The A118G Single Nucleotide Polymorphism of the μ-Opioid Receptor Gene (OPRM1) Is Associated with Pressure Pain Sensitivity in Humans

Roger B. Fillingim,* † Lee Kaplan,* Roland Staud,* Timothy J. Ness,‡ Toni L. Glover,* Claudia M. Campbell,* Jeffrey S. Mogil,§ and Margaret R. Wallace*

Abstract: Responses to painful stimuli are characterized by tremendous interindividual variability, and genetic factors likely account for some proportion of this variability. However, few studies have identified genetic contributions to experimental pain perception in humans. This experiment investigated whether the A118G single nucleotide polymorphism of the μ-opioid receptor gene (OPRM1) was associated with responses to three different experimental pain modalities in a sample of 167 healthy volunteers (96 female, 71 male). Responses to thermal, mechanical, and ischemic pain were assessed in all subjects, and genotyping of OPRM1 was performed, which revealed that the rare A118G allele occurred in 24 females (25%) and 12 males (17%). Statistical analyses indicated that subjects with a rare allele had significantly higher pressure pain thresholds than those homozygous for the common allele. Also, a sex by genotype interaction emerged for heat pain ratings at 49°C, such that the rare allele was associated with lower pain ratings among men but higher pain ratings among women. These data indicate an association of a common single nucleotide polymorphism of OPRM1 with mechanical pain responses and that this genotype may be associated with heat pain perception in a sex-dependent manner.

Perspective: This study examines the association of the A118G SNP of OPRM1 to experimental pain sensitivity. The results indicate that the rare allele is associated with higher pressure pain thresholds. These results support previous contentions that OPRM1 may be a pain-relevant gene; however, replication of these findings is needed.

© 2005 by the American Pain Society

Key words: Genetics, μ-opioid receptor gene, pressure pain threshold, heat pain, ischemic pain, pain perception, sex differences.
found an association of a δ-opioid receptor gene (OPRD1) SNP with thermal pain responses among men but not women, consistent with the results of a previous linkage mapping study in mice. Zubiet et al reported that an SNP of the catechol-O-methyltransferase gene (COMT) was associated with both pain report and pain-induced brain μ-opioid receptor binding during experimental muscle pain induction. Thus, there is some limited direct evidence of genetic contributions to human pain responses.

Uhl et al suggested that another likely candidate gene that may be associated with pain sensitivity in humans is the μ-opioid receptor gene (OPRM1) (MIM: 600018), located on human chromosome 6q24-q25. The A118G polymorphism of OPRM1—in which the adenine nucleotide located at position 118 is replaced by a guanine—is relatively common, with at least one rare G allele (ie, AG or GG genotypes) occurring in approximately 20% to 30% of the population. This nucleotide substitution results in a change of amino acid at position 40 (in exon 1) of the μ receptor protein from asparagine to the negatively charged aspartate. Moreover, some functional consequences of this SNP have been reported, in that the A118G variant μ-opioid receptor shows greater binding affinity for β-endorphin, which represents a potential mechanism whereby this SNP may alter pain sensitivity. This gene ranks highly on all three criteria recently proposed for prioritizing candidate genes in association studies for pain: strength of evidence supporting involvement in pain processing, frequency of the specific variant, and likelihood that the SNP affects function. However, to date, no studies have reported an association between the A118G SNP and baseline pain responses in humans. Therefore, we compared responses to three experimental pain procedures among healthy volunteers with at least one rare allele to responses among those with two common alleles (ie, AA genotype) at the A118G SNP. On the basis of the previous finding that the variant receptor enhances binding affinity for β-endorphin, we hypothesized that individuals possessing one or more rare alleles would exhibit diminished sensitivity to evoked pain. Because genetic influences on psychologic processes have been widely reported and because psychologic factors are known to affect pain perception, we also examined several pain-related psychologic variables that have been associated with pain responses in previous research.

Methods

Subjects

Subjects included 167 healthy volunteers (96 female, 71 male) recruited by posted advertisements for a study examining sensory effects of analgesic medications. All participants were healthy nonsmokers and were free of clinical pain, psychiatric disturbance, substance abuse, or use of centrally acting medications. Subjects refrained from any over-the-counter medication use for at least 24 hours before testing. Forty-seven (47%) of the women were taking oral contraceptives. Subjects were paid $50 per experimental session for their participation.

