Published in final edited form as:

Pain. 2016 April ; 157(4): 858–867. doi:10.1097/j.pain.0000000000000449.

Modification of *COMT*-dependent pain sensitivity by psychological stress and sex

Carolina B. Meloto[a], **Andrey V. Bortsov**[b], **Eric Bair**[c,d], **Erika Helgeson**[d], **Cara Ostrom**[d], **Shad B. Smith**[c,e], **Ronald Dubner**[f], **Gary D. Slade**[g], **Roger B. Fillingim**[h], **Joel D. Greenspan**[f], **Richard Ohrbach**[i], **William Maixner**[c,e], **Samuel A. McLean**[b], and **Luda Diatchenko**[a,*]

[a]The Alan Edwards Centre for Research on Pain, McGill University, McGill University Genome Building, Montreal, QC, Canada

[b]Department of Anesthesiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[c]Center for Pain Research and Innovation, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; Department of Endodontics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[d]Department of Biostatistics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[e]Center for Translational Pain Medicine, Duke University, Durham, NC, USA

[f]Department of Neural and Pain Sciences, University of Maryland School of Dentistry, Baltimore, MD, USA; Brotman Facial Pain Center, University of Maryland School of Dentistry, Baltimore, MD, USA

[g]Center for Pain Research and Innovation, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; Department of Dental Ecology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[h]Department of Community Dentistry and Behavioral Science, University of Florida, College of Dentistry, and Pain, Research and Intervention Center of Excellence, Gainesville, FL, USA

[i]Department of Oral Diagnostic Sciences, University at Buffalo, Buffalo, NY, USA

Introduction

Catechol-O-methyltransferase (COMT) is a widely expressed enzyme responsible for the O-methylation and deactivation of catechol-containing compounds, including epinephrine, norepinephrine, and dopamine [21]. Hence, COMT is an important regulator of extracellular concentrations of key neurotransmitters involved in numerous neurological functions, including pain perception. Despite being extensively influenced by environmental factors, the genetic component of pain is substantial and its heritability ranges from 22–60% [29, 30]. *COMT* is currently one of the most studied genes in pain genetics research [23], due to its critical involvement in pain transmission pathways.

*Corresponding author. Address: 740 Avenue Dr Penfield, Room 2200, Montreal, QC H3A0G1, Canada. Tel.: 514 398-2878; fax: 514 398-8900. luda.diatchenko@mcgill.ca (L. Diatchenko).

The *COMT* gene carries many common single nucleotide polymorphisms (SNPs) along its sequence. The most investigated single SNP is rs4680 (*val*^{158}*met*) [19], whose *met* allele is associated with greater pain sensitivity [44]. This SNP is also integrated into one of the three major functional *COMT* haplotypes initially identified by our study group [9], together with SNPs rs6269 and rs4633, and rs4818. The most frequently occurring haplotype, consisting of alleles ATCA (48.7%), is associated with intermediate pain responsiveness and named APS for average pain sensitivity. The second most frequent (GCGG, 36.5%) haplotype is associated with lowest pain responsiveness and named LPS (low pain sensitivity). The least frequent haplotype (ACCG, 10.5%) is associated with highest responsiveness to pain and named HPS (high pain sensitivity) [9]. Similar frequencies of *COMT* haplotypes and their contribution to different pain-related phenotypes have also been reported in other studies [14, 22, 31, 40, 42]. Although the molecular genetic mechanisms whereby the APS and HPS haplotypes regulate COMT activity are different [19, 27], both encode reduced enzymatic activity compared to LPS. Subsequently, elevated levels of epinephrine activating beta adrenergic receptors was demonstrated as the mechanism whereby reduced COMT activity leads to exacerbated pain perception [28].

Numerous studies have investigated the association between *COMT* genetic variants and multiple pain phenotypes, but results have not always been consistent [39]. Among many non-genetic reasons that may account for this lack of consistency and have been discussed elsewhere [2], genetic effects are often modified by interactions with environment and sex, especially for genetic variants of substantial minor allele frequency and modest effect size [24].

Among environmental factors, exposure to stress and its consequent release of epinephrine [10, 38] is likely to affect the same pain pathways as COMT [28]. Additionally, *COMT* is less expressed in females [5, 7, 21, 35, 43], which may account for its sexually dimorphic effects [3, 16, 34]. We thus hypothesized that the relationship between common functional *COMT* genetic variants, such as the *COMT* haplotypes, and pain would be greatly influenced by stress and sex. We therefore investigated if *COMT* haplotypes, stress, and sex interact to modify sensitivity to various pain modalities ranging from experimental pain to clinical acute pain.

Material and Methods

Phenotypic data were obtained from two independent multicenter cohorts fully described elsewhere [20, 32, 36] and briefly described here. Approvals of the institutional review boards were obtained from each of the sites and data-coordinating centers participating in the *OPPERA* (Orofacial Pain: Prospective Evaluation and Risk Assessment) and in the *post-MVC* (motor vehicle collision) studies. All subjects participating in both studies provided signed informed consent.

OPPERA: study setting and participants

The *OPPERA* cohort consists of males and female study volunteers from multiple ethnicities and aged between 18 to 44 years who were enrolled at four U.S. study sites. It was primarily designed to identify determinants of temporomandibular disorders (TMD) [20]. Between

May 2006 and November 2008, subjects from Baltimore (MD), Buffalo (NY), Chapel Hill (NC), and Gainesville (FL) were recruited to participate in the study by advertisements, e-mails, flyers, and word of mouth. In total, 3,263 TMD-free controls and 185 chronic TMD cases were enrolled. Detailed demographic description of the full cohort has been previously reported [36]. After removing participants who were missing genetic data or other critical phenotypic data, 2,972 were considered eligible for this study. The mean age of the included study subjects was 26.9 years, consisting in 1,274 (42.9%) males and 1,698 (57.1%) females. The cohort is racially diverse, with 1,578 (53.1%) non-Hispanic white subjects, 845 (28.4%) African-Americans, 259 (8.7%) Asians, 203 (6.8%) Hispanics, and 87 (2.9%) subjects of other or mixed races.

OPPERA: phenotype collection

In this cohort, subjects completed a 3-hour clinic visit that included questionnaires, blood sample collection, clinical examination, quantitative sensory testing (QST) and measures of blood pressure, heart rate and heart rate variability, the latter done at rest and following orthostatic and psychological challenge. Since measures of response to repeated thermal stimuli provide the strongest association with *COMT* haplotypes [3, 8], we focused on these measures in the present study.

A detailed description of the QST procedures have been provided elsewhere [15]. Briefly, thermal pain sensitivity was assessed using a commercially available thermal stimulator (Pathway; Medoc; Ramat Yishai, Israel). Prior to testing, subjects were instructed to verbally rate their peak pain intensity after each pulse using a 0–100 numerical scale, where “0” represented “no pain” and “100” represented “the most intense pain imaginable”. Participants were also told that they would receive a series of 10 pain-evoking thermal stimuli in a row, and would be asked to report their peak pain intensity after each stimulus. Practice trials were administered to provide the participant a sense of the timing of stimulus delivery and to verify understanding of the protocol. The thermode (5.73 cm^2) was manually placed on the skin of the ventral forearm at a temperature of 38°C, and 10 temperature pulses were given at 2.4 to 2.5 second inter-stimulus intervals. For the first series of stimuli, the peak temperature was 46°C, with a ramp rate of 20°C/second, and a hold time of 750 msec at the peak temperature. Following this, the thermode was moved to another location on the forearm, and the same protocol was conducted with a peak temperature of 48°C. This was followed by another test series with a 50°C peak temperature at a third location. For each temperature, a total of ten response measures to repeated thermal stimuli were collected. The data were used to compute three analytic variables: *first pulse*, defined as the peak pain intensity to the first of 10 thermal pulses at each of the three temperatures; area under the curve (*AUC*), defined as the overall responsiveness to the 10 repeated thermal stimuli within each of the series; and *maximum pain*, defined as the highest peak pain intensity reported by the subject during each of the three series of stimuli (Supplementary Figure 1).

After the pain sensitivity tests, a cognitive test (STROOPx2) challenging subjects to name the color of a word that is printed in a different color (e.g., the word "red" printed in blue, in which case “blue” would be the correct answer) was conducted. At the end of this, subjects

were asked to rate how stressful they found the entire experimental session to be using a 0–100 numerical rating scale (NRS) where “0” represented “not at all stressful” and “100” represented “extremely stressful”. The NRS value indicated by the subject was used as the measure of stress in this study, since it was the stress assessment carried out closest in time to the QST session and most importantly, we trust that it best represents the subject’s global stress level during the entire QST session.

