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Pain modality- and sex-specific effects of *COMT* genetic functional variants

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Abstract

The enzyme catechol-O-methyltransferase (COMT) metabolizes catecholamine neurotransmitters involved in a number of physiological functions including pain perception. Both human and mouse *COMT* genes possess functional polymorphisms contributing to inter-individual variability in pain phenotypes such as sensitivity to noxious stimuli, severity of clinical pain and response to pain treatment. In this study, we found that the effects of *Comt* functional variation in mice are modality-specific. Spontaneous inflammatory nociception and thermal nociception behaviors were correlated the most with the presence of the B2 SINE transposon insertion residing in the 3'UTR mRNA region. Similarly, in humans, *COMT* functional haplotypes were associated with thermal pain perception and with capsaicin-induced pain. Furthermore, *COMT* genetic variations contributed to pain behaviors in mice and pain ratings in humans in a sex-specific manner. The ancestral *Comt* variant, without a B2 SINE insertion, was more strongly associated with sensitivity to capsaicin in female versus male mice. In humans, the haplotype coding for low COMT activity increased capsaicin-induced pain perception in women, but not men. These findings reemphasize the fundamental contribution of COMT to pain processes, and provide a fine-grained resolution of this contribution at the genetic level that can be used to guide future studies in the area of pain genetics.

Introduction

Catechol-O-methyltransferase (COMT) is an enzyme that degrades catecholamines including epinephrine, norepinephrine and dopamine. Thus it represents a critical protein that contributes to maintaining the homeostasis set points for a variety of diverse biological systems including, but not limited to pain perception, mood, cognition, and responses to both physical and emotional stressors. About a decade ago, Zubieta et al [1] reported that a common, functional, single nucleotide polymorphism (SNP) of *COMT*, Val158Met, modulates pain perception and brain responses to pain in healthy volunteers. The

associations of this SNP with affective disorders has been an area of active investigation since 1997 [2] and Val158Met is considered to be the main functional *COMT* variant. It has been shown that the substitution from a valine (Val) to methionine (Met) at position 158 leads to a three- to four-fold reduced activity of the COMT enzyme by lowering protein stability [3–5]. More recently, Diatchenko et al. [6] identified three common *COMT* haplotypes, which further define the level of COMT activity via an effect on translation. Although these haplotypes include the Val158Met SNP, haplotypes are associated with a more profound change in COMT activity (up to twenty-fold difference) and correlate strongly with variations in human pain perception [7], whereby lower enzymatic activity is associated with higher sensitivity to painful stimuli.

In recent rodent studies, three independent laboratories have shown that in inbred mouse strains, a *Comt* allele, ComtB2i, defined by the presence of 3'-UTR B2 SINE element, affects *Comt* expression and multiple behaviors, including nociceptive behaviors [8–10]. The presence of the B2 SINE element is associated with higher COMT activity and, analogous to human studies, with lower pain sensitivity.

Since the initial findings of Zubieta et al. [1], functional *COMT* alleles, SNPs or haplotypes, have been examined in over 40 independent association studies of human pain [11] and have been shown to be associated with several pain conditions including musculoskeletal pain, fibromyalgia, orafacial pain, chronic headaches, postsurgical pain, irritable bowel syndrome and the perception of pain evoked by multiple experimental procedures. However, not all findings have been replicated, and not all pain conditions tested were affected (see detailed review in Belfer & Segall [12]). The inconsistencies in the associations between *COMT* polymorphisms and pain perception have been addressed by Loggia et al [14]. They showed that the ability to detect the effect of *COMT* on pain processing seemed to depend on the presence of: 1) a sufficiently robust challenge to the pain processing system and/or 2) the recruitment of pain-inhibition mechanisms that appear to rely on CNS COMT activity. Lack of replication, however, can also be explained by other factors related to study design and study population [15], such as differences in age range, ethnicities, phenotyping tools, or phenotype definitions between studies can substantially influence the results of genetic analysis. Finally, insufficient sample size can significantly decrease statistical power to detect true associations (for example, the study of 42 women who have undergone mastectomy with breast reconstruction revealed only a trend but not significant association between occurrence of persistent pain and *COMT* Val158Met SNP (P = 0.06 [16]).

Here, using both animal and human genetic studies, we investigate and discuss the architecture of *COMT*'s contribution to the basic mechanisms of pain processing. We demonstrate that the impact of *COMT* on pain substantially depends on the modality of the noxious stimulus and the individual's sex.

Materials and Methods

Pain Modalities and *Comt* alleles in mice

Data from the "Heritability of Nociception Project", which is publically available on the Jackson Laboratory's Mouse Phenome Database website (http://www.jax.org/phenome; Project: Mogil 1), were used as the source data for the findings reported in this manuscript. Twelve standard "J" inbred mouse strains were tested using a battery of common behavioral assays of nociception. Detailed experimental methods, protocol and comparative results can be found in previous publications [17–19] and at The Jackson Laboratory's Mouse Phenome Database website. For the analyses described in the studies below, we downloaded mean data for each strain (individual data per mouse unavailable) from 20 nociception assays, excluding data for the C57BL10/J strain because a complete data set was not available. In

addition, data from the 129/J strain were excluded from our analysis because of the previously identified genetic heterogeneity resulting in ~129 substrains [20]. Five of the strains carried the mutant *Comt*^{B2i} allele: A, AKR, BALB/c, C57BL/6, and SM, and five strains carried the ancestral *Comt*$^{+}$ allele: C3H/He, C58, CBA, DBA/2, and RIIIS.

Two to six pain assays comprised each pain modality (e.g., spontaneous responses to inflammatory irritants, chemically-induced thermal hypersensitivity, thermal sensitivity, mechanical sensitivity, sensitivity as measured by tail withdrawal and sensitivity to neuropathic pain). Tail withdrawal was separated from the other thermal assays because the tail flick is a spinally-mediated reflex, and evidence exists for an analgesic role of elevated catacholamines within the spinal cord [33]. In each pain assay, the data reported for each individual strain was transformed into a *z*-score using the formula $z = (\chi - \mu)/\sigma$, where χ is the strain mean score of the assay, μ is the mean score of all ten strains in the assay, and σ is the standard deviation across all strains in the assay. Each strain was assigned a *z*-score for each nociception assay. To determine the contribution of the *Comt*^{B2i} allele to pain sensitivity per modality, the sum *z*-scores obtained from summing 2–6 nociception assays in 5 strains with *Comt*^{B2i} and *Comt*$^{+}$ alleles, respectively, were tested for statistical significance using a one-tailed, unpaired *t*-test (GraphPad Prism, version 5.00 for Windows, GraphPad Software, San Diego, California USA). An overall strain *z*-score per strain was calculated by summing *z*-scores across each pain modality, where higher (more positive) numbers reflected higher sensitivity to pain.

