Research Paper

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Influence of catechol-O-methyltransferase Val158Met on fear of pain and placebo analgesia

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Abstract

Higher levels of fear have been shown to partly explain individual differences in placebo analgesic responding. The catechol-O-methyltransferase (COMT) rs4680 Val158Met polymorphism has been associated with both increased placebo analgesia and increased fear-related behavior, in what appears to be inconsistent findings in the literature. The aim of the study was therefore to investigate placebo analgesia and fear-related processes with regard to the COMT genotype, to sort out whether the Met-allele is associated with increased placebo analgesia or increased fear of pain (FOP). A 3 Group (Emla, placebo and natural history) by 5 Test (2 pretest, 3 posttests) mixed design was used (N = 223). A contact heat-evoked stimulator was used to induce pain, and FOP was quantified with the Fear of Pain Questionnaire-III. Saliva was obtained for genotyping. As expected, we observed a significant interaction of test by group ($P < 0.01$), with lower pain report in the placebo group compared with the natural history group ($P < 0.01$). There was a main effect of the COMT genotype on fear of medical pain ($P = 0.032$), and Met-allele carriers reported significantly higher fear of medical pain compared with the Val-allele ($P = 0.044$). We observed no effect of the COMT genotype on mean pain-level report or placebo analgesia. Thus, we conclude that the Met-allele seems to be associated with the negative emotional process of fear, but not with placebo analgesia.

Keywords: Placebo analgesia, Fear of pain, Genetics, COMT rs4680 Val158Met, Polymorphism

1. Introduction

Placebo analgesia is defined as the reduction of pain after administration of an inert treatment in combination with an expectation that the treatment will reduce the pain.[20] The literature describes both responders and nonresponders to placebo treatment,[10,11,39] and genetic factors may be important in explaining individual differences in placebo analgesia. In a clinical perspective, identifying responders and nonresponders can improve treatment outcome in pain conditions, as treatment approaches can be individually customized.[16]

It has been suggested that the placebo response may be mediated by activation of reward processing, ie, central monoamine pathways.[9,16] Because of dopamine's (DA) important role in reward processing, DA has been the primary focus in understanding the genetic aspect of reward processing in the placebo response.[15,19,39] Catechol-O-methyltransferase (COMT) is an enzyme that metabolizes catecholamines—and modulates adrenergic, noradrenergic, and dopaminergic signaling. Several single-nucleotide polymorphisms in the gene encoding the COMT enzyme may affect expression of the catecholamines. One such genetic variant is the COMT Val158Met rs4680 that causes a substitution of valine (Val) to methionine (Met) at codon 158. This substitution affects enzyme activity in which individuals homozygous for the Met-allele have a 3 to 4 times reduced enzyme activity compared to those homozygous for the Val-allele. Thus, the Met-allelic variant is associated with higher DA levels,[16] which may affect reward mechanisms.

Reward expectations are known to induce release of DA in the nucleus accumbens.[33] Scott et al.[33] examined the role of reward expectations in formation of placebo analgesia. They found that increased levels of DA because of reward expectations were proportional to placebo-induced DA levels after placebo administration. Because COMT polymorphism is central to the level of DA, COMT is also suggested to be involved in placebo analgesia.[39] For example, Yu et al.[39] found a higher correlation of placebo analgesic effect in healthy subjects with the Met-allelic variant, compared to the other allelic variants.

The COMT genotype has also been associated with anxiety and fear. Wendt et al.[38] found that individuals expressing the COMT Met-allele had delayed fear extinction and deficient fear inhibition compared to Val/Met and Val-allele. Norrholm et al.[30] found that individuals expressing the Met-allele experienced increased fear-potentiated startle during fear conditioning compared to the other allelic variants. Taken together, the Met-allele has been reported to display stronger placebo analgesia[39] and more fear-related behavior.[30,38]

Interestingly, earlier studies suggest that high fear of pain (FOP) is negatively related to placebo analgesia.[22] Furthermore, experimentally induced fear has been found to abolish the effect of a placebo on pain.[24] These findings conflict with either Yu et al,'s[39] finding that subjects with the COMT Met-allele displayed larger placebo responses, or Wendt et al.'s[38] finding of more

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fear-related behavior in subjects with the Met-allele. Thus, the aim of the study was to investigate placebo analgesia and fear-related processes in carriers of the COMT rs4680 Met-allele, to investigate what appear to be inconsistent findings in the literature.

2. Methods

2.1. Subjects

The participants were recruited through announcements at the University campus. All participants signed an informed consent before they were enrolled in the study. In the consent form, it was specified that if the participants previously or presently had experienced any severe medical conditions or chronic pain conditions, were pregnant, had cutaneous injuries to the arms or hands, or used prescribed medications with the exception of contraceptives, participation were not allowed. The participants received a gift certificate of 200 NOK as compensation for participation. The study was conducted in accordance with Helsinki declarations and was approved by the Regional Committee for Medical Research in North Norway (project number 2013/966). A total of 327 healthy volunteers c were included in the experiment. For this article, 223 participants (142 females and 81 males) had complete data for pain and FOP and were included from the original sample.