General Experimental Procedures

All subjects participated in two experimental sessions separated by at least 2 days. The data presented here represent baseline pain responses obtained as part of a continuing investigation of opioid analgesia. For women, all sessions were conducted during the follicular phase of the menstrual cycle, between days 4 and 10 after the onset of menses. For 36 of the women the two sessions occurred within 2 to 7 days in the same menstrual cycle, and for 59 of the women the two sessions occurred in separate menstrual cycles and were separated by approximately 4 weeks. For men, sessions were separated by equal intervals, with 34 men being tested within one week and 38 being tested approximately 4 weeks apart. At a separate introductory session, approximately 1 week before the experimental sessions were conducted, all subjects provided verbal and written informed consent and completed a series of health and psychologic questionnaires. All sessions were conducted by two experimenters, either two female or one female and one male. Each experimental session started with insertion of an intravenous cannula from which 5 mL of blood was drawn, followed by a 15-minute rest period, during which blood pressure and heart rate were monitored. Next, baseline experimental pain testing was performed, including assessment of thermal pain, pressure pain, and ischemic pain (described in detail below). All procedures were approved by the University of Florida’s Institutional Review Board.

Pain Testing Procedures

The following experimental pain procedures were conducted before drug administration. Pressure and thermal pain were delivered first in counterbalanced order, separated by a 5-minute rest period. Ischemic pain always occurred last to reduce the possibility of carryover effects. Before each pain procedure, digitally recorded instructions were played for the subject.

Pressure Pain Threshold

A hand-held algometer (Pain Diagnostics and Therapeutics, Great Neck, NY) was used to assess the pressure pain threshold. Mechanical pressure was applied with use of a 1-cm² probe. An application rate of 1 kg per second was used because this rather slow application rate reduces artifact associated with reaction time. Subjects were instructed to report when the pressure first became painful. Pressure pain thresholds (PPT) were assessed at three sites: the center of the right upper trapezius (posterior to the clavicle), the right masseter (approximately midway between the ear opening and the corner of the mouth), and the right ulna (on the dorsal forearm, approximately 8 cm distal to the elbow) with the order of site presentation counterbalanced. Pressure pain thresholds were assessed three times at each site, and the average of the three assessments was computed and used in subsequent analyses.
Thermal Pain Threshold and Tolerance

The first thermal procedure involved assessment of heat pain threshold and tolerance. Contact heat stimuli were delivered with use of a computer-controlled Medoc Thermal Sensory Analyzer (TSA-2001, Ramat Yishai, Israel), which is a Peltier-based stimulator. Temperature levels were monitored by a contactor-contained thermistor and returned to a preset baseline of 32°C by active cooling at a rate of 10°C per second. The 3 × 3–cm contact probe was applied to the right ventral forearm. In separate series of trials, warmth thresholds, heat pain thresholds, and heat pain tolerances were assessed with use of an ascending method of limits. From a baseline of 32°C, probe temperature increased at a rate of 0.5°C per second until the subjects responded by pressing a button to indicate when they first felt pain and when they no longer felt able to tolerate the pain. This slow rise time was selected as a test of pain evoked mainly by stimulation of C-nociceptive afferents, as has been previously demonstrated.42,53 Four trials of heat pain threshold (HPTH) and heat pain tolerance (HPTo) were presented to each subject. The position of the thermode was altered slightly between trials (although it remained on the ventral forearm) to avoid either sensitization or response suppression of cutaneous heat nociceptors. For each measure, the average of all four trials was computed for use in subsequent analyses.