Capsaicin model in male and female mice

Mice were obtained from Jackson Laboratory (Bar Harbor, ME) and bred in-house for a limited number of generations. The following strains were used (all 'J' substrains): A, AKR, BALB/c (BALB), C3H/He (C3H), C57BL/6 (B6), C57BL/10 (B10), C58, CBA, DBA/2 (D2), RIIIS, and SM.

As described by Lariviere et al. [17], mice were habituated for 30 min to observation cylinders (30 cm diameter × 30 cm high) before being lightly restrained, injected in the plantar hind paw with 2.5 mg capsaicin (in 20 μl of 2% dimethyl sulfoxide in saline), and returned to the observation cylinders. The amount of time spent licking the injected paw was measured for 15 min after injection. Strain means from male mice were presented in Lariviere et al. [17]; data from female mice are presented here for the first time.

Capsaicin model in healthy human volunteers

University of Pittsburgh (Pitt) cohort description—The Pitt study sample consisted of 35 healthy subjects (87.5% female; nonsmokers; 18–45 years old; self-reported ethnicity =75.1% Caucasians, 16.6% African Americans, 8.3% Asians; 95.9% of non-Hispanic origin).Exclusion criteria included finding spicy foods objectionable, current minor infections (e.g., colds, or skin lesions), current acute or chronic pain, and other conditions that may affect pain or taste sensitivity. All subjects provided informed consent, and the University of Pittsburgh Institutional Review Board approved all study procedures. Capsaicin (Sigma, 98%) was dissolved in 95% ethanol to 6000 ppm, and diluted to 0.004 M and 0.0004 M in 70% ethanol; then 0.3 ml of the solution was applied to the malar surface of the cheek in a 1 × 1 cm gauze pad covered by a self-adhesive plastic film to insure skin contact and prevent evaporation (2 pads per concentration). A two-step increase in concentration was used: 24 min of a low concentration (0.0004 M) followed by 22 min of a high concentration (0.004 M). Subjects rated the sensation produced after the first minute and thereafter every 3 min, using a standard, non-modulus, free magnitude estimation (FME; ratings are of any number of the subject's choice, with larger numbers indicating a more intense sensation). Finally, a 10 cm line visual analogue scale (VAS) was used to rate the

burn sensation experienced at the end of the 46 min and allow for scaling of the FME ratings across subjects.

John Hopkins (JH) cohort description—The John Hopkins study sample consisted of 108 healthy adults (51.9% female; mean age = 28.61 (± 8.55SD); self-reported ethnicity =50.9% Caucasians, 35.2% African American; 13.9% Asians; 94.4% of non-Hispanic origin). Eligibility criteria included having no pain condition or medical disorders; participants were excluded from participation if they had an active alcohol or drug abuse problem or if there was present use of narcotics, antidepressants, anticonvulsants, or muscle relaxants. All subjects provided informed consent, and the Johns Hopkins Institutional Review Board approved all study procedures.

Capsaicin application methods were similar to procedures published previously [21–23]. A topical cream consisting of 0.5 g of 10% capsaicin was applied to a 6.25-cm^2 area on the dorsal aspect of the right hand and was evenly spread on the skin. An occlusive dressing (Tegederm™) was placed over the site to maintain the capsaicin within the area of application. Since topical capsaicin-induced pain varies strongly as a function of skin temperature [21], a contact thermode (Medoc US, Minneapolis, MN, USA), held at a constant temperature of 38 °C, was maintained over the area. Post - application, pain ratings gradually increased and plateaued within 30 min of applying the capsaicin [21]. Pain rating data were collected over a 90 min interval. Participants rated their pain continuously on a computerized 0–100 visual analog scale (0 = no pain and 100 = most intense pain imaginable). In addition, the participants were prompted every 30 s asking whether the value on the screen represented their current rating. This task was added to ensure that subjects were attentive to the assessment procedures.

Thermal and pressure stimulation modelsin humans

Thermal pain phenotyping was conducted in a human cohort recruited for a case-control study of temporomandibular joint disorder (TMD) at the University of North Carolina at Chapel Hill as described elsewhere [51]. This cohort consisted of 198 healthy controls and 200 TMD cases identified by Research Diagnosis Criteria (RDC) exam for TMD. All subjects in the TMD case-control study were Caucasian females, age 18–45. Subjects who granted informed consent and supplied a sample of whole blood for DNA analysis were included in the genetic analysis.

Quantitative Sensory Testing (QST) in this cohort was performed similarly as described previously [51]. For thermal pain assessments, contact heat stimuli were delivered using a computer-controlled Medoc Thermal Sensory Analyzer (TSA-II, Ramat Yishai, Israel), a Peltier-element-based stimulator with a 15 × 15 mm surface area. Thermal threshold and tolerance were measured on the left ventral forearm using an ascending method of limits. Thermal pain threshold was defined as the temperature (°C) at which the participant first perceived heat pain, whereas thermal pain tolerance was defined as the temperature (°C) at which the participant could no longer tolerate the pain. The temperature increased from a baseline of 32 °C with a 0.5 °C/s rate of rise until the participant responded by pressing the button. Average thermal threshold and tolerance values were calculated from four trials used to assess threshold values and four trials used to assess tolerance values. Individual trials were conducted with a 30-s inter-stimuli interval at different sites of the ventral forearm.