OPPERA: genotyping

Genomic DNA was purified from blood samples using Qiagen Extraction Kits from Cogenics, Inc (now Integrated Laboratory Systems, Morrisville, NC). Genotypic data were obtained using the Pain Research Panel (Algynomics, Chapel Hill, NC), a chip-based platform that consists of 3,295 SNPs representing 358 genes known to be involved in systems relevant to pain perception [37]. *COMT* SNPs rs6269 and rs4818, and SNPs rs4633 and rs4680, are in complete linkage disequilibrium (LD) in the Caucasian population. In the Pain Research Panel, SNPs rs6269 and rs4633 tag SNPs rs4818 and rs4680, respectively, and were thus used for *COMT* haplotypes reconstruction using HaploView [1].

Post-MVC: study setting and participants

The *post-MVC* study is a prospective multicenter cohort of 948 European American males and females aged 18 to 65 years [32]. The primary goal of this study was to gain new insights into the pathophysiology of persistent pain and psychological sequelae after minor MVC. Individuals presenting to one of the participating research network emergency department (ED) sites within 24 hours after a minor MVC were recruited. Participating sites included the Baystate Medical Center, Massachusetts General Hospital, North Shore University Hospital, Shands Jacksonville Hospital, Spectrum Health Butterworth Hospital, St. Joseph Mercy Hospital, and William Beaumont Hospital (2 sites). A research assistant stationed in each of the eight study site EDs approached potentially eligible patients and they were asked to participate in the study after the voluntary nature of their participation, risks and benefits of the study had been explained. Following consent, study participants completed study procedures described below. From the 10,629 individuals who presented to the ED within 24 hours after a minor MVC, 1,416 were considered eligible, 969 agreed to participate in the study, and 948 completed the evaluation. The mean age of study participants was 36 years, of which 575 (61%) were females and 373 (39%) were males.

Post-MVC: phenotype collection

For this study, we focused on two phenotypes collected within 24 hours after the MVC: *pain intensity*, defined as the overall pain intensity at the time of evaluation, and *distress level*, as determined by the Peritraumatic Distress Inventory [6]. Current pain intensity was assessed using a numeric rating scale (NRS) ranging from 0 (‘no pain’) to 10 (‘worst pain possible’). If the participants reported pain, they were also asked whether the pain was related to the MVC; only MVC-related pain (greater than 99% of all pain reported) was included in the present analyses. Peritraumatic distress (score range from 0 to 52) was assessed using the Peritraumatic Distress Inventory (PDI). The PDI is a reliable and valid instrument for measuring distress during the peritraumatic period [6, 41].

Post-MVC: genotyping

Blood (8.5cc) was collected using PAXgene DNA storage tubes. Blood samples were then refrigerated at the study site and shipped in batches every 2 weeks to Beckman Coulter Genomics, Inc, Morrisville, NC. DNA purification was performed using the PAXgene™ blood DNA kit (Qiagen, Valencia, CA). Genotyping was performed in batches using the Sequenom platform (Sequenom, Inc., San Diego, CA). The LD between *COMT* SNPs was explored by calculating Levontin D' and squared correlation r^2 using HaploView [1]. Haploblocks were estimated using the method of confidence intervals [12]. *COMT* haplotypes and their population frequencies were estimated using the expectation-maximization algorithm implemented in HaploView and were then verified using Bayesian estimation of haplotype frequencies implemented in HAPLOTYPE procedure (SAS version 9.2, SAS Institute, Cary, NC) [18]. Detailed linkage disequilibrium and haplotype structure of *COMT* in this cohort have been reported elsewhere [4], and corresponded to those identified in the discovery *OPPERA* cohort and in the original publication [9].

Data analysis

The association between pain sensitivity and *COMT* haplotype, stress, and sex in the *OPPERA* cohort was evaluated using regression models. Given that the *COMT* haplotypes affect pain sensitivity in both healthy control and TMD-case subjects, both control and TMD-case subjects were included in the analysis. The outcome variable in each model was one of the measures of thermal pain sensitivity (first pulse, AUC, or maximum pulse at 46°C, 48°C, or 50°C). Prior to fitting the model, each outcome variable was normalized to have mean 0 and standard deviation 1 to ensure that regression coefficients for the different outcomes had comparable scales. Predictors in the model included a dummy variable for female sex, a dummy variable for study site, the participant's self-reported stress rating, and dummy variables for the number of APS or HPS haplotypes carried by each participant. The LPS haplotype was treated as the reference group, so the count of LPS haplotypes was not included in the model. All two-way and three-way interactions were also included in the model. To control for the effects of population stratification due to racial heterogeneity in the *OPPERA* cohort, ancestral eigenvectors were computed for each *OPPERA* participant using the Eigenstrat software [33]. The first six eigenvectors were included as covariates in the model. Inverse probability weighting was used to adjust for the case-control study design (which caused chronic TMD cases to be overrepresented in our sample). Variance estimates were computed using weighted generalized estimating equations to compute the sandwich estimator of the variance [26]. The prevalence of chronic TMD in the population was assumed to be 5% [17]. The threshold of statistical significance was set at P=0.05. The finding was further highlighted when P<0.006, because it is consistent with a strict Bonferroni correction for tests of nine thermal pain measures. However, this degree of correction is overly cautious, given that the nine measures were correlated. Later, this analysis was repeated using only the count of HPS haplotypes in the model (APS was not included). In other words, the second series of models included the count of HPS haplotypes as a predictor variable but not the count of APS haplotypes. Considering that TMD-case subjects are more likely to be female and to carry the HPS haplotype, which may not be

sufficiently controlled for by inverse probability weighting, another series of models using only the count of HPS haplotypes and control subjects was conducted.

The predicted values of the second series of models were plotted against the count of HPS haplotypes. Three fixed values of self-reported stress were considered, namely 0, 10, and 20 (the latter two values corresponding roughly to the 50^{th} and 75^{th} percentile of the distribution of self-reported stress), and the predicted values were calculated separately for males and females and for each of the two values of stress. When calculating these predicted values, the study site was assumed to be North Carolina and the values of all six ancestral eigenvectors were set to be 0. Each pain measure was also plotted against self-reported stress separately for those with 0, 1, or 2 copies of the HPS haplotype, and a loess curve was calculated and plotted to visualize the association between stress and pain. The analysis was also repeated separately for males and females.

A final set of models repeated the analysis separately for males and females but treated stress as a dichotomous variable. Rather than using the 0–100 self-reported stress rating as a covariate, this covariate was replaced with a dummy variable that was equal to 1 if the participant reported any stress during the procedure (i.e., a stress rating of greater than 0) and 0 otherwise. Contrasts were used to evaluate the effect of the HPS haplotype in both the “stressed” and “non-stressed” groups.

The associations between *COMT* haplotypes and pain outcomes in the *post-MVC* cohort were assessed using general linear models. Study site was included in these models as a set of dummy variables to account for potential genetic heterogeneity between study recruitment centers. Interactions between *COMT* haplotypes, peritraumatic distress, and patient sex on acute pain severity were assessed by introducing the corresponding product terms into the model. Significance of the model parameter estimates was evaluated using the t-statistic. In order to evaluate the stability of the interaction estimate, we re-fitted the model in 1000 bootstrap samples, and output the 95% confidence intervals for the interaction term estimate.

Results

OPPERA cohort: experimental thermal pain sensitivity

Data for this study were collected from 2,972 female and male subjects who were enrolled in the *OPPERA* cohort. In the present study, we have investigated if the effect of *COMT* haplotypes on pain sensitivity can be modified by interactions with stress and sex, by means of a three-way interaction analysis. The distribution of study subjects according to sex, stress level, and *COMT* haplotypes can be found in Table 1. Because thermal pain, especially measures of response to repeated thermal stimuli, is the sensory domain most strongly associated with *COMT* haplotypes [3, 8], different measures of response to repeated thermal stimuli were tested (Supplementary Figure 1). This analysis was conducted with all three *COMT* haplotypes in the model (LPS, APS, and HPS), using the LPS haplotype and male sex as reference haplotype and sex, respectively. Subjects’ ratings of how stressful they found the entire QST session to be was used as the stress measure, since it was the closest

stress assessment to the thermal pain sensitivity procedures. The median stress score was 10, the interquartile range went from 1–20, and 24.5% of subjects reported a score of zero.