2.2. Design

A 3 group (Emla cream, placebo cream and natural history) × 5 test (2 pretests, 3 posttests) double-blind, between-group design with repeated measures was used. The participants were randomly assigned to 1 of the 3 groups. The group receiving the Emla cream was included in the design to assure that the experimenters were blinded. Thirty one participants (9.4%) were assigned to the Emla group and data from this group were not included in the analyses. The experimenters were students at the clinical psychology program at the Department of Psychology, UiT The Arctic University of Norway, and they were experienced in preforming experimental laboratory testing.

2.3. Outcome measures

Pain was induced by contact heat stimulation (30 × 30 aluminum contact thermode, Pathway; Medoc, Israel) attached to the left volar forearm. Pain was measured continuously during stimulation by a 0 to 100 Computerized Visual Analogue Scale (COVAS) (Medoc, Israel), where 0 equaled no pain sensation and 100 equaled the most intense pain sensation imaginable. The temperature used in the pre- and posttest was calibrated for each participant at VAS = 60.

Subjective stress was measured by 2 adjective pairs from the Norwegian translation of the Short Adjective Check List (SACL),[25] similar to previous studies in our laboratory.[1–3,22–24] The adjective pairs were tense-relaxed and nervous-calm. A rating of 0 = completely relaxed/calm and 10 = maximum tension/nervousness was used. Stress was measured prior to the pretest and immediately after the posttests.

Fear of pain was measured by the Fear of Pain Questionnaire-III (FPQ-III).[27] The FPQ-III is a 30-item self-report questionnaire related to specific situations that is associated with pain. There are 3 subcategories that record fear specific to severe pain (such as breaking your neck), minor pain (such as biting your tongue while eating) and medical pain (such as having an item that is stuck in your eye removed by a doctor). Each item is rated on a 5-point Likert scale, where 1 = no fear at all and 5 = extreme fear. The questionnaire has been used in several previous studies and has demonstrated good internal consistency and test-retest reliability.[27,32]

2.4. Pain stimulation

Stevens's power equation VAS = $b(t\text{-}t_0)^{c35}$ was used to predict the stimulus intensity needed to produce a rating of VAS = 60. In this equation, b is a scaling factor, t is the stimulus temperature, t_0 is the intercept, where VAS is assumed to be zero which was set to 35°C, and c is the exponent which defines the shape of the stimulus response function that was estimated based on the 7 calibration tests.[29] The duration of the stimulations was 10 seconds from when the thermode reached the target temperature (43°C-47°C) until the start of the return to baseline at 32°C. The temperature of the thermode increased/decreased by 10°C/s. The calibration procedure was similar across participants, and the temperature that was calculated to be VAS = 60 was used throughout the experiment.

The pretests 1 and 2 in the experimental procedure had a duration of 15 seconds from the time when the thermode reached the target temperature until the start of the return to baseline at 32°C. The temperature of the thermode increased/decreased by 10°C/s. The interval between pretest 1 and pretest 2 was 30 seconds. The posttests 1, 2, and 3 had the same temperature, duration, and intervals as the pretests.

2.5. Placebo cream

E45 Cream (E45 Cream: Crookes Healthcare, Nottingham, United Kingdom) was chosen as placebo cream. A dose of 3 g was used for each participant. The E45 was chosen because it is similar to Emla cream in color, odor, and consistence. Emla cream is an analgesic cream with a 5% eutectic mixture of 25 mg/mL lidocaine and 25 mg/mL prilocaine in an oil-water emulsion cream.

2.6. Blood pressure and heart rate

Blood pressure and heart rate were measured with a standard electronic blood pressure device (Microlife, Widnau, Switzerland). Systolic/diastolic blood pressure and heart rate were registered before the calibration procedure, after pretests and after the posttests.

2.7. Genotyping

Collection of saliva and extraction of genomic DNA were done using Oragene DNA sample collection kit (DNA Genotech Inc. Kanata, Ontario, Canada) according to the manufacturer's instructions. The genotyping was carried out using a predesigned TaqMan assay for COMT (Applied Biosystems, Foster City, CA). Approximately, 10 ng genomic DNA was amplified in a 5 μL reaction mixture in a 384-well plate containing 1× TaqMan genotyping master mix (Applied Biosystems) and 1× assay mix, the latter containing the respective primers and probes. The probes were labeled with the reporter dye FAM or VIC to distinguish between the 2 alleles. After initial denaturation and enzyme activation at 95°C for 10 minutes, the reaction mixture was subjected to 60 cycles of 95°C for 15 seconds and 60°C for 1 minute. The reactions were performed on an ABI 7900HT sequence detection system. Negative controls containing water

instead of DNA were included in every run. Genotypes were determined using the SDS 2.2 software (Applied Biosystems). Approximately, 10% of the samples were re-genotyped and the concordance rate was 100%.

2.8. Procedure

The experiment took place inside a steel cubicle (2.8 × 2.8 m), where the participants were placed in a comfortable chair. The cubicle was shielded from sound and electricity, and the temperature was kept at 20°C. When the participants arrived, they were informed about the experiment. They were told that the purpose was to investigate how the effect of an analgesic cream influences the experience of pain and how genetic factors influence treatment of pain. They were also told that they would either get the analgesic cream or an inert placebo cream. However, the participants were not told what treatment they would receive or whether they participated in the natural history group. The participants then signed the consent form and filled out the FPQ-III. After, they were asked to rate subjective stress according to the SACL, and blood pressure was measured.