Following heat pain assessments, the temporal summation of heat pain was assessed. Participants were asked to rate the intensity of pain evoked by ten brief heat pulses applied consecutively to the ventral forearm. Participants provided verbal ratings between 0–10 for non-painful warm sensations and from 20 (representing pain threshold) to 100 (representing the most intense pain imaginable). The ten consecutive heat pulses were delivered with a 1.5

second interpulse interval delivered from a base temperature of 38 °C. The pulse ramp rate was ~10 °C/second, and the peak thermal pulse duration was ~1.5 seconds. Two different test stimuli, one set to 47 °C and one set to 50 °C, were used to generate two temporal summation curves. The participants responses to the first initial heat pulse in a train was used as a measure of heat pain sensitivity to suprathreshold heat pain stimuli and the verbal responses to the first three heat pulses were used to assess the rate of rise of heat pain (i.e., temporal) for each train as described by Bhalanget al [51].

Pressure pain threshold (PPT) was assessed over the right and left temporali, masseter, and trapezius muscles, temporomandibular joint (TMJ) and lateral epicondyles with a pressure algometer (Pain Diagnosis and Treatment, Great Neck, New York, USA). The PPT was defined as the amount of pressure (kg) at which the participant first perceived the stimulus to be painful. Participants verbalized their responses by saying “stop” when the onset of pain was perceived. One pretrial assessment was performed at each site followed by additional assessments until two consecutive measures differing by less than 0.2 kg were obtained. The maximum number of assessment at each site was five. Pressure stimuli were delivered at an approximate rate of 1 kg/s. The cutoff pressure for all sites was 5 kg. The values from the right and left sides were averaged to obtain one PPT value at each anatomical site.

COMT genotyping in humans

Pitt and JH cohorts—Genomic DNA was purified using QIAampTM DNA Blood Kit (Qiagen, Valencia, CA, USA). Five *COMT* SNPs, rs2097603, rs6269, rs4633, rs4818, and rs4680, were genotyped using pre-designed assays from Applied Biosysthems (ABI, Foster City, CA). Genotyping was performed by the 5’ nuclease method [27] using fluorogenic allele-specific probes. The PCR reaction mixture consisted of 2.5 ml Master Mixture (ABI), 100 nM detection probe for each allele, 900 nM forward and 900 nM reverse amplification primers, and 20 ng genomic DNA in a total reaction volume of 25 ml. Allele-specific signals were distinguished by measuring endpoint 6-FAM or VIC fluorescence intensities at 508 nm and 560 nm, respectively, and genotypes were generated using Step One Plus System and Software (ABI). Genotyping error rate was directly determined by re-genotyping 25% of the samples, randomly chosen, for each locus. The overall error rate was <0.005. Genotype completion rate was 0.98.

TMD case-control cohort—Genotyping of *COMT* markers was part of a larger-scale genotyping effort where samples were genotyped on the Algynomics (Chapel Hill, NC) Pain Research Panel, a dedicated chip-based platform utilizing the Affymetrix MegAllele technology. The Pain Panel assesses 3,295 single nucleotide polymorphisms (SNPs) representing 358 genes known to be involved in systems relevant to pain perception (see method details in Smith et al [25]). Samples were dropped from the analysis for genotyping completeness rate <0.95, or if there were discrepancies between self-reported race or sex and genotypic results; for identified family clusters, only a single representative from each family was included. SNPs were filtered based on call rate <0.95, discordance rate between repeated samples >0.99, minor allele frequency < 0.01, and Hardy-Weinberg $p<10^{-5}$. Genotype completion rate in the cleaned datasets was >0.99.

Data analysis

Allele frequencies were calculated in each sample and Hardy–Weinberg Equilibrium (HWE) test were performed using PLINK v.1.07 [28]. Single marker and haplotype analyses were conducted for the associations between *COMT* markers and capsaicin-induced or thermal-induced pain scores. All analyses were adjusted for significant. Correction for multiple testing was not performed since only one gene was tested, and all variables are related.

Pitt and JH cohorts

The Johns Hopkins and Pitt capsaicin-sensitivity data were combined for analysis. Multivariable regression analysis was performed to test for association of each *COMT* SNP with the dependent variable after adjustment for possible effects of covariates including study site (JH or Pitt), age, ethnicity and sex. A p-value of 0.05 was utilized as the cut-point for retention in the final model. Haplotype phasing and analysis was performed in PLINK [28]; common haplotypes (frequency > 0.01) were designated as Low Pain Sensitivity (LPS), Average Pain Sensitivity (APS), and High Pain Sensitivity (HPS) as described in [6]. Association analysis was performed between common haplotypes and dependent variables using a generalized linear model framework modeling genetic (i.e. haplotype) and environmental (i.e. covariates) effects simultaneously. Due to the relatively small numbers of the HPS haplotype, a set of conditional haplotype tests were performed which tested each haplotype separately. In these conditional haplotype tests, a test statistic was calculated from a comparison between a null model consisting of all haplotypes, and an alternate model with unique effects of the tested haplotype and a reference group of all other haplotypes. The model constructed for haplotype analysis incorporated sex, age, study site and ethnicity as covariates.

UNC TMD case-control cohort

Since the UNC cohort is a case-control study of TMD, any association test between an intermediate phenotype (including measures of experimental pain sensitivity) and genotype will be biased without adjusting for the case-control nature of the data. This adjustment was performed by using weighted generalized estimating equations as described in Monsees et al. [30]. The prevalence of chronic TMD in the population was assumed to be approximately 5%, http://www.nidcr.nih.gov/DataStatistics/FindDataByTopic/FacialPain/PrevalenceTMJD.htm). For each association test between a pain sensitivity measure and the *COMT* haplotypes, an omnibus-Wald test statistic was calculated to test the null hypothesis that the mean paint ratings were equal in the six haplotypes. A Wald test statistic was also calculated to test the null hypothesis that the mean rating of each specific haplotype was equal to the mean rating of the LPS/LPS haplotype.

Results

Pain Modality-Specific Effects of Murine *Comt*

We first tested if the *Comt* SINE status is associated with pain sensitivity in a battery of animal pain assays that constitute a number of fundamental nociceptive modalities. We compared sensitivity in 20 nociception and hypersensitivity assays previously collected in 10 inbred mouse strains. Importantly, only data from male mice were available from the public dataset. We organized the data into six pain modalities: spontaneous responses to inflammatory irritants, chemically induced thermal hypersensitivity, thermal sensitivity, mechanical sensitivity, sensitivity as measured by tail withdrawal, and sensitivity to neuropathic pain. We calculated z-scores for each of these modalities as described above.