In the *OPPERA* cohort, the three-way interaction was not significant for any of the measures of thermal pain sensitivity tested (Supplementary Table 1). Nonetheless, the two-way interaction between HPS haplotype and stress was significant for all temperatures of the "*AUC*" and "*maximum pain*" measures, such that the effect of stress on pain decreased as the number of HPS haplotypes increased (Table 2). Four of these interactions were significant even after strict Bonferroni correction ($P<0.006$). Two-way interactions between APS haplotype and stress, between APS or HPS haplotype and sex, and between stress and sex were not significant for any of the measures of thermal pain sensitivity tested (Table 2). Additionally, the main effects of stress and sex, but not *COMT* haplotypes, were associated with all measures of thermal pain sensitivity tested, with stress and female sex being significantly associated with increased thermal pain sensitivity (Table 2).

Because neither the main effect of APS haplotype nor any of its interactions were statistically significant, a new analysis was conducted excluding the APS haplotype from the model, and focusing on the count of HPS haplotype. The two-way interaction between HPS haplotype and stress was statistically significant for all temperatures of the "*AUC*" and "*maximum pain*" measures, such that presence of the HPS haplotype conferred a weaker association between stress levels and pain (Table 3). Virtually the same results were obtained when only control subjects were included in the analysis (Supplementary Table 2). Thus, in order to increase the power of generalization of our study, all subsequent analyses were done including both controls and TMD cases. The weakening effect of the HPS haplotype on the association between stress levels and pain is illustrated in Supplementary Figure 2, which displays the relationship between pain sensitivity and stress in individuals carrying 0, 1, or 2 copies of the HPS haplotype for one representative measure of thermal pain sensitivity (*AUC* at 48°C). The relationship between thermal pain sensitivity and stress in individuals carrying no copies of this haplotype was characterized by a steep slope, in which thermal pain sensitivity increased with the increased report of stress. This slope though became gradually flatter as the number of copies of the HPS haplotype increased. Additionally, the two-way interaction between stress and sex was significant for "*AUC*" and "*maximum pain*" at 50°C, such that the association between stress and pain was weaker for females compared to males (Table 3). The two-way interaction between HPS haplotype and sex was not statistically significant for any of the measures of thermal pain sensitivity tested (Table 3). Also, the main effect of the HPS haplotype was statistically significant at virtually all temperatures for the "*AUC*" and "*maximum pain*" measures. HPS haplotype count, stress, and female sex were associated with higher thermal pain sensitivity (Table 3).

Results of the analysis including only the HPS haplotype in the model also suggested that the main effects of stress and female sex, both contributing to increased thermal pain sensitivity, may be superseded by the interaction between them. This finding needs to be interpreted with caution because the interaction effects were seen for the measures of "*AUC*" and "*maximum pain*" at 50°C. Since stress and female sex are independently associated with higher thermal pain sensitivity, it might be that at 50°C, the highest temperature used in the QST procedures, females reporting stress reach the plateau of their pain sensitivity quicker

than males not reporting stress. Consequently, the increasing effect of stress on pain sensitivity of females becomes lower possibly due to ceiling effects, as denoted by the effect of the two-way interaction between stress and gender for "*AUC*" and "*maximum pain*" at 50°C (Table 3).

Finally, we dichotomized our research subjects by their report of stress and compared the effect of the HPS haplotype on thermal pain sensitivity in individuals who reported no stress to individuals who reported any stress for each sex separately (Supplementary Table 3). These results showed that in females or males reporting no stress, there was a strong dose-dependent effect of the HPS haplotype, in which each additional copy of the haplotype was associated with higher pain sensitivity for all measures of thermal pain sensitivity tested (solid lines, Figure 1 and Supplementary Figure 3). This association, however, was absent in females or males reporting any stress, and its increasing effect on pain sensitivity leading to such a loss of effect is also shown in Figure 1 and Supplementary Figure 3. Figure 1 also illustrates that females are generally more sensitive to thermal pain, as denoted by the upward lift in the thermal pain sensitivity curve of females compared to that of males.

Altogether, results of the analyses performed for experimental thermal pain sensitivity indicate that HPS haplotype, stress, and sex contribute to pain sensitivity and their main effects may be further modified by interactions between them. Specifically, the effect of stress on pain sensitivity is modified by its interaction with the HPS haplotype, so that the increasing effect of stress on pain decreases as the number of copies of the HPS haplotype increases. Additionally, even though the effect of this interaction shows no difference between sexes, the response curves of females and males are shifted relative to each other due to the higher general pain sensitivity of females. With these results in mind, we then tested the effect the interaction among HPS haplotype, stress, and sex on an independent cohort of individuals with clinical acute pain.

Post-MVC cohort: clinical acute pain

For this study, data were collected from 948 female and male subjects enrolled in the *post-MVC* cohort. Here, we tested the effect of the interaction among HPS haplotype, stress, and sex on clinical acute pain severity. The distribution of study subjects according to sex, stress level, and *COMT* haplotypes can be found in Table 4. Clinical acute pain severity was defined as the rating of subjects of their overall pain intensity (0–10 NRS) when presenting at the ED. Analysis was done including only the HPS haplotype in the model, and using males as the reference sex. It included a set of dummy variables for study site, to adjust for potential heterogeneity of the study population between sites. Peritraumatic distress was included in the model as the Peritraumatic Distress Inventory (PDI) total score. The median PDI score was 18, the interquartile ranged from 12–26, the minimum value was of 0 and maximum of 48, and 1.5% of subjects reported a score of zero.

This analysis revealed that the effect of the three-way interaction among HPS haplotype, stress, and sex on clinical acute pain severity was statistically significant (p=0.0097, Table 5, Model 1). In order to clarify how HPS haplotype, sex, and stress interact to modify clinical acute pain sensitivity, we tested the effect of the interaction between HPS haplotype and stress on females and males separately (Models 2–3, Table 5). We observed that the effect of

the HPS haplotype on pain sensitivity was modified by stress in males only, as denoted by the significant interaction between HPS haplotype and stress in males (p=0.013), but not in females (p=0.484).

We then dichotomized the peritraumatic distress score into high distress (PDI score > 18) and low distress (PDI score ≤ 18) and compared the effect of the count of HPS haplotype on pain sensitivity in males with *low* distress, males with *high* distress, females with *low* distress and females with *high* distress (Table 5, Models 4–7). This analysis demonstrated a significant effect of the HPS haplotype on acute pain severity only in males with *low* stress (p = 0.008). In this group, we observed a 1.2-point increase in pain severity per each copy of the HPS haplotype. This effect is illustrated in Figure 2, which shows a significant dose-dependent effect of the HPS haplotype on pain sensitivity among males with *low* stress only; the effect of HPS haplotype is absent in males with *high* stress and in females with both *low* and *high* stress. Finally, we evaluated the association between distress (treated as a continuous PDI score) and pain severity separately in males and females carrying zero vs 1 or 2 HPS haplotypes. We observed no association between distress and pain in males with one or two copies of HPS haplotype (regression coefficient beta = 0.007, SE = 0.048; p = 0.88) and a strong positive association in males without HPS haplotype (beta = 0.076; SE = 0.014; p < 0.0001). In females, the association between distress and pain severity was significant and was not affected by the presence or absence of HPS haplotype (beta = 0.077, SE = 0.023, p = 0.001 in females with 1 or 2 copies of HPS haplotype; beta = 0.054, SE = 0.012, p < 0.001 in females with no HPS haplotypes).

Overall, similar to what was observed in the *OPPERA* cohort relative to experimental thermal pain sensitivity, analyses of this clinical acute pain cohort demonstrate that HPS haplotype, stress, and sex contribute to acute pain after MVC. Specifically, the effect of stress on pain sensitivity is modified by its interaction with the HPS haplotype and sex, so that the increasing effect of stress on pain report decreases as the number of copies of the HPS haplotype increases in males but, unlike what was observed in the *OPPERA* cohort, virtually absent in females.

Discussion

Results obtained for both the experimental thermal pain and clinical acute pain studies demonstrate that HPS haplotype, stress, and sex interact with each other and modify pain sensitivity. Based on our results, we suggest that stress and sex may impact the ability to detect COMT-dependent pain sensitivity due to their effects on the adrenergic system. Specifically, because low COMT activity (HPS haplotype) and exposure to stress can increase the epinephrine load in the system, an increase to pain sensitivity may also result. This relationship, however, may reach a plateau under a wide range of physiological conditions. This suggests that COMT-dependent pain sensitivity can be ideally observed in states in which this relationship has not yet reached such a plateau, making the detection of this association somewhat dependent upon factors that can affect this relationship. Inferences based on our results are summarized on Figure 3.

