Original Experimental

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The association between selected genetic variants and individual differences in experimental pain

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Abstract

Objectives: The underlying mechanisms for individual differences in experimental pain are not fully understood, but genetic susceptibility is hypothesized to explain some of these differences. In the present study we focus on three genetic variants important for modulating experimental pain related to serotonin (SLC6A4 5-HTTLPR/rs25531 A>G), catecholamine (COMT rs4680 Val158Met) and opioid (OPRM1 rs1799971 A118G) signaling. We aimed to investigate associations between each of the selected genetic variants and individual differences in experimental pain.
Methods: In total 356 subjects (232 low back pain patients and 124 healthy volunteers) were genotyped and assessed with tests of heat pain threshold, pressure pain thresholds, heat pain tolerance, conditioned pain modulation (CPM), offset analgesia, temporal summation and secondary hyperalgesia. Low back pain patients and healthy volunteers did not differ in regards to experimental test results or allelic frequencies, and were therefore analyzed as one group. The associations were tested using analysis of variance and the Kruskal-Wallis test.
Results: No significant associations were observed between the genetic variants (SLC6A4 5-HTTLPR/rs25531 A>G, COMT rs4680 Val158Met and OPRM1 rs1799971 A118G) and individual differences in experimental pain (heat pain threshold, pressure pain threshold, heat pain tolerance, CPM, offset analgesia, temporal summation and secondary hyperalgesia).
Conclusions: The selected pain-associated genetic variants were not associated with individual differences in experimental pain. Genetic variants well known for playing central roles in pain perception failed to explain individual differences in experimental pain in 356 subjects. The finding is an important contribution to the literature, which often consists of studies with lower sample size and one or few experimental pain assessments.

Keywords: experimental pain; genetic susceptibility; pain modulation; pain sensitivity.

Introduction

Assessments of experimental pain are assumed to be of clinical value in management of pain patients, but the underlying mechanisms for individual differences in experimental pain are not fully understood and needs to be better addressed. Assessments of experimental pain may include tests for pain sensitivity, e.g. pain threshold and pain tolerance, or tests that assess the dynamic function of pain modulation, e.g. conditioned pain modulation (CPM), offset analgesia, temporal summation and secondary hyperalgesia.

Increased pain sensitivity has been associated with numerous pain disorders [1–3] and is regarded as one of the characteristics in central sensitization of the nervous system [4]. CPM represents reduced pain perception of a painful stimulus (test-stimulus) when a second painful stimulus (conditioning stimulus) is inflicted and is

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assumed to measure inhibitory pain modulation [5]. CPM has also been associated with pain disorders [6] and has been shown to predict the development of pain [7–9] and treatment response [10, 11]. Offset analgesia is another measure of inhibitory pain modulation, where a disproportionate decrease in pain perception is seen after a small decrease in stimulus intensity [12]. Similar to CPM, offset analgesia has been associated with pain disorders [13–15]. Tests that reflect central sensitization in pain disorders are temporal summation, which represents an increase in pain perception despite no change in stimulation intensity [16, 17] and secondary hyperalgesia, which is present if the tissue beyond an area of tissue damage (primary hyperalgesia) becomes hypersensitive [18, 19].

One of the underlying mechanisms for individual differences in experimental pain is genetic susceptibility. Many genetic variants are assumed to be important for modulating pain perception, but genetic variants related to serotonin (5-HT), catecholamine and opioid signaling have been of particularly interest and extensively studied due to their physiological function [20]. However, results from studies examining association between these variants and individual differences in experimental pain in humans are conflicting [21–25] and more studies are needed to elucidate whether these genetic variants can explain individual differences in experimental pain. Therefore, the present study aimed to investigate associations between each of the selected genetic variants; *SLC6A4* 5-HTTLPR/rs25531 A>G, *COMT* rs4680 Val158Met and *OPRM1* rs1799971 A118G, and individual differences in experimental pain.

Methods

Study design

The present study used data from a prospective cohort study of acute low back pain patients admitted to a hospital (n=232) [26, 27]. The present study was a cross-sectional study using socio-demographic data assessed through questionnaires, blood samples collected at hospital admission, and data from experimental pain testing performed six weeks after hospital admission. Similar data have been collected from healthy volunteers participating in studies at the same laboratory as the low back pain patients (n=124) [27–29]. The present study combined data from the low back pain patients and healthy volunteers.

A written informed consent was obtained prior to participation. The study was approved by the regional committee for medical and health research ethics in Norway (project number: 2010/2927, 2012/1108) and was conducted in accordance with the Declaration of Helsinki. Healthy volunteers received a gift certificate of NOK 250 for participation.

