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REGULAR ARTICLE

Effects of functional polymorphisms of opioid receptor mu 1 and catechol-O-methyltransferase on the neural processing of pain

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Aim: Pain is reconstructed by brain activities and its subjectivity comes from an interplay of multiple factors. The current study aims to understand the contribution of genetic factors to the neural processing of pain. Focusing on the single-nucleotide polymorphism (SNP) of opioid receptor mu 1 (OPRM1) $A^{118}G$ (rs1799971) and catechol-O-methyltransferase (COMT) $val^{158}met$ (rs4680), we investigated how the two pain genes affect pain processing.

Method: We integrated a genetic approach with functional neuroimaging. We extracted genomic DNA information from saliva samples to genotype the SNP of OPRM1 and COMT. We used a percept-related model, in which two different levels of perceived pain intensities ("low pain: mildly painful" vs "high pain: severely painful") were employed as experimental stimuli.

Results: Low pain involves a broader network relative to high pain. The distinct effects of pain genes were observed depending on the perceived pain intensity. The effects of low pain were found in supramarginal gyrus, angular gyrus, and anterior cingulate cortex (ACC) for OPRM1 and in middle temporal gyrus for COMT. For high pain, OPRM1 affected the insula and cerebellum, while COMT affected the middle occipital gyrus and ACC.

Conclusion: OPRM1 primarily affects sensory and cognitive components of pain processing, while COMT mainly influences emotional aspects of pain processing. The interaction of the two pain genes was associated with neural patterns coding for high pain and neural activation in the ACC in response to pain. The proteins encoded by the OPRM1 and COMT may contribute to the firing of pain-related neurons in the human ACC, a critical center for subjective pain experience.

Keywords: *brain and pain, catechol-O-methyltransferase, opioid receptor mu 1.*

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Pain is a highly subjective experience.[1] Individual differences in pain come from an interplay of multiple factors.[2–5] Pain experience can be partially explained by the pain gene, a gene whose polymorphisms act on the gene expression or its function in a way that influences pain response.[6] There are two potent candidates for pain genes. First, the opioid receptor mu 1 (OPRM1) single-nucleotide polymorphism (SNP) involves the μ-opioid receptor (MOR) activity by changing its sensitivity to opioids and affects the clinical effects of opiate drugs.[7–10] The OPRM1 $A^{118}G$ (rs1799971) codes an adenine-to-guanine substitution that leads to the replacement of the amino acid asparagine with the negatively charged aspartate at a putative N-glycosylation site of MOR.[7] Second, the catechol-O-methyltransferase (COMT) gene encodes the enzyme catechol-O-methyltransferase, which metabolizes catecholamines. The COMT $val^{158}met$ (rs4680) involves the substitution of valine with methionine at codon 158. The two pain genes have been known for inconsistent effects on pain behavior. For example, the OPRM1 G-allele carriers showed higher pain sensitivity relative to A/A homozygotes.[11–13] However, some researchers did not observe the effect of $A^{118}G$ SNP[9,14,15] but found effects of the other opioid receptor genes (e.g. OPRD and OPRK) on pain response.[16] For the COMT, *met/met* homozygotes had higher pain sensitivity compared with *val* carriers.[17–19] By contrast, several studies found not only that the $val^{158}met$ variation is not associated with pain response[20,21] but also that COMT rs6268, rather than rs4680, influences pain ratings.[22]

The behavioral inconsistency reflects the gap between gene and pain percept. If we can objectively measure pain, the pain gene may better explain pain experience. One possible method is to examine neural processing of pain. Pain processing was thought to have two subsystems: (i) the lateral system consists of the thalamocortical projection to the primary/secondary somatosensory cortices (SI/SII) and posterior insula (INS) to subserve the sensory component of pain; and (ii) the medial system encompasses the anterior cingulate cortex (ACC) and prefrontal cortex involving the motor, cognitive, and emotional components of pain.[23] Accumulating evidence has suggested that pain emerges from a complex and dynamic interaction of the sensory, cognitive, and emotional processes of the widely distributed brain networks, rather than the divided two systems.[24–28] Using the percept-related model, in which the perceived pain intensity is used

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as experimental stimuli and different components of pain can be separately tested, neuroimaging studies have reported robust neural activations in various brain regions depending on perceived pain intensity[29–34]: mildly painful stimuli (henceforth referred to as low pain) induced the blood oxygen level–dependent (BOLD) signal changes in a broader neural network than severe pain (henceforth, high pain),[30,33] while both low and high pain induced neural activations in the INS, SII, and inferior/middle frontal gyrus (IFG/MFG).

Attempts to understand the effects of the pain genes started with *in vivo* positron emission tomography. Although $A^{118}G$ variants did not affect the endogenous opioid release induced by pain, the vulnerability of G-allele carriers indicates an overall decrease in MOR availability and less responsive neurotransmitter systems in the thalamus, nucleus accumbens, ventral pallidum, and amygdala.[14] By contrast, the $val^{158}met$ polymorphism affected the activation of the μ-opioid system and μ-opioid–binding potentials in the same subcortical regions: *met/met* showed a reduced activation of the MOR system and elevated baseline MOR-binding potentials.[17] Furthermore, results from functional magnetic resonance imaging (fMRI) studies suggest pain gene effects in a perceived pain intensity–dependent manner. Compared with OPRM1 A/A, G-allele carriers exhibited increased neural activation in the precentral gyrus (PrCG)[15,34] in response to pain and had a less pronounced opioid analgesic effect on the SI, SII, and posterior INS, which involve sensory component of pain intensity.[35] COMT *met/met*, relative to *val* carriers, exhibited greater BOLD response in the ACC, SII, INS, and amygdala for painful stimuli.[19] Moreover, a study using the percept-related model showed COMT pain gene effects for high pain on the periaqueductal gray, hippocampus (HC), lingual gyrus (LING), calcarine cortex (CAL), precuneus (PCUN), cuneus, superior and middle occipital gyrus (SOG/MOG), and cerebellum (Cere).[32]