Based on our findings, stress is one of the key factors producing such an effect, and therefore, impeding the ability to detect the HPS haplotype contribution to pain sensitivity. Because exposure to stress leads to a tonic increase in the release of epinephrine [38], it contributes to enhancement of adrenergic system activity, thereby increasing the epinephrine load in the system in proportion to the intensity of the stressor event. In our study, the stressor event in the *OPPERA* cohort was the quantitative sensory testing (QST) session itself, which is likely to produce a lower stress response (i.e., release of epinephrine) than a motor vehicle collision. Consequently, if the exposure to stress is strong enough to saturate the adrenergic system, the association between the HPS haplotype and pain sensitivity will no longer be observable. Indeed, our findings show no significant association between the HPS haplotype and pain sensitivity in subjects under stress in both the *OPPERA* and *post-MVC* cohorts (Figure 3, *OPPERA*-any stress and *post-MVC*-high stress lines). Interestingly, experimental pain testing has been reported before as a stressful factor, masking genetic association results [25].

Another factor that may impact the detection of COMT-dependent pain sensitivity is sex, as indicated by the significant interaction among HPS haplotype, stress, and sex in the *post-MVC* cohort. Females are known to express less COMT due to estrogenic down-regulation [5, 7, 21, 35, 43], and thus to exhibit higher baseline COMT-dependent pain sensitivity. Consequently, at the same epinephrine level, females should show higher pain sensitivity than males (Figure 3). This means that pain sensitivity would reach its plateau faster in females and thus, the association between the HPS haplotype and pain sensitivity would no longer be observable at lower levels of epinephrine in females compared to males. Indeed, our findings demonstrate no significant association between the HPS haplotype and pain sensitivity even in females categorized as low stress, but exposed to a strong stressor event, as in the *post-MVC* cohort (Figure 3, *post-MVC*-low stress line). On the other hand, for females in the *OPPERA* cohort, pain sensitivity was likely still within the linear range of its relationship with epinephrine load. As a result, the effect of the HPS haplotype on pain sensitivity was still detectable (Figure 3, *OPPERA*-no stress line).

Our findings suggest that both the HPS haplotype and stress, the former coding for low COMT activity and the latter leading to release of epinephrine, may contribute to augmenting the epinephrine load in the system and thus, to heighten pain sensitivity. Consequently, the association between the HPS haplotype and pain sensitivity would be more easily observed if the adrenergic system has not yet been overloaded with epinephrine. Otherwise, COMT-dependent pain sensitivity may have already reached its plateau, masking the contribution of the HPS to pain phenotypes. In light of these observations, we believe that it is important to maintain the stress level at minimum or to control for stress level when investigating the association between the HPS haplotype and pain phenotypes.

Furthermore, the present findings indicate that controlling for stress may be even more critical for the detection of the association between the HPS haplotype and pain sensitivity in females, as the relationship between epinephrine load and pain sensitivity may plateau faster than in males. This means that, in case of an increase in the epinephrine load, the association between the HPS haplotype and pain sensitivity would be masked faster in females, potentially obfuscating associations between *COMT* genetic variants and pain

phenotypes. Thus, we believe that it is also crucial to separate females from males or to adjust for gender when investigating the association between the HPS haplotype and pain phenotypes.

Such stress and sex-dependent effects are not unique to the *COMT* gene. For instance, SNP rs10877969 in the vasopressin-1A receptor gene (*V1AR*) has been shown to influence capsaicin pain levels exclusively in male subjects reporting stress. Subsequent experiments in mice confirmed this male-specific interaction of V1AR and stress, and demonstrated that vasopressin activates endogenous analgesia mechanisms unless they have already been activated by stress [25].

It should be noted that the stress measures used both in the *OPPERA* and *post-MVC* cohorts are not identical, nor are they pure measures of stress. In the *OPPERA* cohort, psychological stress was measured at the end of the entire QST session and was operationalized by subjects' numerical ratings of how stressful they found that session to be. It is uncertain how much of this rating is accounted by the subjects' stress response to the the pain experienced during the QST session or the challenge experienced during the end-of-the-session cognitive test. Considering that subjects had never experienced a QST session before, it is conceivable that they might be feeling stressed in response to the instructions to the QST session and fear of the upcoming pain-evoking stimuli. In fact, in a similar experimental noxious stimuli test for vasopressin agonist responses to capsaicin-induced pain, subjects expressed much higher stress response in the first testing session than in the second one [25]. In the *post-MVC* cohort, stress was measured using the Peritraumatic Distress Inventory, in which subjects rate the extent to which items such as life threat, loss of control, helplessness/anger were experienced during and immediately after the MVC. The questionnaire was administered to the subjects in the ED, that is, at the same time they were asked to rate their overall pain intensity. Hence, it is also uncertain how much of the stress score is accounted by the stressfulness of the MVC versus the stress triggered by MVC-related pain. However, even if these stress measures are not ideal, we believe that they are, at least in part, a function of the subjects' underlying stress reactivity. Additionally, because the primary goal of both cohorts was not to investigate the causal relationship between pain and stress, their designs do not allow us to make conclusions regarding the directionality of effects. That is, although pain was the outcome variable in our regression models, it is equally plausible that pain may cause stress.

Few recent studies have provided evidence that *COMT* genetic variants interact with other psychological phenotypes that potentially have a stress reactivity component. The HPS haplotype was shown to be a robust predictor of persistent shoulder pain when combined with pain catastrophizing [13, 14]. In another study, the *COMT met* allele interacted with pain catastrophizing to produce more pain on days when pain catastrophizing was higher [11]. In these studies low activity COMT variants were additive to catastrophizing, which may reflect the fact that the measures were related to trait rather than state stress reactivity. The interaction between *COMT* genotypes and catastrophizing reinforce the importance of epinephrine load for pathophysiology of pain states.

Finally, we propose that the presented gene (*COMT*) – environment (stress) – sex relationship deepens the current understanding of the pathophysiology of the COMT contribution to pain phenotypes. Clinically, we believe that combined – *COMT* haplotype, stress level, and sex – may represent an important risk factor contributing to heighten pain sensitivity in different pain conditions.

In conclusion, our findings indicate that future studies aiming to investigate the relationship between *COMT* haplotypes and pain phenotypes – and most likely other conditions in which epinephrine contributes to the pathophysiology of pathological states – need to be aware that stress and sex are important components of this relationship. In fact, the strongest association between HPS haplotype and pain was observed in the subgroup of males who reported no stress, which comprises the smallest subgroup analyzed (n = 331). In other words, in order to be able to identify the association of *COMT* haplotypes with a given phenotype or condition, one should ideally ensure that phenotypes are being collected within the linear range of this relationship, before such effects have already caused COMT dependent pain sensitivity to plateau (Figure 3). Further development of new statistical approaches are required that will allow to measure association between genetic variants and behavioral phenotypes in a dynamic manner rather than a static one.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank all participants of the *OPPERA* and *post-MVC* cohorts for their contribution. We also would like to acknowledge Dr. Bruce Weir for his contribution for the genetic design of the *OPPERA* cohort. This work was funded by the Canadian Excellence Research Chairs (CERC) Program (http://www.cerc.gc.ca/home-accueil-eng.aspx), grant CERC09 to LD; by the National Institute of Dental and Craniofacial Research (http://www.nidcr.nih.gov/), National Institutes of Health (NIH), grants U01DE017018 to LD WM GDS RBF JDG RO and R01DE016558 to LD; by Pfizer Research Funds (http://www.pfizer.com/responsibility/grants_contributions/grants_and_contributions) to LD; and by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Award Number R01AR056328 to SM. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Drs. Slade, Fillingim, Smith, Maixner, and Diatchenko have equity ownership in Algynomics, Inc., the commercial provider of the genotyping platform used in this article. Drs. Slade, Maixner, and Diatchenko are consultants to the company; Drs. Maixner and Diatchenko are on the Board of Directors. Drs. Maixner and Diatchenko are inventors on a patent application related to COMT diplotypes and pain sensitivity that has been licensed to Proove Biosciences. These relationships have been reviewed in conjunction with this research and are under management by UNC–Chapel Hill.