The participants were placed in the chair inside the cubicle, and instructed how to use the COVAS. The thermode was attached to the volar forearm, and the experimenter left the cubicle. During the experiment, the experimenters sat outside in a larger room monitoring the apparatus for control of the experimental events. The calibration procedure was then preformed to set the participants temperature for VAS = 60. After 4 of 7 stimulations, the contact thermode was moved 2 cm from the original spot towards the elbow, to avoid hyperalgesia. After the calibration procedure, the participants were told that the experiment would begin.

The stimulation program for the 2 pretests was then initiated and the participants reported pain level using the COVAS for each stimulation. When the pretests were completed, the experimenter entered the cubicle and removed the thermode. The cream was applied on the participants' volar forearm, between the elbow and the wrist. The participants in the placebo group were told, "the cream that will be applied to your arm reduces pain. The substance in the cream is used as a local anesthetic in many pain-reducing remedies and is effective in treatment of heat pain." The participants were also told that there would be a break for a few minutes so that the cream would get some time to produce the analgesic effect. In the natural history group, no cream was applied and no information about the treatment was provided. The participants were told that there would be a break of a few minutes and that they could relax and wait for the experiment to continue. Subjective stress and blood pressure were measured and the experimenter left the cubicle.

After a 17-minute break, the left over cream was removed with water napkins, and the thermode was attached 2 cm from the last stimulation spot towards the elbow, where the cream had been applied. The experimenter then initiated the 3 posttests. The participants reported the pain level with COVAS for each stimulation. When the posttests were completed, subjective stress and blood pressure was measured, and the thermode was removed. Saliva samples were then collected from the participants. The saliva samples were kept in a refrigerator until it was shipped to the DNA laboratory analysis. The experimental procedure had a total duration of approximately 45 minutes. **Figure 1** presents an overview of the experimental procedure.

2.9. Response scoring

The placebo analgesic effect is defined as suggested by Hoffman, Harrington, and Fields,[17] as the mean pain reduction after placebo treatment on a group level, compared to a natural history group that does not receive any treatment. The placebo analgesic response is referred to as an individual response to placebo treatment.

2.10. Statistical analysis

The statistical analyses were performed with SPSS 24.0 (IBM SPSS, Chicago, IL). Repeated measures data for pain and stress were analyzed with linear mixed models (LMM). Linear mixed models were chosen over repeated measures analysis of variance (ANOVA) because LMM can be used with data in which residuals do not have constant variance across group-levels. Furthermore, LMM do not require independent error terms and are less affected by missing data compared to standard general linear models.[6,34] Hence, a repeated LMM was used to analyze the pain data. The repeated LMM was performed with an autoregressive covariance structure (AR1) and included COMT, Group, and Test as fixed factors and the 3 subscales of FOP and sex of the participant as continuous predictors. Catechol-O-methyltransferase was analyzed with 3 levels (COMT = Val/Val, Val/Met, Met/Met). The following interactions were tested: Test by Group, Group by COMT, Test by Group by COMT. An allele-dependent model was assumed as genotype model based on the allele frequency distribution. The effect of the Met/Met allele was expected to be twice the effect of the Val/Met-allele, when compared with the Val/Val-allele. Separate univariate LMM were used to test the effect of FOP on placebo analgesia and the effect of COMT on FOP.

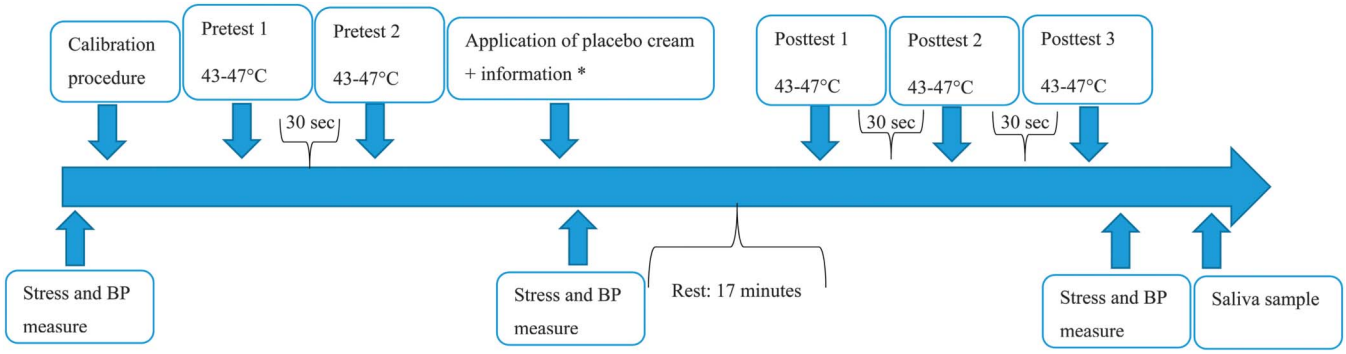

Figure 1. Overview of the procedure. The pain stimulus temperature was calibrated to VAS=60 before the pretests. The duration of the procedure was approximately 45 minutes. *Participants in the natural history group received no cream and no information. BP, blood pressure.

The participants were assumed to induce significant individual variance, and the individual variance was treated as the only random effect in the repeated measures analysis. *P* values for comparisons of levels within interactions were adjusted for multiple comparisons with Bonferroni contrast corrections. The *P* values shown in the results for comparisons of levels within interactions are the adjusted *P* values. An alpha value of 0.05 was used for all analyses.