The present study aimed to disentangle the relationships between the two pain genes (i.e. OPRM1 and COMT) and the neural processing of perceived pain intensity. Using multivariate pattern analysis (MVPA), we investigated the different neural representations of low and high pain by analyzing the BOLD signal pattern across voxels while individuals rate the intensities of painful stimuli delivered by cuff pressure algometry to the left calf muscle. To verify any brain areas that were sensitive to low and high pain, we conducted searchlight-based MVPA and tested the null hypothesis of no difference in brain network between low and high pain should be true. Using MVPA and univariate analyses, we investigated whether the two pain genes differentially affected the neural activations for low and high pain. Specifically for high pain, we expected that: (i) G-allele carriers have higher neural activation in the pain-related regions than A/A for OPRM1; and (ii) *met* carriers have greater activation than *val/val* for COMT.

Materials and Methods

Participants

A total of 105 individuals (54 women; aged 19–46 years) participated in this study (Table 1). The participants were physically healthy with intelligence quotients ≥ 80, as assessed using the Wechsler Intelligence Scale.[36] Exclusion criteria included: (i) a history of brain injury, epilepsy, or psychiatric disorders; (ii) current use of psychotropic or pain medication; and/or (iii) left-handedness. This study was approved by the institutional review board of the University of Fukui in accordance with the ethical standards in the Declaration of Helsinki, and all participants provided written informed consent prior to the experiment.

Table 1. Demographic description of the participants

	n (% female)	Age, mean (SD)	FSIQ, mean (SD)
Participants	105 (51.42%)	27.2 (7.3)	111.3 (11.73)
Genotype OPRM1 rs179971			
A/A	37 (54.05%)		
A/G	48 (50%)		
G/G	20 (55%)		
COMT rs4680			
met/met	10 (70%)		
met/val	45 (51.11%)		
val/val	50 (48%)		
Genotype subgroups			
(1) OPRM1 A/A + COMT *met* carriers	18 (55.56%)		
(2) OPRM1 A/A + COMT *val/val*	19 (52.63%)		
(3) OPRM1 G carriers + COMT *met* carriers	37 (54.05%)		
(4) OPRM1 G carriers + COMT *val/val*	31 (45.16%)		

COMT, catechol-O-methyl transferas; FSIQ, full score IQ; OPRM1, opioid receptor mu 1.

DNA extraction and SNP genotyping

Saliva samples were collected using a self-collection kit (OG-500 Oragene; DNA Genotek, Inc., ON, Canada). We used a standard phenol-chloroform method to extract genomic DNA from the saliva samples using the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany). Using TaqMan genotyping assays (Applied Biosystems, Foster City, CA, USA), we genotyped OPRM1 $A^{118}G$ (rs1799971) and COMT $val^{158}met$ (rs4680) SNPs. All samples were genotyped *via* real-time polymerase chain reaction (PCR) analysis using the StepOnePlus System (Applied Biosystems, Waltham, MA, USA). Reactions were performed in 10-μL volumes containing 9 ng genomic DNA, 0.25 μL Tris-EDTA buffer, 0.25 μL of each TaqMan probe, and 5 μL TaqMan PCR Master Mix. The PCR cycling conditions comprised one cycle at 95°C for 20 s, followed by 40 cycles at 95°C for 3 s and at 60°C for 20 s. For each amplification, we used 4.5 μL high-performance liquid chromatography-grade water containing Master Mix as a negative PCR control. Genotype discrimination was then performed using StepOnePlus System software version 3.0.1.

Experimental design and materials

Calibration and pain assessment

Before commencing the experiment, the participants underwent a pain calibration session using a computer-controlled cuff pressure algometer (Rapid Cuff Inflation System E20 AG101; Hokanson, WA, USA). A cuff (13 × 85 cm) was mounted on the gastrocnemius of the participant's left leg, and rapid cuff inflation was applied. The participants were instructed to score their own pain levels for low and high pain using the visual analog scale (VAS), which ranged from 0 (no pain) to 20 (most severe pain imaginable), corresponding to "4–5" and "18–19," respectively. Participants were acquainted with the pain stimuli and rating procedures during the pain calibration session. The results of cuff pressure levels were used as MRI session pain stimuli for each participant.

Pain cuff task and functional imaging

While performing a pain cuff task, each participant underwent functional imaging to measure neural activity in the brain. Prior to scanning, the two levels of stimulus intensity were briefly recalibrated based on the results of the pain calibration session. For the pain cuff task, the participants received 12-s-long tonic pain stimuli that were presented and the rated pain scores using a two-button response pad for VAS rating (Fig. 1c). The experiment consisted of two sessions,