An overall summation of z-scores obtained from all six modalities was significantly associated with the $Comt^{B2i}$ allele (p=0.025, Figure 1), such that the absence of B2 SINE element, which codes for lower COMT activity, was associated with greater pain behaviors. We next tested which pain modalities were responsible for this association. Strains of the $Comt^{B2i}$ allele were significantly less sensitive to pain in the spontaneous responses to inflammatory irritants modality (p=0.0005, Figure 1). Five out of 6 pain tests contributing to this overall significance were associated with SINE element with $p < 0.05$, and the early phase of formalin-induced pain was marginally insignificant ($p = 0.07$, Supplementary Table 1, Supplementary Figure 1). Near significance was also found for thermal nociception (hot

plate and Hargreaves tests; $p = 0.0595$; Figure 1) where latency of heated hindpaw withdrawal in Hargreaves test was independently significantly and associated with the presence of the B2 SINE element ($p = 0.03$, Supplemental Table 1). While significance was not found with other modalities, an overall trend was observed such that the mean pain z-score of five mice strains with the *Comt*^{B2i} allele was lower, signifying less pain behavior, than the mean of five strains with the ancestral *Comt*$^{+}$ allele. An exception was observed, however, for the relationships with neuropathic pain (Figure 1).

Sex-Specific Effects of Murine *Comt*

We then examined the effects of sex on the *Comt* contribution to murine pain phenotypes. Whereas the "Heritability of Nociception" series reports results from only male mice, we conducted an independent study with both male and female standard inbred mice using the capsaicin pain model, one of the assays in the spontaneous inflammatory nociception modality.

We examined correlations between the presence or absence of the *Comt*^{B2i} allele with mean capsaicin-induced pain response (Figure 2), and assessed the genetic correlation among inbred strains to determine if males and females share a similar sex-related genetic determination [49, 50]. In females, the relationship between the *Comt*^{B2i} allele and capsaicin-induced pain is clear and highly significant, such that strains that are B2 SINE-negative are more sensitive: Spearman $r = 0.87$ and Pearson $r = 0.80$, both are statistically significant ($p < 0.05$, one-tailed *t*-test). A general linear model (GLM) applied to individual data with strain nested within the *Comt*^{B2i} allele confirmed this pattern and found a highly significant main effect of the *Comt*^{B2i} allele in females ($p < 0.004$). In males, however, capsaicin licking and presence of the *Comt*^{B2} allele were only modestly related: Spearman $r = 0.52$ and Pearson $r = 0.48$, both are not statistically significant, with no significant main effect of the *Comt*^{B2i} allele on capsaicin pain sensitivity (GLM, $p = 0.14$). Thus, these results indicate that while the *Comt*^{B2i} allele can have a significant effect on capsaicin pain regardless of sex (GLM with both sexes: sex effect, $p = 0.51$, *Comt*^{B2i} allele effect, $p = 0.03$), the heritable differences among strains are highly related to the *Comt*^{B2i} allele in females, but not in males.

Association of human *COMT* variants with capsaicin-induced pain

We next tested whether the murine findings are also applicable for human pain sensitivity. The sensitivity to capsaicin-induced pain, which is an assay in the Spontaneous Inflammatory Nociception modality in mice, was measured in humans. In the combined (Pitt + JH) cohort no single SNP was associated with pain scores (Supplementary Table 2). The Val158Met SNP showed a trend towards lower post 30 min pain ratings in subjects with one or more A alleles ($p = 0.07$, Beta = −6.75). This result is in the opposite direction than expected because lower COMT activity associated with the Met substitution should lead to greater pain sensitivity.

A significant effect of the HPS and APS haplotypes was observed at the post 30 min time point ($p = 0.04$ for both, adjusted means 67.8 and 57.5 respectively; Figure 3, Table 1). The LPS haplotype was not significantly associated with capsaicin-induced pain in this cohort. Subjects carrying the HPS haplotype showed the greatest difference and were the most sensitive to capsaicin. This effect was significant for females ($p = 0.04$ and $p = 0.02$, adjusted means 69.8 and 56.9, respectively), but not for males ($p = 0.43$ and 0.54, respectively). It is also evident from the analysis that males did not contribute to the overall significance of the association, consistent with effect of the sex of mice on *Comt* contribution to pain in mice.

Association of human *COMT* variants with thermal and pressure pain

The association between thermal and pressure stimuli with *COMT* genotypes in a human cohort of healthy volunteers was also examined.

Similar to what was observed for the responses to capsaicin-induced pain, no SNP in the *COMT* central haploblock (Supplementary Table 3) was significantly associated with pain scores, including the Val158Met SNP. It should be noted that only SNPs rs6269 and rs4633 were analyzed in this cohort because the other two SNPs that define the central *COMT* haploblock [6, 7] did not pass genotyping QC. However, because SNPs rs6269 and rs4818 and SNPs rs4633and rs4680 are in perfect linkage disequilibrium in Caucasians [7], a comprehensive assessment of the haploblock and Val158Met was still possible with SNP rs4633 used as a marker of Val158Met variation. SNP rs165599 was associated with the thermal pain ratings to the initial first pulse from the train of 10 pulses delivered at 47 °C ($p = 0.02$) or 50 °C ($p = 0.04$). This suggests that this SNP contributes to the variation in responses to thermal pain sensitivity in humans, which is consistent with the modifying effect of the genetic structure of the 3'UTR of *COMT* in the central haploblock [52].

Next, we constructed the three major haplotypes for the central *COMT* haploblock using SNPs rs6269 and rs4633 (Table 2, Supplementary Tables 4,5). In general, no association was observed between *COMT* haplotypes and the pressure pain algometer ratings. However, it is interesting to note that the HPS/HPS haplotype was slightly more sensitive than the LPS/LPS haplotype for pressure pain ratings obtained from the masseter muscle (mean difference = −0.52, $p = 0.04$). No other significant associations were observed between any of the pressure pain algometer ratings and *COMT* haplotypes.