3. Results

3.1. Descriptive statistics

The mean (SD) pain-level score was 52.40 (SD 18.43, min 7.00, max 92.40). The mean for systolic blood pressure was 126.23 (SD 11.63, min 101.33, max 177.33). The mean for heart rate was 68.02 (SD 11.42, min 44.00, max118.67). Seventy-five participants were COMT genotype Met/Met, 100 participants were genotype Val/Met, and 45 were genotype Val/Val. No deviation from the Hardy-Weinberg equilibrium was observed. This suggests that there are no systematic differences in the allele frequencies in the present sample compared to the European Caucasian population. Thus, the frequency of the COMT allelic variants is consistent with the frequency in European Caucasians (the allele frequency database). Three participants were not genotyped because of incomplete saliva samples. Descriptive statistics and outcome variables for each genotype are presented in **Table 1**.

3.2. Pain and placebo analgesia

The LLM revealed a significant effect of Test, ($F_{(4, 538.49)} = 62.86$, $P < 0.001$) and Test by Group ($F_{(4, 538.49)} = 10.82$, $P < 0.001$) with lower pain report in the placebo group compared to the natural history group in test 4 and test 5 ($P < 0.01$). The contrasts did not show other significant differences in pain between the placebo and the natural history group. There was no effect of COMT ($F_{(2, 207.57)} = 0.72$, $P = 0.49$) or Group ($F_{(1, 207.58)} = 0.07$, $P = 0.80$). Likewise, there were no significant interaction effect of COMT by Group ($F_{(2, 207.58)} = 1.99$, $P = 0.14$) or Test by Group by COMT ($F_{(16, 538.49)} = 0.97$, $P = 0.49$). There was an effect of sex of the participant on mean pain level, because of females reporting higher pain intensity compared to males ($F_{(1, 206.99)} = 7.19$, $P = 0.008$). The random effect parameter showed that the participants varied significantly in pain reports (Variance = 282.19, SE = 31.55, Wald Z = 8.95, $P < 0.001$), suggesting that individual differences significantly affected pain reports. The pain-level reports between the placebo group and the natural history group are presented in **Figure 2**. The main effects and interaction effects of pain on the independent variables and continuous predictors are presented in **Table 2**. The pain-level reports between the COMT allelic variants are presented in **Figure 3**. Fear of medical pain was a significant predictor for pain ($F_{(1, 206.99)} = 4.91$, $P = 0.03$), whereas fear of severe pain ($P = 0.69$) and fear of minor pain ($P = 0.91$) had no significant impact on pain level.

3.3. Catechol-O-methyltransferase, fear of pain, and placebo analgesia

The univariate linear mixed models revealed that there was an association between COMT and fear of medical pain ($F_{(2, 204.98)} = 4.64$, $P = 0.032$). There was no association between COMT and fear of severe pain ($F_{(2, 204.98)} = 0.13$, $P = 0.716$) or fear of minor pain ($F_{(2, 204.98)} = 0.01$, $P = 0.909$). The contrasts showed that individuals with the Met-allele reported significantly higher fear of medical pain compared to the Val-allele ($P = 0.044$). The Bonferroni-adjusted contrasts did not show any other significant differences (**Fig. 4**).

To test the effect of fear of medical pain on the placebo effect, and whether COMT had a moderating effect on the relationship between FOP and the placebo effect, a univariate LMM was fitted. Placebo analgesia was constituted as the change in pain from the first pretest to the last posttest in the placebo group compared to the natural history group. Catechol-O-methyltransferase and the fear of medical pain variable were used as predictors in the LMM. The LMM showed that high fear of medical pain was significantly associated with reduced placebo analgesia ($F_{(1, 226.92)} = 15.12$, $P < 0.001$). There was no effect of

Table 1

Descriptive statistics and outcome variables for each genotype.

Natural history group	COMT Met/Met		COMT Val/Met		COMT Val/Val	
	N = 40 (27: 13) *		N = 50 (28: 22)		N = 23 (17: 6)	
	Mean	SD	Mean	SD	Mean	SD
Pain-level report	53.91	21.02	54.88	17.36	49.50	22.16
Systolic BP	125.23	10.83	126.99	11.78	127.81	13.13
Heart rate	69.48	9.66	65.80	7.23	66.64	8.23
Placebo group	**COMT Met/Met**		**COMT Val/Met**		**COMT Val/Val**	
	N = 35 (22: 13)		N = 50 (34: 16)		N = 22 (13: 9)	
	Mean	SD	Mean	SD	Mean	SD
Pain-level report	47.30	17.58	52.70	15.54	55.55	19.66
Systolic BP	125.27	10.18	125.85	11.67	127.36	14.85
Heart rate	67.55	16.43	69.01	11.78	70.16	14.95
Total	**COMT Met/Met**		**COMT Val/Met**		**COMT Val/Val**	
	N = 75 (49: 26)		N = 100 (60: 40)		N = 45 (30: 15)	
	Mean	SD	Mean	SD	Mean	SD
Pain-level report	50.78	19.62	53.55	16.56	52.46	20.96
Systolic BP	125.21	10.39	126.56	11.59	127.59	13.84
Heart rate	68.26	13.30	67.42	9.76	68.41	12.00
Cohens *d*/Hedges *g*†	0.34		0.13		−0.29	

* N (female: male).
† Effect size of the placebo treatment.