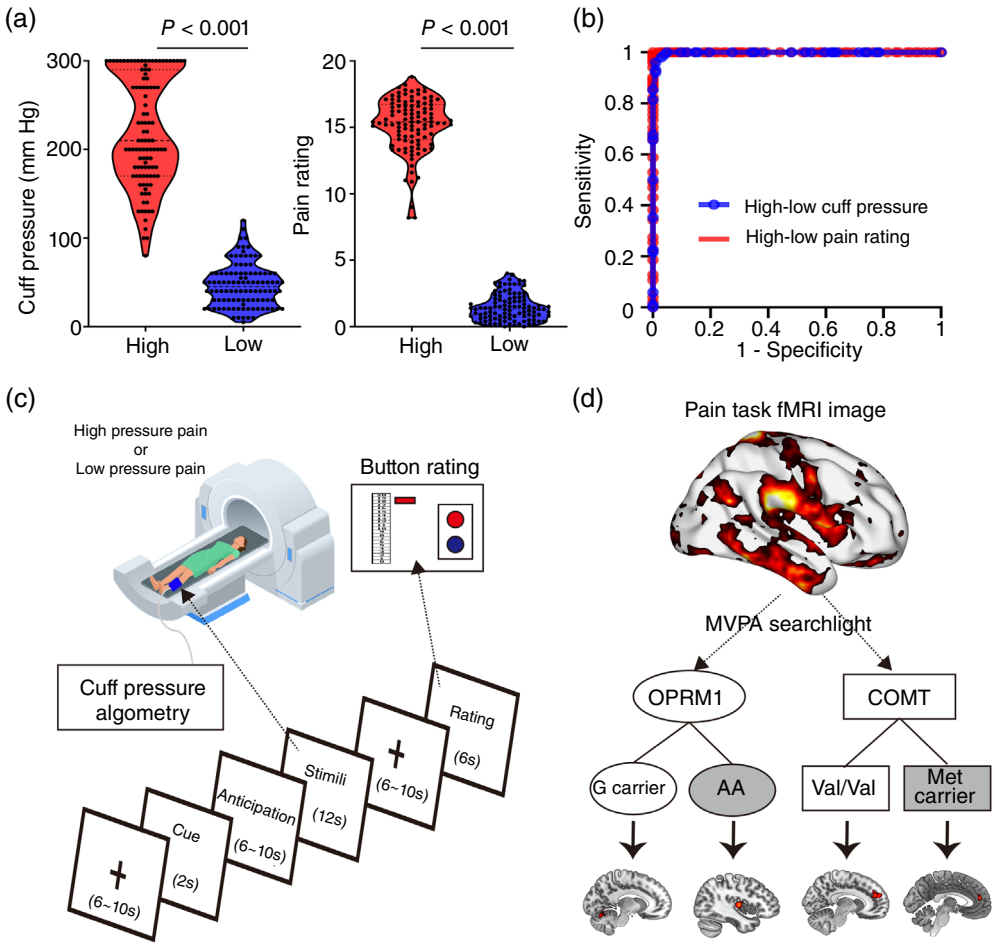

Fig. 1 Pain rating results of the pain cuff task: (a) left = difference in cuff pressure in mm Hg between high and low pain; right = difference in pain rating between high and low pain on the visual analog scale (0–20). (b) Receiver operating characteristic curve comparing high-low cuff pressure and pain rating. (c) Experimental paradigm for the pain cuff task. (d) Multivoxel pattern analysis (MVPA) searchlight procedure depending on the types of pain gene. COMT, catechol-O-methyltransferase; fMRI, functional magnetic resonance imaging; OPRM1, opioid receptor mu 1.

each of which included 10 trials with five low and five high pain stimuli. One trial had six components: (i) a black cross sign presented in the participants' visual field (6 ∼ 10 s with temporal jitter); (ii) a 2-s-long cue; (iii) an anticipatory signal in the visual field (6 ∼ 10 s); (iv) a 12-s-long sentence of "Pain is coming." presented in visual field at pain stimulus onset; (v) a cross sign at the stimulus offset (6 ∼ 10 s); and (vi) a rating where the participants used a magnetic resonance–compatible fiber optic response device (Current Designs Inc., Philadelphia, PA, USA) to report perceived pain intensity (Fig. 1c).

Image acquisition

All MRI data were acquired using a GE 3T Signa positron emission tomography/MRI with an eight-channel head coil was performed at the University of Fukui Hospital. A high-resolution anatomical T1-weighted anatomical MRI was performed (repetition time [TR] = 6.38 ms, echo time [TE] = 1.99 ms, flip angle = 11°, field of view [FOV] = 256 mm, number of slices = 172, voxel dimension = 1.0 × 1.0 × 1.0 mm^3). Task-based volumes were acquired using T2*-weighted gradient-echo planar imaging sequences by 2 sessions (number of slices = 39, thickness = 3.0 mm, volume = 220, gap = 0.5 mm, TR = 2 s, TE = 24 ms, fractional anisotropy = 80°, FOV = 192 × 192 mm^2, in-plane resolution = 64 × 64 pixels, pixel dimension = 3 × 3 mm^2).

Functional MRI data preprocessing and univariate analysis

Data were preprocessed in a conventional manner using the SPM 12 (www.fil.ion.ucl.ac.uk/spm/)[37] involving the following steps: (i) data conversion from DICOM images to nifti files; (ii) slice-timing correction by interpolating to the middle of each volume acquisition period; (iii) realignment of functional images to the very first volume of the entire scan to eliminate motion artifacts; (iv) coregistration of functional and anatomical images; (v) spatial normalization to the Montreal Neurological Institute (MNI) space with a resampled voxel size of 2 × 2 × 2 mm^3; and (vi) smoothing with an isotropic Gaussian kernel of 8 mm full width at half maximum. The last step was eliminated for MVPA. After the preprocessing, we obtained parameter estimate (β) images in association with low and high pain conditions. Using a general linear model approach, the experimental regressors for the design matrix were modeled with boxcar function and general linear model convolved with a canonical hemodynamic response function. The model included realignment parameters to regress out variance due to motion. To remove low-frequency drifts, each time series was high-pass filtered with a 128 s cutoff. We created contrast images for each participant, which were further used to create images of group contrast at the second level.