Study population

Patients were recruited from the Department of Neurology at Oslo University Hospital in Norway between January 2013 and June 2018. Inclusion criteria were age 18 years or older, acute low back pain with or without radiating pain, pain rated ≥4 on an 11 point numeric rating scale (NRS) (0='no pain', 10='worst pain imaginable'). Healthy volunteers were recruited by advertisement at local hospitals and colleges/universities in Oslo, Norway. Inclusion criteria were men and women self-reported to be healthy, aged 18–60 years. Exclusion criteria for patients and healthy volunteers were non-Caucasian heritage (mother or father), inability to understand spoken or written Norwegian, not currently working, previous or current alcoholism or substance abuse, regular use of neuroleptics and tricyclic antidepressants, pregnancy, breastfeeding, psychiatric or somatic diseases making the person unsuitable for inclusion, spinal fracture, malignancy, infection, cauda equina syndrome, rapidly progressive neurologic deficits or chronic pain defined as pain rated ≥4 on an NRS for ≥3 month in the last two years.

Experimental pain testing

The experimental pain testing procedure consisted of standardized tests for sensitivity (pressure pain thresholds, heat pain threshold and heat pain tolerance) and for pain modulation (CPM, offset analgesia, temporal summation and secondary hyperalgesia). Subjects were blinded to the study hypothesis and readouts from the stimulation instruments. A pretest was performed to familiarize subjects with the stimulations and pain intensity rating procedures. Subjects continuously rated the pain intensity on a computerized 10 cm horizontal visual analog scale (VAS) (left end (0 cm): 'no pain', right end (10 cm): 'worst pain imaginable') by scrolling the wheel on a computer mouse in all constant heat stimulations if not otherwise described. See supporting information TableS1 for instrumental details of the different tests.

Pressure pain threshold: To assess pressure pain threshold, the experimenters manually increased pressure (5 N/s) on muscle trapezius with a 1 cm^2 pressure algometer (AlgoMed, Medoc, Ramat Yishai, Israel). The subjects rated their pain by moving a knob along a 10 cm VAS on a box. The left side of the line represented 'no pain', and the right side line represented 'worst pain imaginable'. The subjects were instructed to not move the knob until pain was first experienced. Assessments were performed bilaterally and an average value of the two assessments was used in the analyses.

Heat pain thresholds and tolerance: Heat pain threshold and heat pain tolerance were assessed with gradually increasing the temperature during stimulation on the distal volar aspect of the right forearm with a 30 × 30 mm Peltier thermode (baseline temperature: 32 °C, increase: rate 2 °C/s, decrease rate: 8 °C/s) (Pathway model ATS, Medoc, Ramat Yishai, Israel). When assessing heat pain threshold, subjects were instructed to stop the increase in temperature by clicking on a computer mouse when they felt the first sensation of pain. When assessing heat pain tolerance, subjects were instructed to click on the computer mouse when they could not tolerate the increasing temperature any longer. The temperature was automatically stopped

at 52 °C for safety reasons. If the subject did not reach its threshold before 52 °C, this temperature was noted as the threshold. The tests were repeated three times and an average value was used in the analyses.

Pain6 calculation: A temperature aimed to reflect pain intensity equal to approximately 6 cm on 10 cm VAS (Pain6) was used during the tests for pain modulation. In order to estimate the Pain6 temperature for each individual, 2 °C was subtracted from an average of three tests of pain tolerance (see section heat pain thresholds and tolerance). The estimated temperature was thereafter tested with a 30 s heat stimulus with a 30 × 30 mm Peltier thermode (baseline temperature: 32 °C, increase rate: 1 °C/s, decrease rate: 8 °C/s) (Pathway model ATS, Medoc, Ramat Yishai, Israel) on the left thenar eminence. If the first 20 s of the stimulation was rated outside 4–9 cm on a 10 cm VAS, the temperature was adjusted accordingly.