Temporal Summation of Thermal Pain

After a 5-minute rest period, the temporal summation procedure was conducted. This procedure involved administration of brief, repetitive, suprathreshold heat pulses to assess first and second pain and temporal summation of the latter.41 Subjects rated thermal pain intensity of 10 repetitive heat pulses applied to the right dorsal forearm. The target temperatures were delivered for less than 1 second, with a 2.5-second interpulse interval during which the temperature of the contactor returned to a baseline of 40°C. Subjects were asked to rate the peak pain for each of the 10 heat pulses. Because subjects vary in their responses to heat pain, we examined temporal summation at two different stimulus intensities. This increased the likelihood that at least one set of stimuli would be at least moderately painful yet tolerable for the majority of subjects. Therefore, two sets of target temperatures, 49°C and 52°C, were used. Subjects were instructed to verbally rate the intensity of each thermal pulse on a numeric rating scale as previously described.16 on which 0 represented no sensation, 20 represented a barely painful sensation, and 100 represented the most intense pain imaginable. Subjects were told that the procedure would be terminated when they reported a rating of 100, when 10 trials had elapsed, or when they wished to stop. The average of the 10 ratings was determined for each subject and used in subsequent analyses.

Modified Submaximal Tourniquet Procedure

After the first two pain procedures, a 5-minute rest period was observed, after which subjects underwent the modified submaximal tourniquet procedure.23,39 The right arm was exsanguinated by elevating it above heart level for 30 seconds, after which the arm was occluded with a standard blood pressure cuff positioned proximal to the elbow and inflated to 240 mm Hg with a Hokanson E20 Rapid Cuff Inflator (Bellevue, Wash). Subjects then performed 20 handgrip exercises of 2 seconds’ duration at 4-second intervals at 50% of their maximum grip strength. Subjects were instructed to report when they first felt pain (ischemic pain threshold [IPTH]) and then to continue until the pain became intolerable (ischemic pain tolerance [IPTo]), and these time points were recorded. Every 30 seconds subjects were prompted to alternately rate either the intensity or unpleasantness of their pain by use of joint numeric (0-20) and verbal descriptor box scales. An uninformed 15-minute time limit was observed. In addition to IPTH and IPTo, two total pain scores were created, one for pain intensity and one for pain unpleasantness, by summing all ratings obtained during the procedure. To replace missing values created by subjects terminating the procedure before the time limit, the last rating provided was carried forward.

Psychologic Questionnaires

Positive and Negative Affect Scale

The Positive and Negative Affect Scale (PANAS) is a 20-item scale. Respondents indicated the frequency with which they generally experience 10 positive (eg, “excited”) and 10 negative (eg, “nervous”) feelings.51 This is a well-validated measure of positive and negative affect, and with this instructional set it reflects mood states that are relatively stable over time.29,51 It yields two scores, one for positive affect and one for negative affect.

Coping Strategies Questionnaire

The Coping Strategies Questionnaire (CSQ) is the most commonly used instrument to assess pain coping.44 The catastrophizing scale comprises 6 items (eg, “It’s awful and I feel that it overwhelms me”), and participants report how frequently they have these thoughts and feelings in response to pain on a 7-point Likert scale from 0 (“Never do that”) to 6 (“always do that”). The CSQ’s catastrophizing subscale has been well validated and is among the most widely used measures of catastrophizing.45 CSQ scales have been associated with recent pain complaints among healthy individuals,13,28,29 and the catastrophizing scale has been associated with experimental pain responses in healthy subjects.11,13,17,19

Kohn Reactivity Scale

The Kohn Reactivity Scale (KRS) consists of 24 items that assess an individual’s level of reactivity or central nervous system arousability.26 It has been recently used as a measure of the construct of hypervigilance.34 This measure has been shown to correlate negatively with pain tolerance5 and has been reported to have adequate internal consistency, ranging from α of .73 to .83.26
The Pennebaker Inventory of Limbic Languidness (PILL) assesses the frequency of the occurrence of 54 common physical symptoms and sensations and appears related to the construct of somatization or to the general tendency to endorse physical symptoms. It has been reported to have high internal consistency (0.88) and adequate test-retest reliability (0.70 over 2 months).40 Recently, it has been used as a measure of hypervigilance in patients with fibromyalgia. These patients demonstrated lower pressure pain thresholds and tolerances and higher scores on the PILL compared with patients with arthritis and pain-free control subjects.34