Multivoxel pattern analysis

We used a linear support vector machine (SVM)[38] with regularization parameter $C = 1$ applying two toolboxes: (i) PRoNTo allows multivariate regions of interest (ROIs) analysis based on statistical pattern recognition techniques[39] and (ii) Decoding Toolbox, adopting decoding approach, which provides searchlight analysis that creates an interpretable map of classification accuracies.[40] For each session, we extracted the β images that were used as input for the classifiers. Classification accuracy was calculated using the leave-one-session-out procedure to examine the model's generalization capability (Fig. S1).

For ROI-based MVPA, an SVM was trained and tested separately on the whole brain, and each ROI was generated based on the 90-parcel automated anatomical labeling template.[37] For the statistical

test of classification accuracies among ROIs, the results were corrected at $q = 0.05$, following the Benjamini–Hochberg procedure. We extracted the β values from the most informative voxels to examine whether the classification accuracies of low and high pain were determined by positive BOLD signals, negative BOLD signals, or their combination. Through a linear SVM, each voxel gains weight, indicating its importance in the classification. The most informative voxels were those whose weights exceeded ± 2 SDs in the group-level analysis. We calculated the percentages of voxels with positive and negative β values and averaged β values for each ROI.

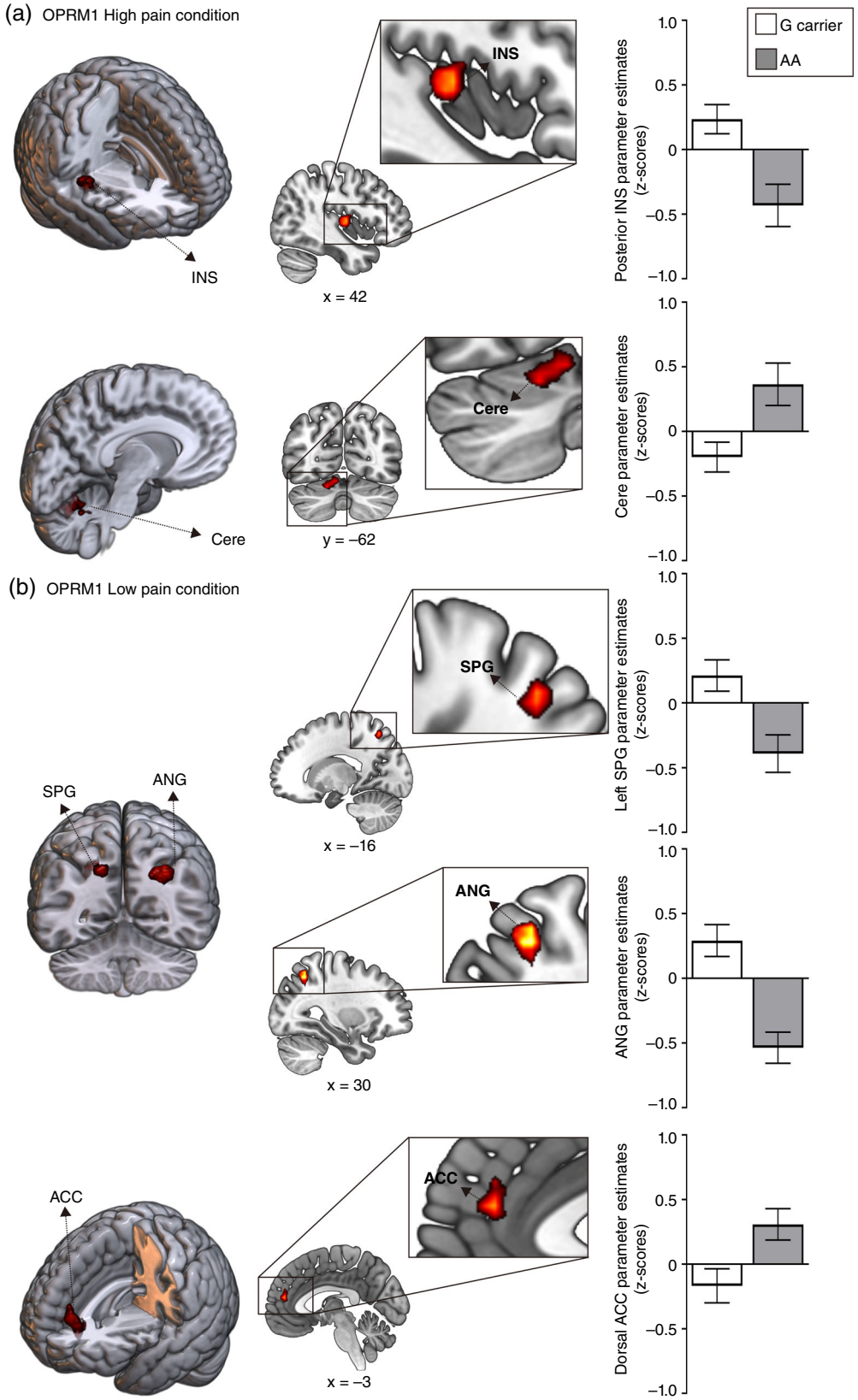

Fig. 2 Opioid receptor mu 1 (OPRM1) $A^{118}G$ polymorphism and brain activation. (a) Effects of OPRM1 on high pain perception. (b) Effects of OPRM1 on low pain perception. ACC, anterior cingulate cortex; ANG, angular gyrus; Cere, cerebellum; INS, insula; SPG, superior parietal gyrus.

In the searchlight MVPA, a searchlight with a 4 mm radius sphere traveled across the entire brain and took each voxel in the volume as the searchlight center. A linear SVM was trained and tested for each sphere, as described above, and the classification accuracy score was assigned to the center of the voxels. In the first-level analysis, the classification accuracy map of the whole brain of each participant was spatially smoothed (6 mm full width at half maximum), and the maps were subjected to random-effect group analysis. The resulting T-map indicates the statistical significance of the voxel-wise accuracies against a chance-level accuracy of 50%. The T-map was thresholded at $q < 0.001$.