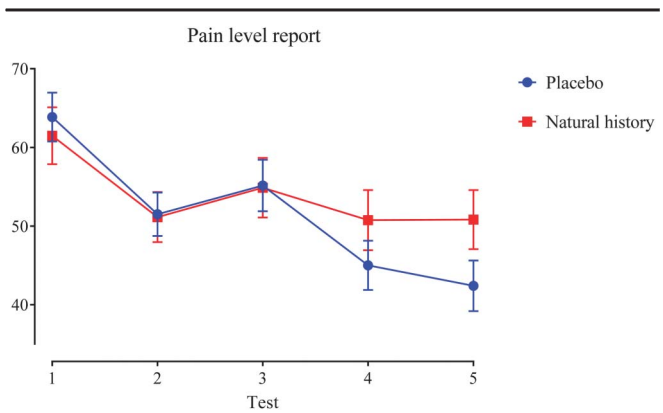

Figure 2. Pain-level report and 95% CI across tests in the natural history and the placebo groups.

Table 2

The main effects and interaction effects of pain on the independent variables and continuous predictors.

	Df	Denominator df	F	P
Test	4	532.91	60.05	<0.01*
Sex	1	204.98	7.54	0.007*
Group	1	205.62	0.066	0.797
COMT	2	205.61	0.563	0.570
FPQ FS	1	204.98	0.133	0.716
FPQ FMI	1	204.98	0.013	0.909
FPQ FM	1	204.98	4.64	0.032*
Test*Group	4	531.92	10.23	<0.01*
Group*COMT	2	205.61	1.914	0.150
Test*Group*COMT	16	531.92	1.09	0.364

* Significant at $P < 0.05$.

Df, degrees of freedom; FPQ FM, fear of pain questionnaire medical pain; FPQ FMI, fear of pain questionnaire minor pain; FPQ FS, fear of pain questionnaire severe pain.

COMT ($F_{(2,\ 226.99)} = 1.12$, $P = 0.30$) or no interaction effect between COMT and fear of medical pain ($F_{(2,\ 226.95)} = 1.04$, $P = 0.36$) on the change in reported pain in the placebo group. Parameter estimates for the univariate LMM are presented in **Table 3**.

There was a main effect of test on stress reports ($F_{(2,\ 470.97)} = 110.76$, $P < 0.001$) showing decreased stress levels from the pretest to the posttests. There were no significant effects of COMT, sex of the participants, or group in the stress data (all F's < 0.55). Fear of medical pain was a significant predictor ($F_{(1,\ 248.24)} = 6.55$, $P = 0.011$) for stress reports, where higher fear of medical pain was associated with increased stress reports.

4. Discussion

In the present study, we investigated how the COMT Val158Met rs4680 polymorphism was associated with FOP, pain, and placebo analgesia. The main finding was that individuals expressing the Met-allele reported significantly higher fear of medical pain compared to the other allelic variants. In addition, we found that participants who reported high fear of medical pain experienced a lower placebo analgesic effect compared to participants low in fear of medical pain.

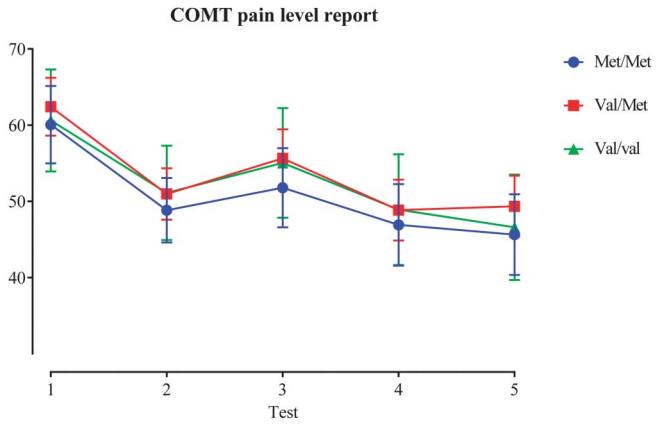

Figure 3. Pain-level report and 95% CI across tests between the COMT genotypes.

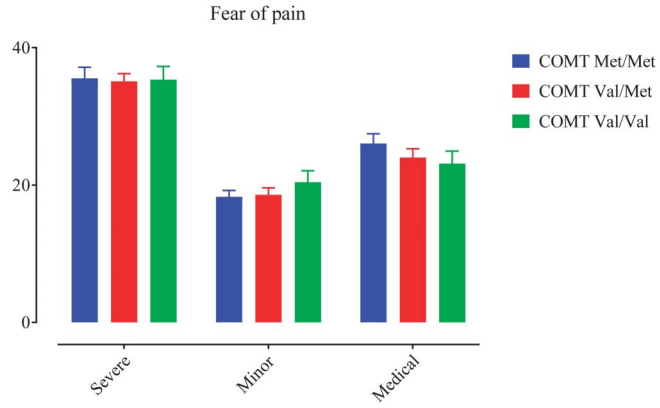

Figure 4. Level of fear of severe, minor, and medical pain and 95% CI between the COMT genotype.

