0.21 [†]	0.04	0.12	0.14	0.15
Anger-In	--	--	0.29 ^{**}	0.04	0.14	0.22 ^{**}	0.20 [†]
Anger-Out	--	--	--	0.26 [*]	0.44 ^{**}	0.67 ^{**}	0.59 ^{**}
BDI	--	--	--	--	0.14	0.35 ^{**}	0.50 ^{**}
STAI	--	--	--	--	--	0.53 ^{**}	0.44 ^{**}
						--	0.65 ^{**}

[†] p<.10
* p<.05
** p<.01

Note: Correlations for continuous measures are Pearson's r; correlations involving A118G group are point-biserial correlations. MPQ-Sensory and MPQ-Affective measures reflect Z-scores aggregated over all pain measures in each study.

Table 3
Summary of hierarchical regression analyses for anger-out and A118G SNP status.

Dependent Variable	Step/Predictor Variables	Change in R ² of Step	p for Change in R ² of Step	B	SE B	p for Predictor Variable
MPQ-Sensory	Step 1: Subject Type	.07	<.02			
	Step 2: Anger-Out	.02	n.s.	0.45	0.18	<.02
	A118G			0.01	0.03	n.s.
	Step 3: Anger-Out X A118G	.05	<.05	0.28	0.23	n.s.
				-0.17	0.08	<.05
MPQ-Affective	Step 1: Subject Type	.02	n.s.			
	Step 2: Anger-Out	.01	n.s.	0.22	0.21	n.s.
	A118G			0.02	0.03	n.s.
	Step 3: Anger-Out X A118G	.08	<.02	0.12	0.26	n.s.
				-0.23	0.09	<.02

Table 4
Summary of hierarchical regression analyses for anger-in and A118G SNP status.

Dependent Variable	Step/Predictor Variables	Change in R ² of Step	p for Change in R ² of Step	B	SE B	p for Predictor Variable
MPQ-Sensory	Step 1: Subject Type	.07	<.02			
	Step 2: Anger-In	.04	n.s.	0.45	0.18	<.02
	A118G			0.00	0.02	n.s.
	Step 3: Anger-In X A118G	.00	<.001	0.26	0.23	n.s.
				-0.01	0.05	n.s.
MPQ-Affective	Step 1: Subject Type	.02	n.s.			
	Step 2: Anger-In	.11	<.02.	0.22	0.21	n.s.
	A118G			0.06	0.02	<.005
	Step 3: Anger-In X A118G	.02	n.s.	0.07	0.25	n.s.
				-0.07	0.05	n.s.