Results

Behavioral differences in low and high pain rating among the participants

Low pain was evoked by a pressure of 5 to 120 mm Hg (mean = 46.72 ± 24.30 mm Hg) to the left calf muscle, and the VAS rating ranged from 0 to 4 of 20 (mean = 1.47, SD = 1.01; median = 1.3, interquartile range = 1.4). High pain required pressure of 80 to 300 mm Hg (mean = 220.20 ± 62.39 mm Hg) and yielded a 8.2 to 18.8 of 20 (mean = 15.22, SD = 2.05; median = 15.4, interquartile range = 2.6) VAS rating. Low and high pain were significantly different for cuff pressure ($P < 0.001$) and pain rating ($P < 0.001$) (Fig. 1a). Receiver operating characteristic curve analysis revealed that the cuff pressure and pain rating significantly differentiated low pain from high pain (area under the curve = 0.998) ($P < 0.0001$) (Fig. 1b). We found no significant difference in groups by pain gene types.

Multivoxel classification of low versus high pain

MVPA discriminated between low and high pain on 90 regions of interest (ROIs) based on the automated anatomical labeling template atlas.[37] The classification accuracies of all ROIs were significantly greater than 50% after correcting for multiple tests (Fig. S1a,b). We examined whether the classification of high versus low pain is associated with neural activation by extracting the β values of the most informative voxels in all ROIs (see Materials and Methods). The accuracies for high pain were significantly greater than those for low pain in bilateral rectus gyrus, MFG and superior frontal gyrus (SFG), middle cingulate cortices, HC, and LING; left ACC and paracentral lobule; right PrCG, CAL, fusiform gyrus, postcentral gyrus (PoCG), superior parietal gyrus (SPG), angular gyrus (ANG), PCUN, and middle temporal gyrus (MTG) at $q = 0.05$.

Whole-brain searchlight MVPA

To further identify any brain regions that were sensitive to low and high pain, we used a searchlight MVPA[41] (Fig. 1d). A 4 mm radius searchlight MVPA detected both low and high pain in the Rolandic operculum (ROL), PoCG, supplementary motor area (SMA), supramarginal gyrus (SMG), and INS. Low pain was detected in more areas than high pain.

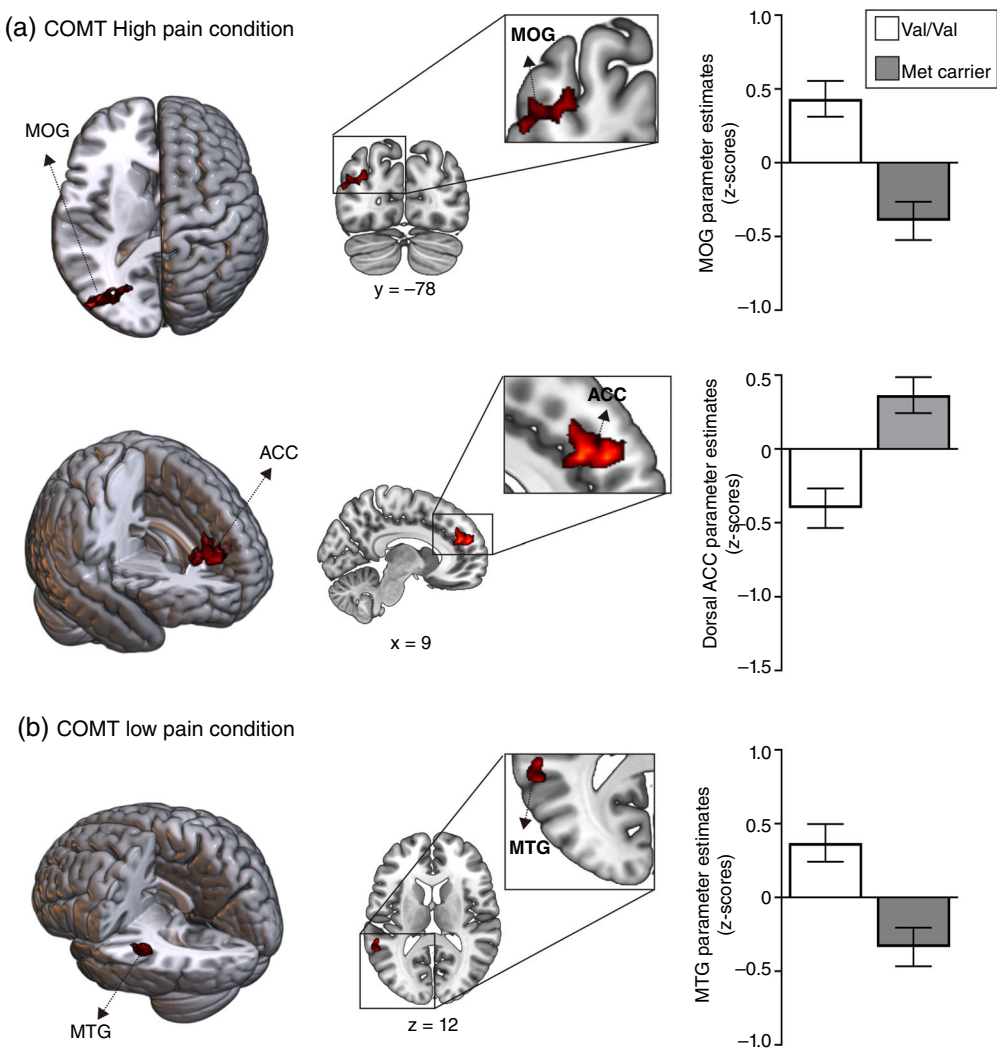

Fig. 3 Catechol-O-methyltransferase (COMT) *val*^{158}*met* polymorphism and brain activation. (a) Effects of COMT on high pain perception. (b) Effects of COMT low pain perception. ACC, anterior cingulate cortex; MOG, middle occipital gyrus; MTG, middle temporal gyrus.