4.1. Pain and placebo analgesia

Whether the COMT polymorphism influences pain modulation is broadly discussed in the literature. Some studies have found that the COMT Met-allele increases level of pain,[40] while other studies have found the opposite effect.[21] Zubieta et al.[40] hypothesized that high activity in the dopaminergic system due to the Met-allele was associated with a lower capacity in μ-opioid receptor binding potential. They performed a PET-scanning of 29 healthy participants and found that subjects with the Met-allele had diminished regional μ-opioid system responses to pain compared to the heterozygotes Val/Met-variant. The opposite effect was found for the homozygotes Val-allele. The conclusion was that Met-allele was associated with higher pain ratings in both sensory and affective pain. Imaging studies provides valid and objective measures, but the findings of Zubieta et al.[40] conflict with the findings of the present study. No significant association was found between the COMT polymorphisms and pain level.

The present study included a high sample size with 223 participants. Likewise, 2 other studies with high sample sizes did not find an association between the COMT polymorphisms and pain sensitivity.[18,28] Kim et al.[18] included 500 participants, and Nicholl et al.[28] included a total sample size of 1475 from 2 population-based cohort studies.

Kim et al.[18] discussed the inconsistent findings of COMT in pain modulation, and suggested that different characteristics in the pain stimuli could influence the role of COMT in pain modulation. Thermal pain and cold temperature, which was employed in Kim et al.,[18] activate nociceptors in the skin. In Zubieta et al.,[40] a hypertonic saline injection was injected in to the masseter muscle. Studies using animal models have also observed that type and duration of the stimulus could have an impact. Loggia et al.[21] hypothesized that the effect of the COMT polymorphism on pain modulation, occurs in settings of repeated pain stimuli. Forty-five healthy participants were tested with thermal heat pain during fMRI-scanning. The pain stimuli were calibrated to low pain (45-46°C) and high pain (48-49°C) intensity. They found that subjects with the Met-allele were more sensitive to repeated stimuli when they were of high intensity. Based on these results, Loggia et al.[21] suggested like Zubieta et al.,[40] that the COMT Met-allele appears to affect pain processing to experimentally induced pain.

Literature on the role of COMT in placebo analgesia is limited. Yu et al.[39] is, to our knowledge, the only study that has investigated COMT in relation to placebo analgesia. They found

Table 3

Parameter estimates for the univariate linear mixed model testing the effect of fear of medical pain on placebo analgesia* in the placebo group.

	B	SE	Df	t	P	95% CI lower	95% CI upper
FPQ MP	1.22	0.42	226.76	2.96	<0.01†	0.40	2.04
COMT Met/Met‡	18.66	13.62	226.97	1.37	0.17	−8.18	45.51
COMT Val/Met‡	19.38	13.0	226.79	1.49	0.14	−6.25	45.01
COMT Met/Met*FPQ MP§	−0.72	0.53	226.87	−1.34	0.18	−1.75	0.33
COMT Val/Met*FPQ MP§	−0.64	0.50	226.86	−1.28	0.20	−1.61	0.34

* Placebo analgesia was constituted as the change in pain reports from the first pretest to the last posttest in the placebo group.
† Significant at $P < 0.05$.
‡ Compared against COMT Val/Val.
§ Compared against COMT Val/Val*FPQ MP.
CI, confidence interval; COMT, catechol-O-methyltransferase; Df, degrees of freedom; FPQ MP, fear of pain questionnaire medical pain; SE, standard error.

that Met-allele was associated with a higher placebo analgesia, compared to the other allelic variants. The present study did not find evidence in support of Yu et al.[39] as no association between COMT and placebo analgesia was detected. However, a non-significant masked effect of COMT on pain level and placebo analgesia cannot be excluded.

The design of Yu et al.[39] differs from the present study. Yu et al.[39] induced placebo analgesia by a conditioning procedure. Placebo analgesia due to conditioning occurs after pairings between a cue signaling low pain (conditioned stimulus) and lower pain stimulus intensity (unconditioned stimulus). A high-cue high pain vs low-cue high pain contrast defined the conditioned placebo analgesic response.[39] Conditioned placebo analgesia may involve unconscious or automatic processes, in addition to conscious processes.[7] The study design could be an explanation to why the findings of Yu et al.[39] conflict with the present findings of no association between COMT and placebo analgesia.

4.2. Fear of pain

Catechol-O-methyltransferase is primarily expressed in cortical regions that are associated with inhibition of the fear response, such as the prefrontal cortex[37] and the hippocampus.[26] The present study showed that individuals expressing the Met-allele displayed higher fear of medical pain compared to the other allelic variants. This is consistent with previous findings.[30,38] To our knowledge, study by George et al.[14] is the only previous study that has included both the FPQ-III and COMT genotyping in a pain study. However, they did not find FOP to be a contributor to clinical pain ratings, so the association between FOP and COMT was not further explored.

Increased fear is associated with reduced placebo analgesic effects[12,22–24] and can to some extent be explained by increased release of cholecystokinin,[5] which is known to abolish opioid-mediated placebo analgesia via antagonism of endogenous opioids.[4] Lyby et al.[24] found that higher FOP, and experimentally induced fear, reduced placebo analgesia. In the present study, we found that high fear of medical pain was a significant predictor for pain level, which indicates that elevated level of fear in a clinical setting could result in increased level of pain. Fear of minor pain and fear of severe pain did not predict pain level or placebo analgesia.