References

1. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2):263–265. [PubMed: 15297300]
2. Belfer I, Segall S. COMT genetic variants and pain. Drugs of today. 2011; 47(6):457–467. [PubMed: 21695287]
3. Belfer I, Segall SK, Lariviere WR, Smith SB, Dai F, Slade GD, Rashid NU, Mogil JS, Campbell CM, Edwards RR, Liu Q, Bair E, Maixner W, Diatchenko L. Pain modality- and sex-specific effects of COMT genetic functional variants. Pain. 2013; 154(8):1368–1376. [PubMed: 23701723]

4. Bortsov AV, Diatchenko L, McLean SA. Complex multilocus effects of catechol-O-methyltransferase haplotypes predict pain and pain interference 6 weeks after motor vehicle collision. Neuromolecular Med. 2014; 16(1):83–93. [PubMed: 23963787]

5. Boudilkova B, Szumlanski C, Maidak B, Weinshilboum R. Human liver catechol-O-methyltransferase pharmacogenetics. Clinical Pharmacology and Therapeutics. 1990; 48(4):381–389. [PubMed: 2225698]

6. Brunet A, Weiss DS, Metzler TJ, Best SR, Neylan TC, Rogers C, Fagan J, Marmar CR. The Peritraumatic Distress Inventory: a proposed measure of PTSD criterion A2. The American journal of psychiatry. 2001; 158(9):1480–1485. [PubMed: 11532735]

7. Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet. 2004; 75(5):807–821. [PubMed: 15457404]

8. Diatchenko L, Nackley AG, Slade GD, Bhalang K, Belfer I, Max MB, Goldman D, Maixner W. Catechol-O-methyltransferase gene polymorphisms are associated with multiple pain-evoking stimuli. Pain. 2006; 125(3):216–224. [PubMed: 16837133]

9. Diatchenko L, Slade GD, Nackley AG, Bhalang K, Sigurdsson A, Belfer I, Goldman D, Xu K, Shabalina SA, Shagin D, Max MB, Makarov SS, Maixner W. Genetic basis for individual variations in pain perception and the development of a chronic pain condition. Human molecular genetics. 2005; 14(1):135–143. [PubMed: 15537663]

10. Dronjak S, Jezova D, Kvetnansky R. Different effects of novel stressors on sympathoadrenal system activation in rats exposed to long-term immobilization. Ann N Y Acad Sci. 2004; 1018:113–123. [PubMed: 15240359]

11. Finan PH, Zautra AJ, Davis MC, Lemery-Chalfant K, Covault J, Tennen H. COMT moderates the relation of daily maladaptive coping and pain in fibromyalgia. Pain. 2011; 152(2):300–307. [PubMed: 21130573]

12. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The structure of haplotype blocks in the human genome. Science. 2002; 296(5576):2225–2229. [PubMed: 12029063]

13. George SZ, Dover GC, Wallace MR, Sack BK, Herbstman DM, Aydog E, Fillingim RB. Biopsychosocial influence on exercise-induced delayed onset muscle soreness at the shoulder: pain catastrophizing and catechol-o-methyltransferase (COMT) diplotype predict pain ratings. The Clinical journal of pain. 2008; 24(9):793–801. [PubMed: 18936597]

14. George SZ, Wallace MR, Wright TW, Moser MW, Greenfield WH 3rd, Sack BK, Herbstman DM, Fillingim RB. Evidence for a biopsychosocial influence on shoulder pain: pain catastrophizing and catechol-O-methyltransferase (COMT) diplotype predict clinical pain ratings. Pain. 2008; 136(1–2):53–61. [PubMed: 17686583]

15. Greenspan JD, Slade GD, Bair E, Dubner R, Fillingim RB, Ohrbach R, Knott C, Mulkey F, Rothwell R, Maixner W. Pain sensitivity risk factors for chronic TMD: descriptive data and empirically identified domains from the OPPERA case control study. The journal of pain : official journal of the American Pain Society. 2011; 12(11 Suppl):T61–T74. [PubMed: 22074753]

16. Harrison PJ, Tunbridge EM. Catechol-O-methyltransferase (COMT): a gene contributing to sex differences in brain function, and to sexual dimorphism in the predisposition to psychiatric disorders. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2008; 33(13):3037–3045. [PubMed: 17805313]

17. Isong U, Gansky SA, Plesh O. Temporomandibular joint and muscle disorder-type pain in U.S. adults: the National Health Interview Survey. J Orofac Pain. 2008; 22(4):317–322. [PubMed: 19090404]

18. Lin S, Cutler DJ, Zwick ME, Chakravarti A. Haplotype inference in random population samples. Am J Hum Genet. 2002; 71(5):1129–1137. [PubMed: 12386835]

19. Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melen K, Julkunen I, Taskinen J. Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry. 1995; 34(13):4202–4210. [PubMed: 7703232]

20. Maixner W, Diatchenko L, Dubner R, Fillingim RB, Greenspan JD, Knott C, Ohrbach R, Weir B, Slade GD. Orofacial pain prospective evaluation and risk assessment study--the OPPERA study. The journal of pain : official journal of the American Pain Society. 2011; 12(11 Suppl):T4–T11. e11–e12. [PubMed: 22074751]
21. Mannisto PT, Kaakkola S. Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacological reviews. 1999; 51(4):593–628. [PubMed: 10581325]
22. McLean SA, Diatchenko L, Lee YM, Swor RA, Domeier RM, Jones JS, Jones CW, Reed C, Harris RE, Maixner W, Clauw DJ, Liberzon I. Catechol O-methyltransferase haplotype predicts immediate musculoskeletal neck pain and psychological symptoms after motor vehicle collision. The journal of pain : official journal of the American Pain Society. 2011; 12(1):101–107. [PubMed: 20688576]
23. Mogil JS. Pain genetics: past, present and future. Trends in genetics : TIG. 2012; 28(6):258–266. [PubMed: 22464640]
24. Mogil, JS.; Diatchenko, L.; Fillingim, R. An introduction to Pain. In: Gold, M., editor. An Updated Review: Refresher Course Syllabus. Seattle: IASP Press; 2014.
25. Mogil JS, Sorge RE, LaCroix-Fralish ML, Smith SB, Fortin A, Sotocinal SG, Ritchie J, Austin JS, Schorscher-Petcu A, Melmed K, Czerminski J, Bittong RA, Mokris JB, Neubert JK, Campbell CM, Edwards RR, Campbell JN, Crawley JN, Lariviere WR, Wallace MR, Sternberg WF, Balaban CD, Belfer I, Fillingim RB. Pain sensitivity and vasopressin analgesia are mediated by a gene-sex-environment interaction. Nature neuroscience. 2011; 14(12):1569–1573. [PubMed: 22019732]
26. Monsees GM, Tamimi RM, Kraft P. Genome-wide association scans for secondary traits using case-control samples. Genetic epidemiology. 2009; 33(8):717–728. [PubMed: 19365863]
27. Nackley AG, Shabalina SA, Tchivileva IE, Satterfield K, Korchynskyi O, Makarov SS, Maixner W, Diatchenko L. Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. Science. 2006; 314(5807):1930–1933. [PubMed: 17185601]
28. Nackley AG, Tan KS, Fecho K, Flood P, Diatchenko L, Maixner W. Catechol-O-methyltransferase inhibition increases pain sensitivity through activation of both beta2- and beta3-adrenergic receptors. Pain. 2007; 128(3):199–208. [PubMed: 17084978]
29. Nielsen CS, Stubhaug A, Price DD, Vassend O, Czajkowski N, Harris JR. Individual differences in pain sensitivity: genetic and environmental contributions. Pain. 2008; 136(1–2):21–29. [PubMed: 17692462]
30. Norbury TA, MacGregor AJ, Urwin J, Spector TD, McMahon SB. Heritability of responses to painful stimuli in women: a classical twin study. Brain : a journal of neurology. 2007; 130(Pt 11): 3041–3049. [PubMed: 17932101]
31. Orrey DC, Bortsov AV, Hoskins JM, Shupp JW, Jones SW, Cicuto BJ, Hwang J, Jordan MH, Holmes JH, Haith LR, Roane BM, Diatchenko L, Cairns BA, McLean SA. Catechol-O-methyltransferase genotype predicts pain severity in hospitalized burn patients. J Burn Care Res. 2012; 33(4):518–523. [PubMed: 22210062]
32. Platts-Mills TF, Ballina L, Bortsov AV, Soward A, Swor RA, Jones JS, Lee DC, Peak DA, Domeier RM, Rathlev NK, Hendry PL, McLean SA. Using emergency department-based inception cohorts to determine genetic characteristics associated with long term patient outcomes after motor vehicle collision: methodology of the CRASH study. BMC emergency medicine. 2011; 11:14. [PubMed: 21943293]
33. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nature genetics. 2006; 38(8):904–909. [PubMed: 16862161]
34. Sannino S, Gozzi A, Cerasa A, Piras F, Scheggia D, Manago F, Damiano M, Galbusera A, Erickson LC, De Pietri Tonelli D, Bifone A, Tsaftaris SA, Caltagirone C, Weinberger DR, Spalletta G, Papaleo F. COMT Genetic Reduction Produces Sexually Divergent Effects on Cortical Anatomy and Working Memory in Mice and Humans. Cerebral cortex. 2014
35. Segall SK, Nackley AG, Diatchenko L, Lariviere WR, Lu X, Marron JS, Grabowski-Boase L, Walker JR, Slade G, Gauthier J, Bailey JS, Steffy BM, Maynard TM, Tarantino LM, Wiltshire T.