Significant searchlight centers for low pain were the SFG (peak at MNI coordinates [9, 30, 48], [0, 24, 42]), MFG ([−42, 36, 21], [−30, 3, 63], [12, 45, 48]), IFG ([−51, 6, 12], [15, 36, 51]), ROL ([−57, 0, 3], [51, −30, 21]), PrCG ([−42, −3, 60], [−36, −3, 66]), PoCG ([−60, −21, 21], [15, −42, 69], [60, −21, 39]), SMA ([−3, 15, 51], [−3, 0, 57]), SPG ([24, −60, 72]), SMG ([−57, −24, 30], [−54, −24, 15]), [63, −27, 27]), MOG ([−18, −105, −6], [−12, −105, 6]), MTG ([−51, −60, 6]), [51, −54, 3], [57, −66, 3]), and INS ([−27, 24, 0]). Significant searchlight centers for high pain were the right ROL ([54, −27, 21]), PoCG ([15, −48, 69], [15, −42, 75], [21, −42, 78]), SMA ([15, 69], [9, 12, 72], [3, −18, 69]), SMG ([66, −24, 27], [63, −21, 30]), [60, −15, 24]), PHG ([24, −18, −33]), INS ([54, 6, 0]), temporal pole (TP) ([57, 9, −3]), and bilateral inferior temporal gyri (ITG) ([48, −39, −21], [−54, −12, −36], [−51, −9, −39]).

Univariate analyses for low and high pain

Univariate analyses checked compliance and differences between the pattern- and voxel-based analyses. Positive associations were found between pain rating and activation in the left PrCG ($r = 0.376$, $P < 0.001$) and the right MOG ($r = 0.392$, $P < 0.001$) for low pain. Negative associations were found in the left PrCG ($r = -0.493$, $P < 0.001$) and Cere ($r = -0.533$, $P < 0.001$) for high pain (Fig. S2b).

Pain gene effects

We conducted full-factorial analysis on the β values calculated from the univariate approach with pain intensity (low vs high) and pain gene (for OPRM1 A/A vs G-allele carriers; for COM Tval/*val* vs *met* carriers) as fixed factors and sex and age as nuisance covariates. The analysis was conducted separately for each pain gene. We performed an exploratory search using an uncorrected threshold of peak level $P < 0.005$ with a minimum of 50 contiguous voxels and then applied a cluster-level correction at a false discovery rate $q < 0.05$. For low pain, OPRM1 G-allele carriers showed significantly higher neural activation in the left SMG and ANG than A/A. Conversely, A/A had significantly higher activation in ACC than G-allele carriers (Fig. 2b). For high pain, significant differences between G-allele carriers and A/A were found in the posterior INS and Cere (Fig. 2a). For low pain, COMT val/*val* exhibited significantly higher activation in the right MTG than *met* carriers (Fig. 3b). For high pain, *val/val* displayed higher activation in the MOG than *met* carriers, while *met* carriers showed higher activation in dorsal ACC (dACC) (Fig. 3a). In addition, we confirmed activations in the insula, ACC, and dACC using an uncorrected threshold of peak level $P < 0.001$ with a minimum of 22 contiguous voxels and then applied a cluster-level correction at a false discovery rate $q < 0.05$ (Table S1). Overlaying the main effects of the two pain genes, we conducted a conjunction analysis to examine any brain regions that both OPRM1 and COMT affect simultaneously. Fourteen voxels in dACC (Fig. 4a) showed significant differences in high pain accuracy and parameter difference for four subgroups: (i) OPRM1 A/A with COMT *met* carriers; (ii) OPRM1 A/A with COMT *val/val*; (iii) OPRM1 G-allele carriers with COMT *met* carriers; and (iv) OPRM1 G-allele carriers with COMT *val/val* (Fig. 4b; Table 1). The subgroup 1 showed higher accuracy for high pain (subgroup 1 = 91.7%, subgroup 2 = 73.7%, subgroup 3 = 79.3%, subgroup 4 = 77.4%; $F = 2.736$, $P = 0.047$) and higher parameter difference (subgroup 1 = 13.1, subgroup 2 = −0.13, subgroup 3 = 5.4, subgroup 4 = 6.15; $F = 3.784$, $P = 0.012$).

Discussion

This study investigated the effects of OPRM1 and COMT polymorphism on neural activities for the differently perceived pain intensity. The wide variability in pain ratings suggests the subjective nature of pain perception. Classification accuracies for low and high pain were above chance level. Compared with the univariate analyses, MVPA revealed noticeably broader brain regions for low and high pain. High pain had significantly greater classification accuracies than low pain. The searchlight MVPA results demonstrated that both low and high pain were processed in the operculo-insular cortex extending from INS to SI/SII, but with distinct neural patterns, suggesting that differentially perceived pain intensity is associated with activities of functionally different neural populations.[42] Low pain consists of a broader network than high pain. Low pain–specific activations were found in the frontal, parietal, occipital, and temporal areas, whereas high pain specificity was found in the temporal regions. The results reflect the multidimensional aspects of pain experience encompassing sensory, discriminative, motor, cognitive, evaluative, emotional, and motivational components.[43] The distinguishable OPRM1 and COMT gene effects are complex and region-specific depending on the perceived pain intensity level. A subset of dACC is affected by both pain genes, suggesting that the proteins encoded by OPRM1 and COMT in dACC may contribute to the firing of pain-related neurons.