Pain patients have been shown to experience large analgesic effects (average effects size of $g = 1.49$) after placebo treatment.[13] Results from the present study suggest that high fear of medical pain reduces the placebo analgesic effect. Several studies have shown that a higher negative effect reduces the analgesic effect of opioids.[8,36] Because of higher fear of medical pain in individuals with the Met-allele, it is possible that patients with the Met-allele experience reduced analgesic effects to placebo treatment, compared with the other allelic variants. Pecina, Love, Stohler, Goldman, and Zubieta[31] stated that a genetic test could provide valuable information about expected treatment effect. By identifying the Met-allele patients, the treatment approaches could be adjusted for a more successful outcome.

Higher FOP was associated with higher reports of stress. This is consistent with earlier findings. Aslaksen and Lyby[1] found that higher levels of fear of medical pain increased stress levels. This is also consistent with other studies from our laboratory.[22–24]

4.3. Limitations

Hall et al.[16] presented several limitations and ethical considerations to genetic testing in placebo studies. They stated that the ability to predict the placebo response assumes that placebo responding is a stable trait. However, the placebo response is influenced by context, earlier experience, history, research design, the practitioner's characteristics and treatment duration. A genetic model to predict placebo analgesia without considering the psychological and environmental factor would be rather limited.

5. Conclusion

The present study found higher fear of medical pain in individuals expressing the Met-allele compared to the other allelic variants, which confirms earlier findings of an association between the COMT Val158Met Met-allele and fear-related behavior.[30,38] Higher placebo analgesic effect was found in participants low in fear of medical pain. The present study did not find that the COMT Met-allele was associated with placebo analgesia. This is inconsistent with an earlier report.[38] Thus, the Met-allele seems to be associated with the negative emotional process of fear but not with placebo analgesia.

Conflict of interest statement

The authors have no conflict of interest to declare.