Genotyping

DNA was extracted from whole blood with use of the Puregene DNA extraction kit (Gentra Inc, Minneapolis, Minn). A 302-base pair fragment of the OPRM1 gene was polymerase chain reaction (PCR) amplified from each sample under standard conditions, with the following primers: OPRM1 5’-GAA AAG TCT CGG TGC TCC TG and OPRM1 3’-GCA CAC GAT GGA GTA GAG GG. This fragment spans the C17T SNP and the A118G SNP. PCR products were purified with use of Millipore Microcon spin filters (Billerica, Mass). Big Dye 2.0 chemistry (ABI, Foster City, Calif) cycle sequencing kit was applied to sequence the PCR products with use of the ABI Prism-R310 sequencer. PCR primers were used as the sequencing primers. The resulting sequences were analyzed with Seq Ed (ABI) or Sequencher software (Gene Codes Corp, Ann Arbor, Mich).

Data Analysis

To reduce error variability, the baseline values from the two sessions were averaged for each subject. Sex and genotype differences were evaluated by individual analyses of variance (ANOVAAs), and frequency differences were examined by \( \chi^2 \) analysis.

Results

Genotyping revealed that the rare A118G allele occurred in 24 female subjects (24%) and 12 male subjects (17%). One woman and one man were homozygous for the rare allele (GG), whereas all others were heterozygous (AG). The homozygous and heterozygous subjects were combined to form the rare allele genotype group. Demographic information for male and female subjects of each genotype is presented in Table 1. Subjects with a
variant A118G allele were significantly younger than those with a common allele ($P < .05$), and women were slightly younger than men ($P = .07$); therefore, age was controlled in all analyses. The ethnic distribution was similar across sex and genotype. Among women, oral contraceptive use was equally distributed across genotype groups.

Data from the heat pain and ischemic pain tasks are presented in Table 2. Women had significantly lower HPTh ($P < .05$) and HPTo ($P < .001$) compared with men, but no main effect of genotype and no sex × genotype interaction emerged ($P > .05$). Women also reported significantly higher heat pain ratings during the temporal summation procedure at both temperatures ($P < .001$). Again, no main effect of genotype was observed ($P > .10$), but the sex × genotype interaction was significant for pain ratings at 49°C ($P < .05$). No significant group differences or interactions emerged for IPTh or IPTo ($P > .10$).

Pressure pain data are presented in Figure 1. A significant main effect of genotype emerged for PPT measured at all three sites (trapezius: $P = .002$, masseter: $P = .023$, and ulna $P = .049$). In all cases, subjects with at least one rare allele had higher thresholds than those with two common alleles. The main effect of sex was significant at all three sites ($P < .001$), with women reporting lower PPTs than men.

Psychologic questionnaire data are presented in Table 3. No significant genotype group differences emerged for the PILL, the KRS, or for active or passive coping assessed with use of the CSQ ($P > .10$). The sex × genotype interaction for the PANAS positive affect scale approached significance ($P = .06$), so that men in the rare-allele group reported lower positive affect than their male common-allele counterparts ($P < .05$), but no genotype effect emerged for women. No group differences emerged for negative affect ($P > .10$).

To examine predictors of pressure pain sensitivity (ie, the average pressure pain threshold across all three sites) by use of a multivariate approach, we performed multiple regression analyses in which demographic factors (sex, age, race), genotype group, and psychologic variables (active and passive coping, positive and negative affect, PILL scores, KRS scores) were all entered simultaneously. To determine whether the predictive models differed for women and men, separate regression analyses were performed for each sex. This also permitted the inclusion of oral contraceptive status in the model for women. The threshold for variable entry and retention in the model was $P < .10$. For men, significant predictors included genotype group, active coping, and KRS scores, accounting for 25.8% of the variance (Table 4). For women, the significant predictors included oral contraceptive use and the PILL score, which accounted for 11% of the variance (Table 4). To determine the total proportion of variance accounted for by OPRM1 genotype, another regression analysis was conducted for the total group of women and men combined. In this analysis, demographic factors (sex, age, race), genotype group, and psychologic variables (active and passive coping, positive and negative affect, PILL scores, KRS scores) were all entered first as one block, and then OPRM genotype was entered. Demographic and psychologic factors together accounted for 28.5% of the variance ($P < .001$). After these variables were accounted for, the OPRM genotype accounted for an additional 2.5% of the variance ($P = .02$).