The Wald test for testing the null hypothesis of no association between *COMT* haplotype and the thermal threshold rating was strongly significant ($p < 0.0001$). The HPS/HPS haplotype showed significantly greater sensitivity than the LPS/LPS haplotype (mean difference = −1.49, $p<0.0001$). None of the other haplotypes were significantly different from the LPS/LPS haplotype. The Wald test for testing the null hypothesis of no association between *COMT* haplotype and the responses to repeated thermal stimuli thermal pain ratings was significant for first-pulse response at both 47 °C ($p = 0.02$) and 50 °C ($p = 0.0008$). Closer examination revealed that participants with the HPS/LPS haplotype had significantly higher pain ratings than participants with the LPS/LPS haplotype at both 47 degrees (mean difference = 22.3, $p = 0.002$) and 50 °C (mean difference = 29.9, $p = 0.0006$). The pain ratings of the participants with the other *COMT* haplotypes were not significantly different from those of the participants with the LPS/LPS haplotype at either temperature. No association was observed between *COMT* haplotype and thermal pain tolerance or temporal summation, defined as difference between maximum VAS response and VAS response to first heat pulse [51], in responses to repeated thermal stimuli at either 47 °C or 50 °C.

Discussion

Pain modality-specific effect of *COMT* functional variants on pain

In this set of investigations, we found that the effects of *COMT* functional variants strongly depend on the modality of the pain test. This can be systematically observed in the results of testing the laboratory inbred mouse strains in a battery of common assays of nociception. We observed the strongest association among all pain modalities for the spontaneous responses to inflammatory irritants modality ($p < 0.001$, Figure 1, Supplementary Table 1). Four out of five pain tests comprising this modality were independently associated with the presence of the $Comt^{B2i}$ allele, and the fifth assay approached significance. In all cases of this modality, the presence of the $Comt^{B2i}$ allele was associated with a lower pain response,

reinforcing the statistical significance of combined test. A strong trend toward significance was observed between the $Comt^{B2i}$ allele for the thermal sensitivity modality ($p = 0.06$, Figure 1). Among the two pain tests contributing to the thermal sensitivity modality, one was independently associated with the $Comt^{B2i}$ allele. The majority of remaining assays showed a similar directionality of the genetic effect although none were statistically significant. In all cases, the presence of the $Comt^{B2i}$ allele was correlated with less pain, with the exception of sensitivity to neuropathic pain. Importantly, only data from male mice were available from the public data base and as demonstrated in our study, COMT activity had a stronger contribution to pain phenotypes in females than in males, at least for capsaicin-induced pain. Consequently, if female mice were tested in this study we could get a stronger association with thermal pain sensitivity, which approached significance in the present analysis.

Similarly to rodent studies, *COMT* functional haplotypes were associated with capsaicin-induced pain responses in humans. Importantly, the direction of the association was consistent between the species – with the HPS haplotype, coding for the lowest COMT activity, associated with the highest sensitivity to noxious stimuli. Furthermore, in the UNC human cohort that has been characterized for several noxious stimuli, the sensitivity to thermal stimuli was strongly associated with *COMT* haplotypes, marginally to pressure muscle pain and was not at all associated with temporal summation of heat pain. These human data reinforce the results from murine experiments that the strongest effect of *COMT* is on the chemical/inflammatory and sensitivity to thermal pain. It is important to recognize that capsaicin pain and thermal stimuli initiate pain through partially shared cellular pathways as they both activate TRPV1 receptor; this is not true for other tests contributing to the spontaneous inflammatory nociception modality.

Since human pain perception is a mosaic of integrated components in domains affected by *COMT* polymorphisms (e.g., sensory–discriminative, aversive-affective and cognitive-evaluative), this gene can be viewed as an obvious candidate in genetic association studies of human pain, which can explain a portion of the inter-individual variability in pain perception, sensitivity, persistence and analgesic efficacy [12]. However, several studies have reported negative results [31, 32]. While it is unclear if these negative findings were caused by sample heterogeneity, missing genetic data from functional haplotypes or other design-related issues, the modality-dependent *COMT* effects observed here provide a new perspective on the inconsistencies of previous studies. Recently, we described the dichotomous effects of *COMT* in neuropathic vs. nociceptive pain modalities due to selective adrenergic signaling [33].The exact mechanism of the greater contribution of *COMT* functional variation to inflammatory and thermal nociception modalities is unclear and further studies are warranted. It may reflect a complex interplay of the differential contributions of enhanced peripheral and central nervous system driven by adrenergic versus dopaminergic signaling that results from low COMT activity [34].

Sex-specific effect of *COMT* functional variants on pain

Another novel finding of this study was the identification of significant sex differences in the effect of *Comt* genetic variants on capsaicin-induced pain in mice and humans. Overall, low COMT activity (as determined by gene functional variants) contributed stronger to capsaicin-induced pain in females compared to males in both species.

There is a growing body of evidence that COMT's effects are sexually dimorphic [35]. In inbred strains of mice, female mice have lower *Comt* RNA expression [8]. Human COMT activity in erythrocytes [36] and the liver [37] is lower in females, possibly because estrogens down regulate COMT expression [38]. In human postmortem prefrontal cortex, COMT activity is 17% lower in females than in males [39]. In human genetic studies, a

lower prevalence of non-migrainous headache was found among women who were homozygotes for Val/Val genotype, but not among men [40].

In general, there are three major scenarios of sex-specific differences in the effect on of a gene on a trait or behavior: sex-specific effects (*COMT* affects only one sex), sex-biased effects (*COMT* affects both sexes but to different degrees), and sex-antagonistic effects (*COMT* affects both sexes but in opposite directions) [41]. Furthermore, the particular gene × sex interaction may be pain modality-specific. Sex-specific effects of *COMT* are usually attributed to its transcriptional regulation by estrogens [38, 42]. However, additional mechanisms may be equally important. We propose two plausible explanations for the sex differences in COMT's effect on pain: 1) compared to males, females have lower COMT levels, and both protein levels [37, 43] and COMT activity are under estrogen regulation. Therefore, females may be primed towards more pain sensitivity; 2) males have additional receptor pathways stimulated by catecholamines that are not as functional in females, resulting in differences in physiological responses to stressors that act to suppress pain processing (e.g., blood pressure responses) [44]. In the latter case, *COMT* polymorphisms will have mixed contributions to pain sensitivity, resulting in the absence of significant associations in males, as was recently demonstrated in a Chinese population sample [45].