Shared network between low and high pain

Both low and high pain processing involve neural activities in the INS, ROL, PoCG, SMA, and SMG. The operculo-insular cortex is functionally associated with pain processing,[44–46] and the SI/SII and INS are known for sensory/discriminative processing of pain intensity.[35,47] Of various pain types, experimentally induced pain activates the INS, ROL, PoCG, and SMA. Activation of SMA is associated with flight reactions to pain.[45,46] Taken together, the operculo-insular cortex plays a role in checking painful inputs and initial coding of experimentally induced pain intensity. Intensity coding is an

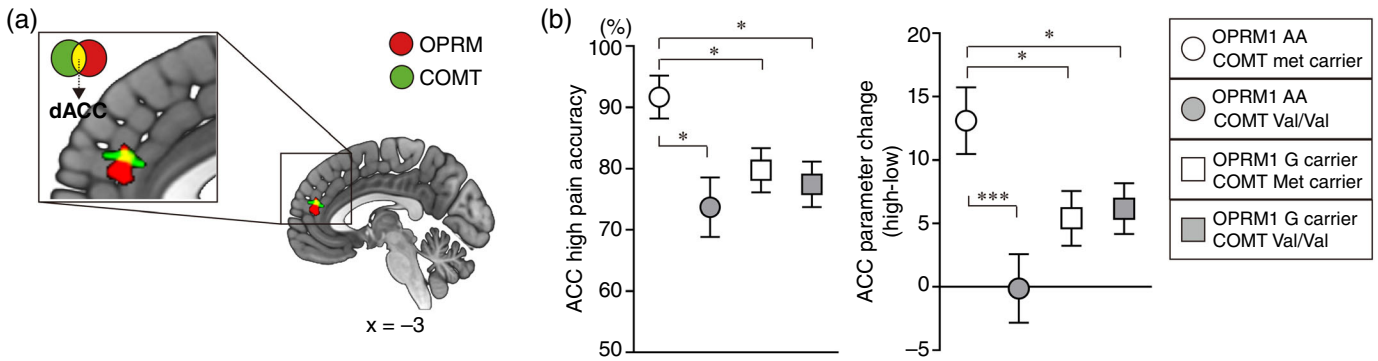

Fig. 4 Overlay of the two pain genes in dorsal anterior cingulate cortex (dACC). (a) Overlay of brain activation in terms of the effects of opioid receptor mu 1 (OPRM1) and catechol-O-methyltransferase (COMT) polymorphism on high pain perception. (b) Difference in high pain accuracy and the high-low parameter difference in dACC among four subgroups: (i) OPRM1 AA homozygotes and COMT *met* carriers; (ii) OPRM1 AA homozygotes and COMT *val/val* homozygotes; (iii) OPRM1 G-allele carriers and COMT *met* carriers; (iv) OPRM1 G-allele carriers and COMT *val/val* homozygotes.

important role of the INS.[48,49] Following the suprasylvian operculum, the INS sequentially responds to pain with a short response latency (*ca*. 40–50 ms).[49] The SII and INS show gradually incremental responses to pain intensity.[49] Significant correlations were observed between the amplitude of the early negative component, mainly generated by the operculo-insular cortex, and perceived pain intensity.[50] Thus, the regions of the operculo-insular cortex, as for the shared network of low and high pain, may play a role in screening input intensity delivered from the peripheral systems and determining whether it is pain, suggesting that the shared network is for sensory/discriminative processing of pain.

Specificity of low and high pain

We found distinguishable neural representations among voxels in the shared network, indicating the functional differences in neural populations.[42] Furthermore, low and high pain had specific sets of regions. Low pain had a broader network than high pain in consistent with previous studies.[30,33] Low pain specificity is determined with neural activities in IFG/MFG/SFG, PrCG, SPG, MOG, and MTG, whereas high pain specificity involves ITG, PHG, and TP. A wide network for low pain seems to reflect the cognitive/evaluative processing of pain. Low pain may require high cognitive demands because low pain stimuli are less salient in the pain intensity decision task and more potent in the activation of the evaluative network in pain processing.[30] By contrast, high pain stimuli are sufficiently potent for the quick cognitive evaluation of painful inputs. In line, our behavioral results showed greater variability in the low pain rating than in the high one. In response to pain, early evoked potentials in the SI and parasylvian areas are followed by a later component in the MFG, which indicates the role of attention.[51] Pain modulation *via* attention involves the INS, SII, MFG, and IFG.[48] In addition, pain anticipation may be involved in the INS, SFG, IFG, and MTG.[52] High pain–specific regions were found in the temporal cortex. Given dysfunction in the temporal lobe in individuals with chronic pain, temporal regions may contribute to pain modulation *via* memory and emotion.[53] Painful stimuli induce robust emotional learning and memory formation, and individuals with medial temporal lesions showed an impaired emotional memory network.[54,55] In pain processing, the emotional component is electrophysiologically distinguishable from the sensory component: early somatosensory response (<200 ms) is followed by a late response (400–500 ms), indicating memory encoding and recall in temporo-occipital junction and TP.[56]

Effects of pain genes on pain

The impacts of pain genes on brain activations are region-specific and complex depending on perceived pain intensity. For low pain, OPRM1 G-allele carriers showed higher activation in the SMG and ANG but lower activation in the ACC relative to A/A. For high pain, G-allele carriers showed higher activation in the INS and lower activation in the Cere. Compared with *val/val*, COMT *met* carriers had lower activation in MTG for low pain, and lower activation in the MOG and higher activation in the ACC for high pain.