**Discussion**

This study examined the association between the A118G SNP of OPRM1 and experimental pain responses in a large sample of healthy young adults. The frequency of rare alleles in our sample was similar to that previously reported. The findings indicate that subjects with one or more rare alleles exhibited lower sensitivity to pressure pain compared with wild-type subjects. Also, a
significant interaction between sex and genotype emerged for heat pain ratings at 49°C, indicating that the rare allele genotype was associated with lower pain ratings among men but higher pain ratings among women. A similar pattern of results emerged for heat pain tolerance but did not reach statistical significance (P = .08). In addition, consistent with previous findings, women reported lower heat pain tolerance, higher heat pain ratings, and lower pressure pain thresholds relative to men. It is important to note that sex was controlled in all analyses of genotype; therefore, the genotype effect is not confounded by sex. There were no significant associations between genotype and psychologic factors, although a marginally significant sex × genotype interaction emerged for positive affect. Multiple regression analysis showed that genotype accounted for a significant proportion of the variance pressure pain sensitivity over and above psychologic factors among men, whereas the multivariate model included only oral contraceptive use and PILL scores in women.

The reasons for the association between the A118G genotype of OPRM1 and mechanical pain sensitivity are not clear. One potential explanation is that the variant receptor has a greater binding affinity for β-endorphin in cell culture; therefore, individuals with the rare allele may realize more effective continuing endorphinergic endogenous pain inhibition (ie, opioid “tone”). This is merely a hypothesis, however, because the A118G SNP might simply be in linkage disequilibrium with the true functional variant, any one of the more than 40 known OPRM1 gene polymorphisms.

That the pattern of associations between genotype and pain perception varied across pain assays should not be surprising. Indeed, previous findings reveal only low-to-moderate correlations among responses to different pain assays, which suggests that disparate factors account for the variability observed in different pain modalities. Analogous studies with inbred mouse strains suggest that this dissociation is genetic, which is supported by recent human data indicating that the TRPV1 genotype was associated with cold pain but not heat pain responses and that the reverse was true for the OPRD1 genotype. Regarding our data, it is possible that the mechanism underlying the association preferentially or exclusively modulates mechanical pain. For example, some evidence suggests that descending opioidergic systems inhibit deep pain more than cutaneous pain does, which would be consistent with OPRM1 genotype being associated with pressure pain but not heat pain. However, an association of OPRM1 genotype with ischemic pain responses might be expected as well. Another possibility is that similar associations with the other pain assays were present but not strong enough to reach statistical significance with the current sample size. Indeed, inspection of the heat pain data among men reveals lower sensitivity in the rare allele group across all measures, with small to moderate effect sizes. Ultimately, more information regarding the specific functional effects of this A118G variant is needed before the mechanisms underlying the association can be directly addressed.

Although no significant interactions with sex emerged for pressure pain thresholds, visual inspection of the means and effect sizes suggests that the association between A118G and mechanical pain sensitivity may be stronger in men than in women. Also, the sex × genotype interaction was significant for heat pain ratings at 49°C and showed a similar direction for heat pain tolerance, although not statistically significant (P = .08), and these findings reflect slightly greater heat pain sensitivity among rare-allele women compared with their consensus female counterparts. Because of the relatively low frequency of the A118G SNP, we may not have sufficient power to determine the sex dependence of the association. However, previous findings in rodents and humans provide evidence of sex-dependent genetic influences on pain and analgesia, including the association of OPRD1 with heat pain responses emerging only in men. It is also intriguing that in a continuing full-genome quantitative trait locus (QTL) mapping study of nociceptive sensitivity in mice, a male-specific linkage is emerging at markers closest to the location of the mouse Oprm gene on chromosome 10 (J. S. Mogil et al, unpublished data). Thus, future examination of genetic contributions to pain sensitivity should include subjects of both sexes and should analyze for sex differences in strength of association.