Comt1 genotype and expression predicts anxiety and nociceptive sensitivity in inbred strains of mice. Genes, brain, and behavior. 2010; 9(8):933–946.

36. Slade GD, Bair E, By K, Mulkey F, Baraian C, Rothwell R, Reynolds M, Miller V, Gonzalez Y, Gordon S, Ribeiro-Dasilva M, Lim PF, Greenspan JD, Dubner R, Fillingim RB, Diatchenko L, Maixner W, Dampier D, Knott C, Ohrbach R. Study methods, recruitment, sociodemographic findings, and demographic representativeness in the OPPERA study. The journal of pain : official journal of the American Pain Society. 2011; 12(11 Suppl):T12–T26. [PubMed: 22074749]
37. Smith SB, Maixner DW, Greenspan JD, Dubner R, Fillingim RB, Ohrbach R, Knott C, Slade GD, Bair E, Gibson DG, Zaykin DV, Weir BS, Maixner W, Diatchenko L. Potential genetic risk factors for chronic TMD: genetic associations from the OPPERA case control study. The journal of pain : official journal of the American Pain Society. 2011; 12(11 Suppl):T92–T101. [PubMed: 22074755]
38. Tai TC, Claycomb R, Siddall BJ, Bell RA, Kvetnansky R, Wong DL. Stress-induced changes in epinephrine expression in the adrenal medulla in vivo. Journal of neurochemistry. 2007; 101(4): 1108–1118. [PubMed: 17394532]
39. Tammimäki A, Männistö PT. Catechol-O-methyltransferase gene polymorphism and chronic human pain: a systematic review and meta-analysis. Pharmacogenetics and genomics. 2012; 22(9): 673–691. [PubMed: 22722321]
40. Tchivileva IE, Lim PF, Smith SB, Slade GD, Diatchenko L, McLean SA, Maixner W. Effect of catechol-O-methyltransferase polymorphism on response to propranolol therapy in chronic musculoskeletal pain: a randomized, double-blind, placebo-controlled, crossover pilot study. Pharmacogenetics and genomics. 2010; 20(4):239–248. [PubMed: 20216107]
41. Udelsman R, Norton JA, Jelenich SE, Goldstein DS, Linehan WM, Loriaux DL, Chrousos GP. Responses of the hypothalamic-pituitary-adrenal and renin-angiotensin axes and the sympathetic system during controlled surgical and anesthetic stress. The Journal of clinical endocrinology and metabolism. 1987; 64(5):986–994. [PubMed: 3031124]
42. Vargas-Alarcon G, Fragoso JM, Cruz-Robles D, Vargas A, Vargas A, Lao-Villadoniga JI, Garcia-Fructuoso F, Ramos-Kuri M, Hernandez F, Springall R, Bojalil R, Vallejo M, Martinez-Lavin M. Catechol-O-methyltransferase gene haplotypes in Mexican and Spanish patients with fibromyalgia. Arthritis research & therapy. 2007; 9(5):R110. [PubMed: 17961261]
43. Xie T, Ho SL, Ramsden D. Characterization and implications of estrogenic down-regulation of human catechol-O-methyltransferase gene transcription. Molecular pharmacology. 1999; 56(1): 31–38. [PubMed: 10385681]
44. Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y, Koeppe RA, Stohler CS, Goldman D. COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. Science. 2003; 299(5610):1240–1243. [PubMed: 12595695]

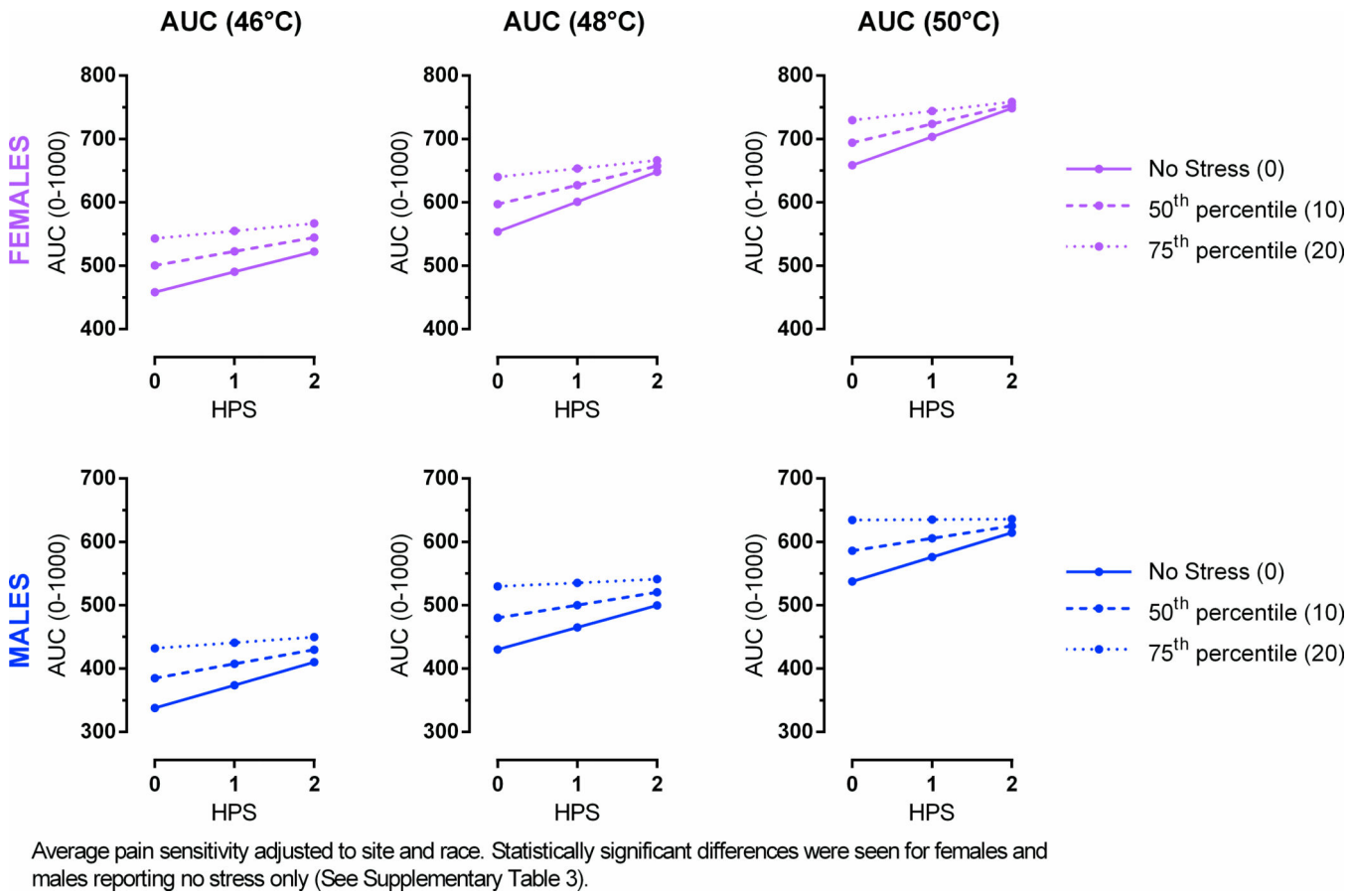
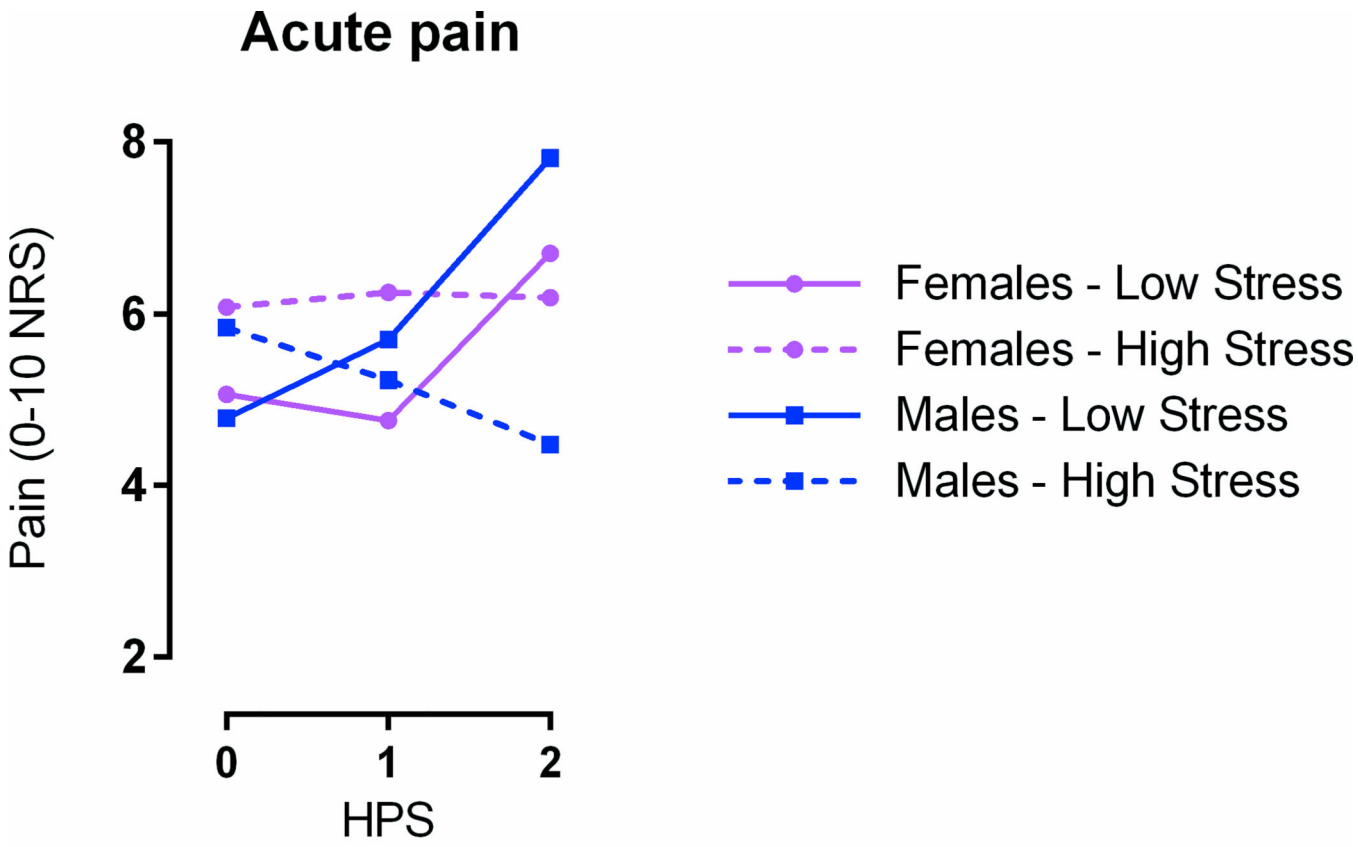