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References

[1] Aslaksen PM, Lyby PS. Fear of pain potentiates nocebo hyperalgesia. J Pain Res 2015;8:703–10.
[2] Aslaksen PM, Vasylenko O, Fagerlund AJ. The effect of transcranial direct current stimulation on experimentally induced heat pain. Exp Brain Res 2014;232:1865–73.
[3] Aslaksen PM, Zwarg ML, Eilertsen HI, Gorecka MM, Bjorkedal E. Opposite effects of the same drug: reversal of topical analgesia by nocebo information. PAIN 2015;156:39–46.
[4] Benedetti F, Amanzio M, Thoen W. Disruption of opioid-induced placebo responses by activation of cholecystokinin type-2 receptors. Psychopharmacology (Berl) 2011;13:791–7.
[5] Benedetti F, Amanzio M, Vighetti S, Asteggiano G. The biochemical and neuroendocrine bases of the hyperalgesic nocebo effect. J Neurosci 2006;26:12014–22.
[6] Clark RC, Shoaib M, Hewitt KN, Stanford SC, Bate ST. A comparison of InVivoStat with other statistical software packages for analysis of data generated from animal experiments. J Psychopharmacol 2012;26: 1136–42.
[7] Colloca L, Benedetti F. How prior experience shapes placebo analgesia. PAIN 2006;124:126–33.
[8] Colloca L, Benedetti F. Nocebo hyperalgesia: how anxiety is turned in to pain. Curr Opin Anaesthesiol 2007;20:435–9.
[9] De la Fuente-Fernandez R, Stoessl AJ. The biochemical bases for reward - Implications for the placebo effect. Eval Health Prof 2002;25:387–98.
[10] Elsenbruch S, Kotsis V, Benson S, Reidick D, Rosenberger C, Schedlowski M, Bingel U, Elke GR. Neural mechanisms mediating placebo analgesia in visceral pain: an fMRI study in placebo responders and non-responder. Gastroenterology 2011;140:S56.
[11] Elsenbruch S, Kotsis V, Benson S, Rosenberger C, Reidick D, Schedlowski M, Bingel U, Theysohn N, Forsting M, Gizewski ER. Neural mechanisms mediating the effects of expectation in visceral placebo analgesia: an fMRI study in healthy placebo responders and nonresponders. PAIN 2012;153:382–90.
[12] Flaten MA, Aslaksen PM, Lyby PS, Bjørkedal E. The relation of emotions to placebo responses. Philos Trans R Soc Lond B Biol Sci 2011;366: 1818–27.
[13] Forsberg JT, Martinussen M, Flaten MA. The placebo analgesic effect in healthy individuals and patients: a meta analysis. Psychosom Med 2017; 79:388–94.
[14] George SZ, Wallace MR, Wright TW, Moser MW, Greenfield WH, 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;136:53–61.
[15] Hall KT, Lembo AJ, Kirsch I, Ziogas CZ, Douaiher J, Jensen KB, Conboy LA, Kelley JM, Kokkotou E, Kaptchuk T. Catechol-O-Methyltransferase val158met polymorphism predicts placebo effects in irritable bowel syndrome. PLoS One 2012;7:1–6.
[16] Hall KT, Loscalzo J, Kaptchuk T. Genetics and the placebo effect: the placebome. Trends Mole Med 2015;21:285–93.
[17] Hoffman GA, Harrington A, Fields HL. Pain and the placebo: what we have learned. Perspect Biol Med 2005;48:248–65.
[18] Kim HS, Neubert JK, Miguel AS, Xu K, Krishnaraju RK, Iadarola MJ, Goldman D, Dionne RA. Genetic influence on variability in human acute experimental pain sensitivity associated with gender, ethnicity and psychological temperament. PAIN 2004;109:488–96.
[19] Leuchter AF, McCracken JT, Hunter AM, Cook IA, Alpert JE. Monoamine oxidase A, catechol-o-methyltransferase functional polymorphisms and the placebo response in major depressive disorder. J Clin Psychopharmacol 2009;29:372–7.
[20] Levine JD, Gordon NC, Fields HL. Mechanism of placebo analgesia. Lancet 1978;2:654–7.
[21] Loggia ML, Jensen K, Gollub RL, Wasan AD, Edwards RR, Kong J. The catechol-O-methyltransferase (COMT) val(158)met polymorphism affects brain responses to repeated painful stimuli. PLoS One 2011;6:1–8.
[22] Lyby PS, Aslaksen PM, Flaten MA. Is fear of pain related to placebo analgesia? J Psychosom Res 2010;68:369–77.
[23] Lyby PS, Aslaksen PM, Flaten MA. Variability in placebo analgesia and the role of fear of pain—an ERP study. PAIN 2011;152:2405–12.
[24] Lyby PS, Forsberg JT, Åsli O, Flaten MA. Induced fear reduces the effectiveness of a placebo intervention on pain. PAIN 2012;153: 1114–21.
[25] Mackay C, Cox T, Burrows G, Lazzerini T. Inventory of measurement of self-reported stress and arousal. Br J Soc Clin Psychol 1978;17: 283–4.
[26] Matsumoto M, Weickert CS, Akil M, Lipska BK, Hyde TM, Herman MM, Kleinman JE, Weinberger DR. Catechol-o-methyltransferase mRNA expression in human and rat brain: evidence for a role in cortical neuronal function. Neurosci 2003;116:127–37.
[27] McNeil DW, Rainwater AJ. Development of fear of pain questionnaire-III. J Behav Med 1998;21:389–410.
[28] Nicholl BI, Holliday KL, Macfarlane GJ, Thomson W, Davies KA, O'Neill TW, Bartfai G, Boonen S, Casanueva F, Finn JD, Forti G, Giwercman A, Huhtaniemi IT, Kula K, Punab M, Silman AJ, Vanderschueren D, Wu FC, McBeth J. No evidence for a role of the catechol-O-methyltransferase pain sensitivity haplotypes in chronic widespread pain. Ann Rheum Dis 2010;69:2009–12.
[29] Nielsen CS, Price DD, Vassend O, Stubhaug A, Harris JR. Characterizing individual differences in heat-pain sensitivity. PAIN 2005;119:65–74.
[30] Norrholm SD, Jovanovic T, Smith AK, Binder E, Klengel T, Conneely K, Mercer KB, Davis JS, Kerley K, Winkler J, Gillespire CF, Bradley B, Ressler KJ. Differential genetic and epigenetic regulation of catechol-o-methyltransferase is associated with impaired fear inhibition in posttraumatic stress disorder. Front Behav Neurosci 2013;7:1–10.
[31] Pecina M, Love T, Stohler CS, Goldman D, Zubieta JK. Effects of the Mu opioid receptor polymorphism (OPRM1 A118G) on pain regulation, placebo effects and associated personality trait measures. Neuropsychopharmacology 2015;40:957–65.
[32] Roelofs J, Peters ML, Deutz J, Spijker C, Vlaeyen JW. The fear of pain questionnaire (FPQ): further psychometric examination in a non-clincal sample. PAIN 2005;116:339–46.
[33] Scott DJ, Stohler CS, Egnatuk CM, Wang H, Koeppe RA, Zubieta JK. Individual differences in reward responding explain placebo-induced expectations and effects. Neuron 2007;55:325–36.
[34] Smith PF. A note on the advantages of using linear mixed model analysis with maximal likelihood estimation over repeated measures ANOVAs in psychopharmacology: comment on Clark et al. (2012). J Psychopharmacol 2012;26:1605–7.
[35] Stevens SS. To Honor Fechner and Repeal His Law: a power function, not a log function, describes the operating characteristic of a sensory system. Science 1961;133:80–6.
[36] Svedman P, Ingvar M, Gordh T. Anxiebo, placebo, and postoperative pain. BMC Anasthesiol 2005;5:9.
[37] Tunbridge E, Burnet PW, Sodhi MS, Harrison PJ. Catechol-o-methyltransferase (COMT) and proline dehydrogenase (PRODH) mRNAs in the schizophrenia, bipolar disorder, and major depression. Synapse 2004;51:112–18.
[38] Wendt J, Neubert J, Lindner K, Ernst FD, Homuth G, Weike AI, Hamm AO. Genetic influences on the acquisition and inhibition of fear. Int J Psychophysiol 2015;98:499–505.
[39] Yu RJ, Gollub RL, Vangel M, Kaptchuk T, Smoller JW, Kong J. Placebo analgesia, reward processing: integrating genetics, personality, and intrinsic brain activity. Hum Brain Mapp 2014;35:4583–93.
[40] Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu YJ, Koeppe RA, Stohler CS, Goldman D. COMT val(158)met genotype affects mu-opioid neurotransmitter responses to a pain stressor. Science 2003;299: 1240–3.