Replication of previous association results

Our current human findings complement our previously published data on the association between functional *COMT* haplotypes and sensitivity to noxious stimuli [6, 7]. As reported previously on a separate human cohort of 202 healthy female volunteers, in this study HPS haplotype was also associated with the highest sensitivity to noxious stimuli. Furthermore, the association between the *COMT* haplotype and thermal pain sensitivity was much stronger than with other pain modalities such as mechanical and ischemic pain. Also, overall algometer pressure pain was not associated with *COMT* haplotypes [7] and, similar to this study, only individual algometer measures obtained at specific anatomical sites reached significance (e.g., pressure threshold at the wrist [46]). Among the measures of thermal heat pain sensitivity, the pattern of association observed in this study is also remarkably consistent with earlier findings [7, 46]: thermal pain threshold showed the strongest association with the *COMT* haplotypes, thermal tolerance showed a weaker association, and thermal pain temporal summation showed no association.

Likewise, similar to the results of previous studies, *COMT* haplotypes were significant predictors of pain sensitivity in both thermal sensitivity and capsaicin-evoked pain assays, but not the Val158Met SNP by itself.

Translational value of the findings

The findings from this study have a number of practical implications. First, modality- and gender-specificity of *COMT* effects should be considered in clinical setting when novel COMT-dependent analgesics are tested, such as propranolol [47, 48]. These considerations may be relevant not only for pain research but also for other complex traits and diseases influenced by *COMT* genetic variation, such as cognitive function or depression. Second, the sex-specific differences in COMT effects on pain, demonstrated here, indicate that sex should be considered differently when evaluating the association between *COMT* genetic variation and pain perception. Regression models to evaluate the association between *COMT* genetic variants and pain sensitivity may fail to detect an association even when a covariate for sex is included in the model. Thus, it may be necessary to consider more complex interactions or stratify by sex when performing such an analysis. It also should be noted that the sex specific effect on pain phenotypes is rather common and not unique for *COMT* [53]. Finally, other biological factors such as age, body mass index, ethnicity and

sleep may further modify the complex relationship between *COMT* genetic variation and pain. Clearly, additional research is needed to facilitate the clinical application of our findings.

In summary, the observed specificity in the genetic effects of genes like *COMT* may be one of the major reasons for difficulties in reproducing findings of genetic influences on pain. This study not only contributes to our understanding of COMT-dependent pain sensitivity but also provides further guidance for future genetic studies of pain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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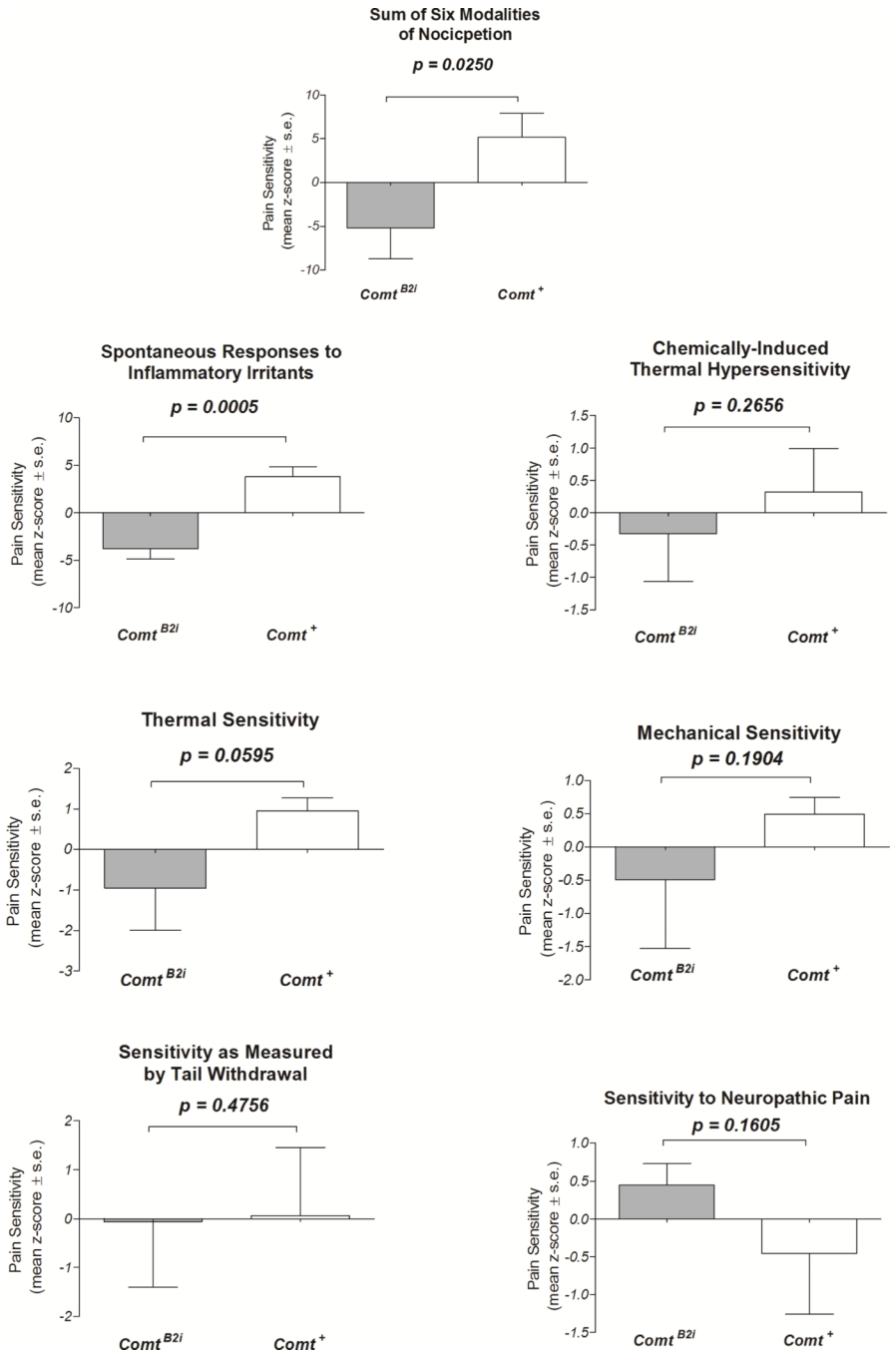

Figure 1.