Although, to our knowledge, an association between OPRM1 genotype and baseline pain responses has not been previously reported, associations of the A118G SNP

### Table 3. Psychologic Measures for Male and Female Subjects by OPRM1 Genotype

<table>
<thead>
<tr>
<th></th>
<th>MALE</th>
<th>FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA (n = 59)</td>
<td>AG or GG (n = 12)</td>
</tr>
<tr>
<td>CSQ active coping*</td>
<td>2.4 (1.0)</td>
<td>2.3 (1.0)</td>
</tr>
<tr>
<td>CSQ passive coping*</td>
<td>0.8 (1.0)</td>
<td>0.5 (0.6)</td>
</tr>
<tr>
<td>PANAS-positive affect</td>
<td>37.8 (5.1)</td>
<td>34.1 (6.5)</td>
</tr>
<tr>
<td>PANAS-negative affect</td>
<td>17.2 (5.1)</td>
<td>15.8 (5.7)</td>
</tr>
<tr>
<td>PILL score</td>
<td>88.6 (16.4)</td>
<td>94.5 (14.0)</td>
</tr>
<tr>
<td>KRS score*</td>
<td>59.0 (12.7)</td>
<td>57.4 (6.1)</td>
</tr>
</tbody>
</table>

*Main effect of sex, P < .05.
to pain-related phenomena have been found. For example, the μ-opioid agonist M6G produced diminished pupil constriction in individuals with the rare allele. More recently, Romberg et al reported that subjects heterozygous for the rare allele M6G had lower analgesic potency than did wild-type individuals. An intriguing case study of two patients with cancer revealed that a patient failing to respond to epidural morphine at doses up to 2000 mg was heterozygous for the rare allele and a meeting abstract reported an apparent association between OPRM1 and alfentanil analgesia. A number of case-control studies have reported the association of OPRM1 and opioid addiction/abuse, although others have failed to replicate these results. A recent study postulates that the pain sensitivity of the individual may affect opioid dependence through the OPRM1 gene. Finally, two studies have demonstrated that individuals with the rare allele display greater cortisol increases after opioid receptor blockade with naloxone. Obviously, continued research is needed to more fully characterize the importance of OPRM1 and other candidate genes in pain and analgesic responses.

The limitations of this study deserve mention. First, because this is the first study associating OPRM1 with experimental pain responses, replication in additional, larger samples will be needed to enhance confidence in the results. Association studies are plagued by the impression of irreproducibility, although a recent meta-analysis suggests that this is largely not due to false-positive studies, genetic heterogeneity (ie, true association in some populations but not others), or publication bias. Rather, many studies (including the current report) are underpowered and thus may miss true associations. Hence, we cannot rule out an association between OPRM1 and heat or ischemic pain. Although the ethnic composition of the wild-type and rare-allele groups was statistically balanced, there are large ethnic variations in A118G allele frequencies (eg, see Crowley et al), and population admixture has been proposed as an explanation for discrepant OPRM1 associations with heroin dependence. The current findings are based on a sample of healthy young adults, and it is not known whether the association between OPRM1 and pain sensitivity would remain if other sources of variability were introduced, such as age or clinical pain. These limitations notwithstanding, the findings indicate that a common SNP of the μ-opioid receptor gene appears to be associated with pressure pain sensitivity. Additional investigation is required to replicate this finding and to determine the underlying mechanisms.

Acknowledgments
This material is the result of work supported with resources and the use of facilities at the Malcom Randall VA Medical Center, Gainesville, Fla. We thank Beth Fisher for her technical assistance.

References
6. Coghill RC, McHaffie JG, Yen YF: Neural correlates of in-


52. Yeomans DC, Pirec V, Proudfoot HK: Nociceptive responses to high and low rates of noxious cutaneous heating are mediated by different nociceptors in the rat: behavioral evidence. Pain 68:133-140, 1996

53. Yeomans DC, Proudfoot HK: Nociceptive responses to high and low rates of noxious cutaneous heating are mediated by different nociceptors in the rat: electrophysiological evidence. Pain 68:141-150, 1996


学霸图书馆
www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，
提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页  文献云下载  图书馆入口  外文数据库大全  疑难文献辅助工具