Figure 1.

Average experimental thermal pain sensitivity for the measures of *AUC* at the temperatures of 46, 48, and 50°C in subjects reporting no stress (stress = 0), the fiftieth (stress = 10) or the seventy-fiftieth percentile (stress = 20) of stress ratings in females and males carrying 0, 1, or 2 copies of the HPS haplotype.



Average pain sensitivity adjusted to site and race. Statistically significant differences were seen for males reporting no stress only. See Table 3.

Figure 2.

Average acute pain sensitivity in low (PDI ≤ 18) or high stress (PDI >18) females and males carrying 0, 1, or 2 copies of the HPS haplotype. The dichotomization is defined by median split.

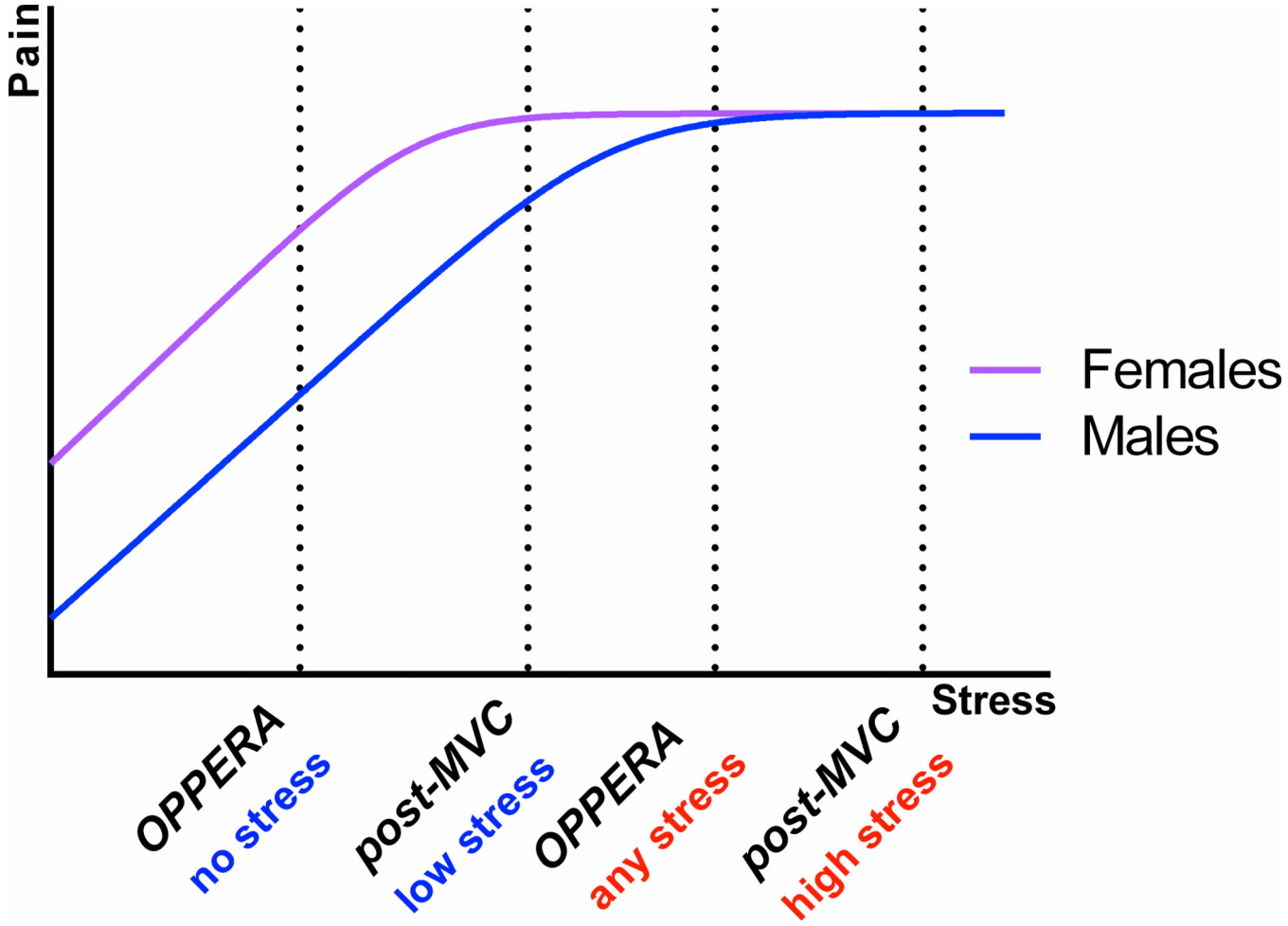


Figure 3.

Summary of the inferences regarding the contribution of the *COMT* haplotype, stress, and sex to pain sensitivity. Both the HPS haplotype and stress lead to increased pain sensitivity that is sex-dependent, likely in an epinephrine-mediated manner. In both sexes, COMT-dependent pain responses reach a plateau when stress reaches a certain level, although the plateau occurs at lower levels of stress in females compared to males. One implication is that, if COMT-dependent pain sensitivity is being investigated out of the linear range of the relationship between stress and pain, studies would not properly delineate the extent to which *COMT* haplotypes contribute to pain perception. The vertical dotted lines represent hypothesized conditions when association results have been assessed in the two cohorts for different stress levels.

Table 1

Number of *OPPERA* cohort subjects (controls and TMD cases) included in this study according to sex, stress level, and number of HPS haplotypes.