Mouse responsiveness to 20 nociceptive assays combined into 6 pain modalities categorized by two *Comt* alleles. Filled bars indicate strain carries the $Comt^{B2i}$ allele. Six modalities of nociception depicted as *z*-scores. Increased sensitivity to pain modality is depicted by positive *z*-score.

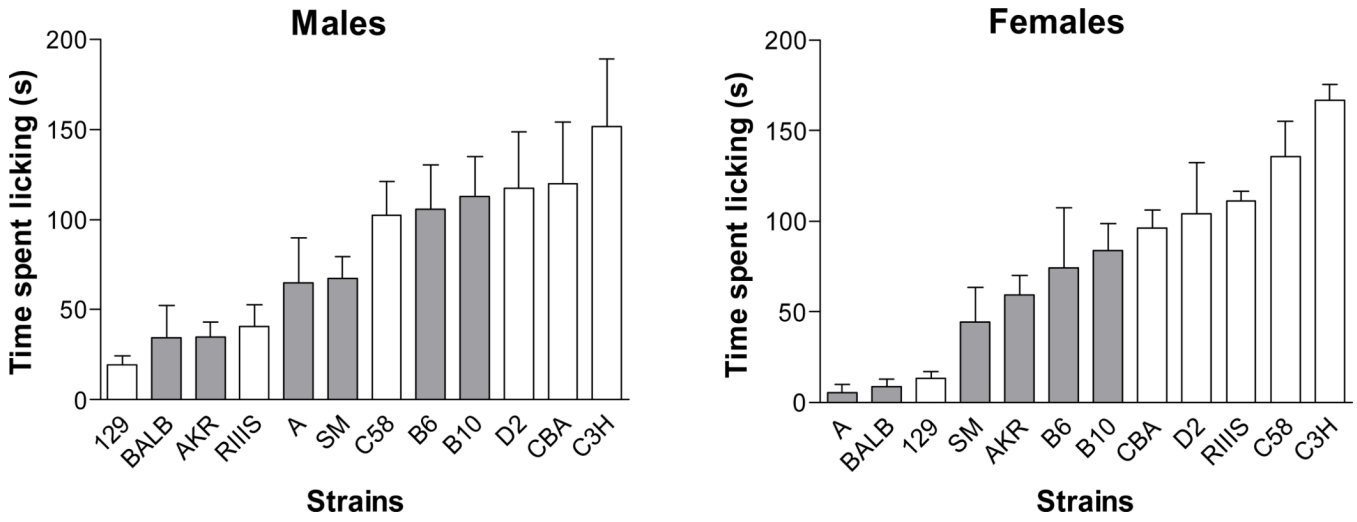

Figure 2.

Mouse responsiveness to capsaicin-induced pain categorized by two *Comt* alleles. Filled bars indicate strain carries the $Comt^{B2i}$ allele. The greater values reflect greater pain sensitivity.

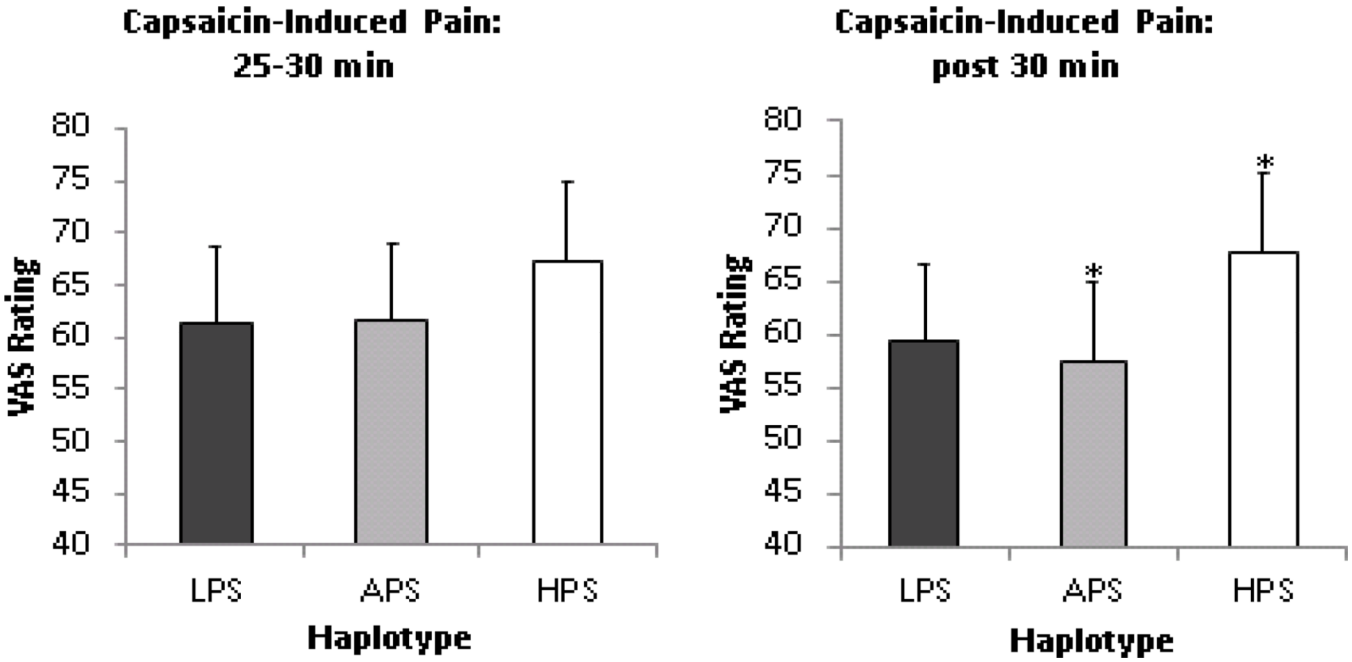

Figure 3.