Effects of OPRM1 $A^{118}G$

Previously, G-allele carriers showed a greater neural activation in pain-related regions than A/A.[15,35,57] Our findings partially supported this: increased activation was found in the INS, SMG, and ANG for G-allele carriers, compared with A/A. The INS is associated with pain intensity coding, indicating sensory processing in pain perception.[35] The postmortem human brain demonstrated the effects of OPRM1 $A^{118}G$ SNP on the SII, another important center of sensory processing.[57] Regarding roles of the SMG and ANG in subserving the interpretation of painful stimuli,[30] OPRM1 effects were found for low pain that involves cognitive/evaluative processing of pain as described above.

In contrast with previous studies, we found that G-allele carriers had significantly lower activation in the ACC. Here, it should be noted that reduced activation in G-allele carriers was observed for low pain. Although the ACC was suggested to mediate the cognitive and affective components of pain,[17] low pain seems to require involvement of a cognitive dimension of pain processing and might suppress involvement of emotional processing in our experimental setting. Therefore, we propose that OPRM1 $A^{118}G$ variants primarily affect the sensory and cognitive processing of pain.

Ample research has indicated that the OPRM1 polymorphism influences various neurotransmitter systems. Specifically, G-allele carriers showed reduced MOR expression in the mouse brain[58,59] and in the human brain.[57,60,61] G-allele carriers have reduced efficacy of MOR signaling *via* altering binding affinity for endogenous opioid peptide,[7] especially in the SII,[57] and affect other neurotransmitter systems (e.g. dopaminergic systems) in response to pain.[14] Considering this, we speculate that G-allele carriers affect the sensory and cognitive dimensions of pain processing due to decreased MOR availability and altered neurotransmitter system in the INS, SII, ANG, and SMA.

Effects of COMT *val*^{158}*met*

The COMT *val*^{158}*met* variants seem to have a stronger influence on neural activities in the key structures for the affective/emotional components of pain (e.g. ACC and temporal cortex). In line with previous studies showing an involvement of ACC in affective/emotional processing of pain,[19,27,31,62] we found higher activation among *met* carriers in ACC for high pain. The ACC was suggested to play an important role in the top-down modulation of pain,[45] potentially mediated by the opioidergic neurotransmission.[63,64] The COMT *val*^{158}*met* variants affect ACC activity, which may mediate the inhibitory pain system, potentially due to the reduction of opioid release.[17] In addition, the ACC showed an opioid-dependent response to pain.[64] Given that the postmortem human prefrontal cortex showed low COMT activity for *met* variants[65] and that low COMT activity elevates catecholamine and activates adrenergic receptors,[66] BOLD responses in the ACC of *met* carriers likely indicate low COMT activity and changes in multiple neurotransmitter systems in pain processing.

Previously, the COMT effects were observed with higher neural activations in pain processing regions for *met* carriers than for *val/val*.[19,32] However, the present study is partially in line with previous findings. For low pain, lower activation in the MTG was observed for *met* carriers, relative to *val/val*. Given that low pain is associated with the MTG deactivation[31] and that negative BOLD signals are related primarily to inhibitory postsynaptic potentials,[67] we suggest that *met* carriers may affect downregulation of inhibitory pain systems in the MTG. For high pain, we found that *met* carriers had lower MOG activation than *val/val* carriers. This may be due to differences in stimulus modality and experimental design.[45] For instance, the significant BOLD response was observed only when pain system was repeatedly and robustly challenged with heat-induced high pain.[32] Considering the temporal changes in pain response, the authors argued that *met* carriers are associated with a slower habituation to pain than *val/val* and the habituation may occur at an early stage of pain processing at the level of the spinal cord.

Regarding the contradicting findings of the present study, it is important to note that the role of the MOG in pain processing has been hypothesized to cognitive dimension,[31] and that the low pain is cognitively demanding. Therefore, we speculate that COMT SNP affects affective/emotional processing of pain.

Pain genes and the dorsal ACC

The most intriguing finding of the present study was that the two genes affect dACC activation, which was not found in behavioral results (Fig. 4). We speculate a trade-off between the two pain genes for the subgroup 1: reduced activation in dACC for COMT *met* carriers may lead to overactivation for OPRM1 A/A. Pain selectivity of ACC has long been in dispute[68,69] because ACC subserves multiple functions.[27,29,62,70] Nonetheless, researchers agree that a small part of dACC is pain selective.[71] Using genetic neuroimaging approaches,

the present study indicates that a subset of dACC might be a pain-selective area where two pain genes interact. We speculate that OPRM1 and COMT may control gene expression in pain-selective neurons in dACC.

Limitations

Limitations of this study include its focus on two pain genes and its failure to investigate the effects of haplotypes. Other pain genes (e.g. CACNG2 gene[6], SCN9A gene[10,27], and 5-HTT gene[72]) or pain phenotypes (i.e. major haplotypes derived from COMT rs6269, rs4633, rs4818, and rs4680) may contribute to individual differences in pain.[18] Furthermore, pain response changes over time, and pain genes may differentially affect the specific time window of pain processing. The experimental design of the current study was not suitable for investigating the temporal dynamics of pain processing. The current findings were restricted to the perception of pain induced by pressure. Finally, the collected data are not large enough to apply holdout procedures for cross-validation of MVPA analysis.

Author contributions

Y.C., H.K., and M.J. designed the research; H.O., H.K., and M.J. performed the research; Y.C., S.L., and M.J. analyzed the data; Y.C., S.L., H.K., and M.J. wrote the manuscript draft; and all authors revised the manuscript.

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Disclosure statement

Hirotaka Kosaka is an editorial board member of the *Psychiatry and Clinical Neurosciences* and a coauthor of this article. To minimize bias, they were excluded from all editorial decision-making related to the acceptance of this article for publication.

Data availability statement

The data presented in this study are available on request from the corresponding authors.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section at the end of this article.