	Female (n=1698)						**Male (n==1274)**					
	***No Stress* (n=396)**			***Stress* (n=1302)**			***No Stress* (n=331)**			***Stress* (n=943)**		
HPS haplotype	***0***	***1***	***2***	***0***	***1***	***2***	***0***	***1***	***2***	***0***	***1***	***2***
Controls	235	129	15	821	317	44	212	105	14	658	225	32
TMD cases	11	5	1	91	25	4	0	0	0	21	7	0
TOTAL	246	135	18	912	343	50	212	106	16	679	233	34

TMD = temporomandibular disorder. “No stress” = NRS stress score of 0; “Stress” = NRS stress score higher than 0.

Table 2

Two-way interactions between *COMT* haplotypes and stress, *COMT* haplotypes and sex, or sex and stress on different measures of experimental thermal pain sensitivity in the *OPPERA* cohort.

	APS*Stress		HPS*Stress		APS*Sex		HPS*Sex		Sex*Stress		APS		HPS		Stress		Sex	
	Coef	***P***	**Coef**	***P***	**Coef**	***P***	**Coef**	***P***	**Coef**	***P***	**Coef**	***P***	**Coef**	***P***	**Coef**	***P***	**Coef**	***P***
First Pulse (46)	0.001	0.5875	0.000	0.9430	−0.020	0.7831	−0.004	0.9709	0.003	0.4530	−0.02	0.6298	0.03	0.7452	0.01	**0.0005**	0.31	**0.0015**
First Pulse (48)	0.000	0.9046	−0.002	0.4279	−0.025	0.7412	−0.027	0.8122	0.002	0.6683	−0.01	0.8823	0.09	0.2856	0.01	**<0.0001**	0.33	**0.0008**
First Pulse (50)	0.000	0.9466	−0.003	0.2411	−0.001	0.9870	−0.089	0.4250	0.001	0.8961	−0.02	0.7310	0.12	0.1729	0.01	**<0.0001**	0.37	**0.0002**
AUC (46)	−0.002	0.3595	−0.006	**0.0234**	−0.032	0.6640	−0.097	0.3652	−0.003	0.3571	0.01	0.8555	0.14	0.1137	0.02	**<0.0001**	0.45	**<0.0001**
AUC (48)	−0.001	0.7716	−0.005	**0.0308**	0.043	0.5726	0.035	0.7314	−0.002	0.5168	−0.04	0.4494	0.09	0.3002	0.02	**<0.0001**	0.40	**0.0001**
AUC (50)	−0.001	0.7233	−0.008	**0.0034**	0.044	0.5905	0.005	0.9647	−0.005	0.1491	−0.03	0.5860	0.14	0.0966	0.02	**<0.0001**	0.41	**0.0001**
Maximum Pain (46)	−0.003	0.2405	−0.007	**0.0045**	−0.053	0.4930	−0.111	0.3158	−0.006	0.1132	0.03	0.5798	0.17	0.0525	0.02	**<0.0001**	0.50	**<0.0001**
Maximum Pain (48)	−0.001	0.7950	−0.007	**0.0048**	0.035	0.6696	0.009	0.9295	−0.003	0.3594	−0.02	0.7762	0.16	0.0785	0.02	**<0.0001**	0.41	**0.0001**
Maximum Pain (50)	−0.001	0.7564	−0.008	**0.0015**	0.014	0.8739	−0.011	0.9173	−0.007	0.0663	0.00	0.9719	0.18	0.0539	0.02	**<0.0001**	0.44	**0.0001**

AUC = area under the curve; (46), (48), and (50) indicate the temperature used in degrees Celsius; Coef = coefficient; *P* = *p*-value. Significant *p*-values (<0.05) are bolded.

Table 3

Three-way interaction among HPS haplotype, stress, and sex; two-way interactions between HPS haplotype and stress, HPS haplotype and sex, and sex and stress; and main effects of HPS haplotype, stress, and sex on different measures of experimental thermal pain sensitivity in the *OPPERA* cohort.

	HPS*Stress*Sex		HPS*Stress		HPS*Sex		Sex*Stress		HPS		Stress		Sex	
	Coef	***P***	**Coef**	***P***	**Coef**	***P***	**Coef**	***P***	**Coef**	***P***	**Coef**	***P***	**Coef**	***P***
First Pulse (46)	−0.002	0.5366	−0.002	0.4339	0.038	0.6562	0.003	0.2522	0.058	0.3628	0.013	**<0.0001**	0.288	**<0.0001**
First Pulse (48)	−0.002	0.5735	−0.003	0.2862	0.048	0.5778	0.002	0.4730	0.086	0.1895	0.014	**<0.0001**	0.310	**<0.0001**
First Pulse (50)	0.000	0.9314	−0.003	0.1217	−0.023	0.7856	0.001	0.8268	0.124	0.0617	0.015	**<0.0001**	0.374	**<0.0001**
AUC (46)	0.001	0.6706	−0.005	**0.0269**	−0.014	0.8620	−0.002	0.4645	0.128	**0.0472**	0.017	**<0.0001**	0.426	**<0.0001**
AUC (48)	−0.001	0.7471	−0.005	**0.0125**	0.044	0.5775	−0.002	0.2498	0.126	0.0521	0.018	**<0.0001**	0.448	**<0.0001**
AUC (50)	0.001	0.6174	−0.007	**0.0015**	0.026	0.7533	−0.005	**0.0163**	0.148	**0.0291**	0.019	**<0.0001**	0.467	**<0.0001**
Maximum Pain (46)	0.002	0.5828	−0.006	**0.0059**	−0.027	0.7509	−0.003	0.1912	0.160	**0.0176**	0.018	**<0.0001**	0.445	**<0.0001**
Maximum Pain (48)	−0.001	0.7727	−0.006	**0.0018**	0.027	0.7412	−0.003	0.1143	0.161	**0.0184**	0.019	**<0.0001**	0.452	**<0.0001**
Maximum Pain (50)	0.001	0.7809	−0.007	**0.0007**	0.033	0.6984	−0.006	**0.0034**	0.144	**0.0460**	0.019	**<0.0001**	0.463	**<0.0001**

AUC = area under the curve; (46), (48), and (50) indicate the temperature used in degrees Celsius; Coef = coefficient; *P* = *p*-value. Significant *p*-values (<0.05) are bolded.

Table 4

Number of *post-MVC* cohort subjects included in this study according to sex, stress level, and number of HPS haplotype.

	Female (n=1561)						**Male**					
	Low Stress (n=974)			***High Stress (n=587)***			***Low Stress (n=770)***			***High Stress (n=476)***		
HPS haplotype	***0***	***1***	***2***	***0***	***1***	***2***	***0***	***1***	***2***	***0***	***1***	***2***
Subjects	661	274	39	395	172	20	537	205	28	333	125	18

"***Low Stress***" = PDI score ≤ 18; "***High Stress***" = PDI score > 18.

Pain. Author manuscript; available in PMC 2017 April 01.

Pain. Author manuscript; available in PMC 2017 April 01.

Table 5

Three-way interaction among HPS haplotype, stress, and sex; two-way interactions between HPS haplotype and stress stratified by sex on clinical acute pain sensitivity in the *post-MVC* cohort.

Model term	Model 1: Full model		Model 2: Females		Model 3: Males		Model 4: Females *Low distress*		Model 5: Females *High distress*		Model 6: Males *Low distress*		Model 7: Males *High distress*	
	Coef (SE)	*P*	Coef (SE)	*P*	Coef (SE)	*P*	Coef (SE)	*P*	Coef (SE)	*P*	Coef (SE)	*P*	Coef (SE)	*P*
HPS	4.27 (1.40)	**0.002**	−0.304 (0.605)	0.616	1.879 (0.619)	0.003	−0.078 (0.419)	0.853	0.151 (0.288)	0.599	0.968 (0.363)	**0.008**	−0.599 (0.581)	0.304
Distress	0.105 (0.031)	**<.001**	0.055 (0.011)	**<.001**	0.078 (0.014)	**<.001**	--	--	--	--	--	--	--	--
HPS*Stress	−0.215 (0.080)	**0.008**	0.017 (0.024)	0.484	−0.094 (0.038)	**0.013**	--	--	--	--	--	--	--	--
Sex	0.597 (0.365)	0.103	--	--	--	--	--	--	--	--	--	--	--	--
Stress*Sex	−2.30 (0.87)	**0.008**	--	--	--	--	--	--	--	--	--	--	--	--
HPS*Sex	−0.025 (0.018)	0.167	--	--	--	--	--	--	--	--	--	--	--	--
HPS*Sex*Stress	0.117 (0.045)	**0.0097**	--	--	--	--	--	--	--	--	--	--	--	--

Coef = coefficient; SE = standard error; *P* = *p*-value. Significant *p*-values (<0.05) are bolded.