Human responsiveness to capsaicin-induced pain categorized by three major *COMT* haplotypes. LPS: haplotype G_C_G_G, APS: haplotype A_T_C_A, HPS: haplotype A_C_C_G. The greater values reflect greater pain sensitivity. VAS – visual analog scale. *P<0.05. Mean scores are adjusted for sex, age, study site, and race. Error bars reflect the standard errors of the mean.

Table 1

Human responsiveness to capsaicin-induced pain categorized by three major *COMT* haplotypes. LPS: haplotype G_C_G_G, APS: haplotype A_T_C_A, HPS: haplotype A_C_C_G. The greater values reflect greater pain sensitivity. The haplotype genetic association results are presented for capsaicin-induced pain at 25–30 and 30 min time points in all subjects and by sex, with means adjusted for sex (in the combined cohort), age, study site, and race.

haplotypes	combined				females				males			
	25–30 min		30 min		25–30 min		30 min		25–30 min		30 min	
	Mean	p	mean	p	Mean	p	Mean	p	Mean	p	Mean	p
HPS	67.38	0.23	67.78	0.04	69.16	0.20	69.84	0.04	66.51	0.72	65.62	0.43
APS	61.57	0.27	57.53	0.04	60.05	0.08	56.92	0.02	65.67	0.83	59.33	0.54
LPS	61.19	0.56	59.24	0.80	63.42	0.98	60.55	0.99	60.14	0.43	58.30	0.75

Table 2

Human responsiveness to heat- and pressure-induced pain categorized by three major *COMT* haplotypes. LPS: haplotype G_C, APS: haplotype A_T, HPS: haplotype A_C. For each QST measurement, a single model is fitted to evaluate its association with haplotypes, where haplotype LPS/LPS is set as the reference level and the other five haplotypes are compared to it. The "intercept" corresponds to the mean QST measurement for haplotype LPS/LPS, and the difference between the other haplotypes and LPS/LPS under the model is summarized by the coefficient (Coef.), standard error (SE), and p-value. Thus, for pressure and thermal threshold and tolerance phenotypes the smaller values of coefficient reflect greater pain sensitivity, but for responses to repeated thermal stimuli, the greater values of coefficient reflect greater pain sensitivity. The overall Wald p-values for the association between haplotypes and each QST measurement are summarized in the last column. The mean of heat- and pressure-induced pain responsiveness categorized by three major *COMT* haplotypes in TMD cases and non-TMD cases are presented in Supplementary Tables 4 and 5.

Phenotype mean		APS/APS	APS/HPS	HPS/HPS	HPS/LPS	LPS/APS	Intercept	Overall P-value
	Coef.	0.0428	−0.0356	−0.2733	−0.0664	0.0616		
	SE	0.1519	0.1946	0.3158	0.3244	0.1461	3.1204	0.9006
pressure pain threshold: temporalis, kg	P-value	0.778	0.8548	0.3868	0.8377	0.6733		
	Coef.	0.1057	−0.1701	−0.5159	0.1824	0.1054		
	SE	0.16	0.2161	0.2481	0.2885	0.1614	2.8429	0.0791
pressure pain threshold: masseter, kg	P-value	0.5088	0.4312	0.0376	0.5273	0.5137		
	Coef.	0.1331	−0.1875	0.0293	0.0821	0.089		
	SE	0.1467	0.2211	0.3371	0.2436	0.145	2.9455	0.7092
pressure pain threshold: TMJ, kg	P-value	0.3642	0.3962	0.9307	0.7362	0.5395		
	Coef.	0.1233	−0.0273	0.2402	0.038	0.2789		
	SE	0.242	0.3391	0.9145	0.36	0.23	4.2498	0.8175
pressure pain threshold: trapezius, kg	P-value	0.6105	0.9357	0.7928	0.916	0.2254		
	Coef.	0.4798	−0.1143	0.1288	0.2205	0.3048		
	SE	0.2197	0.4065	0.8761	0.3591	0.2258	4.7906	0.2624
pressure pain threshold: epicondyle, kg	P-value	0.029	0.7785	0.8831	0.5392	0.1771		
	Coef.	0.276	0.5089	−1.4933	−0.5011	0.2515		
	SE	0.4349	0.5026	0.3624	0.948	0.4126	42.485	<.0001
arm thermal threshold, °C	P-value	0.5257	0.3113	<.0001	0.5971	0.5421		
	Coef.	0.0862	−0.6875	0.2952	−0.6415	0.2272		
	SE	0.268	0.3944	0.4733	0.7673	0.244	47.1548	0.1891
arm thermal tolerance, °C	P-value	0.7477	0.0813	0.5328	0.4031	0.3517		

Phenotype mean		APS/APS	APS/HPS	HPS/HPS	HPS/LPS	LPS/APS	Intercept	Overall P-value
responses to repeated thermal stimuli, first-pulse, at 47°C, VAS	Coef.	−0.5809	5.5119	−3.5093	22.3003	3.4526	20.176	0.0241
	SE	3.0567	6.1415	5.9021	7.3079	2.909		
	P-value	0.8493	0.3695	0.5521	0.0023	0.2353		
responses to repeated thermal stimuli, first-pulse, at 50°C, VAS	Coef.	−0.4886	7.8141	−10.9606	29.8764	3.7357	29.2939	0.0008
	SE	3.8763	7.239	5.7455	8.6974	3.88		
	P-value	0.8997	0.2804	0.0564	0.0006	0.3356		
responses to repeated thermal stimuli, delta, at 47°C, VAS	Coef.	−0.7858	7.4276	−8.7324	1.6549	−2.7372	33.7324	0.2967
	SE	4.1629	7.2302	5.1858	5.9743	3.6867		
	P-value	0.8503	0.3043	0.0922	0.7818	0.4578		
responses to repeated thermal stimuli, delta, at 50°C, VAS	Coef.	−3.3737	0.7861	−8.8556	−15.5333	−4.8554	43.1889	0.3921
	SE	4.6049	7.1308	11.1535	7.6182	4.3739		
	P-value	0.4638	0.9122	0.4272	0.0415	0.267		