Conditioned pain modulation: To assess CPM, a baseline test-stimulus was applied, followed by a 5-min break, before an identical test-stimulus was applied in parallel with a conditioning stimulus. The test-stimulus was a constant heat stimulation from a 30 × 30 mm Peltier thermode (baseline temperature: 32 °C, increase rate: 1 °C/s, decrease rate: 8 °C/s) (Pathway model ATS, Medoc, Ramat Yishai, Israel) with Pain6 temperature for 120 s on the right forearm. The conditioning stimulus was the opposite hand immersed in a 7 °C circulating water bath (LAUDA Alpha RA8, LAUDA-Brinkman LP., New Jersey, USA) with water up to the wrist and the hand held wide open for 120 s or until the pain forced the subject to withdraw the hand from the water bath. After 120 s, subjects were asked to rate the pain intensity of the conditioning stimulus on a 0–10 NRS. To avoid sensitization or habituation of the stimulated area, the area of the baseline test-stimulus and the test-stimulus in parallel with conditioning stimulus was not overlapping. Fifty of the healthy volunteers were part of a subproject and were randomized in regards to stimulation arm. A CPM effect was defined as the difference in average pain intensity between the test-stimulus alone and the test-stimulus in parallel with the conditioning stimulus. The CPM effect was also calculated as a percent change (CPM effect/test-stimulus alone × 100).

Offset analgesia: Two trials with heat stimulation with a 30 × 30 mm Peltier thermode (baseline temperature: 32 °C, increase rate: 1 °C/s, decrease rate: 8 °C/s) (Pathway model ATS, Medoc, Ramat Yishai, Israel) on the right forearm were used to assess offset analgesia. One trial had 30 s constant Pain6 temperature, while the other trial consisted of a three-temperature paradigm; first, heat stimulation was applied with Pain6 temperature for 5 s (T1). Next, the temperature was increased by 1 °C and kept constant for 5 s (T2) before the temperature returned to the initial temperature and kept constant for 20 s (T3). The stimulated area of the two trials was not overlapping to avoid sensitization or habituation of the stimulation area. The order and position of the trials were randomized, and the trials were separated by a 2-min break. Offset analgesia was calculated as the difference in pain ratings between T3-T2 during the three-temperature paradigm compared to the same time interval in the constant stimulation.

Temporal summation: Temporal summation was assessed by heat stimulation with a 30 × 30 mm Peltier thermode (baseline temperature: 32 °C, increase rate: 1 °C/s, decrease rate: 8 °C/s) (Pathway model ATS, Medoc, Ramat Yishai, Israel) on the right forearm with a constant Pain6 temperature for 120 s, except for 50 of the healthy volunteers who were part of a subproject and were randomized in regards to the stimulation arm. Temporal summation was defined as an increase in pain ratings (>0 cm) on a 10 cm VAS from the start (30–40 s) to the end (110–120 s) of the stimulation.

Secondary hyperalgesia: A 5-min heat stimulation of 45 °C with a 30 × 30 mm Peltier thermode (baseline temperature: 32 °C, increase rate: 1 °C/s, decrease rate: 8 °C/s) (Pathway model ATS, Medoc, Ramat Yishai, Israel) was used to create an area of primary hyperalgesia in the center of the volar aspect of the left forearm. After a 2-min break, a von Frey filament (Touch-Test TM Sensory Evaluator, Stoelting, Illinois, USA) was used to map the area of secondary hyperalgesia. The filament was pressed against the skin at 90° angle until the filament bowed, starting at a 5–6 cm distance from the heat stimulation area and repeated every 0.5 cm with 3–4 s intervals in eight directions 45° towards the heat stimulation area. The order of the directions was randomized. Subjects were instructed to look away from the arm and indicate when a prick had a clear change in sensation. This point was then marked with a colored pencil. After all directions were tested, the markings were transferred on to transparency film. The area of secondary hyperalgesia was extracted and calculated with Engauge Digitizer Software, version 10.8.

Genotyping

Blood samples were obtained in 4 ml EDTA tubes and frozen at –80 °C until DNA extraction was performed with QIAamp DNA Blood Kit (n=326) or QIAGEN Autopure LS (n=30) according to the manufacturer's protocol (QIAGEN, Valencia, CA, USA). The genetic variants genotyped were *SLC6A4* 5-HTTLPR/rs25531 A>G, *COMT* rs4680 Val158Met and *OPRM1* rs1799971 A118G. Genotypes were determined using fast quantitative real time polymerase chain reactions (qPCR) (Gene Amp, PCR System 9700, Applied Biosystems, California, USA). PCR amplifications were performed with 384-well plates containing genomic DNA, TaqPath ProAmp Master Mix and TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA) (see supporting information TableS2 for details of the genotyping). Negative controls containing water only were included in every run. Samples with undetermined genotypes were re-genotyped. The overall genotype call rate was 98%.

Regarding the *SLC6A4* 5-HTTLPR/rs25531, we performed gel electrophoresis to determine the long (529 bp) and short (486 bp) allele. Fragments were visualized with ultraviolet light after 2 h separation at 80 V in TAE buffer on a 2.5% agarose gel (MetaPhor™ Agarose, Lonza, Cologne, Germany), containing GelRed (Biotium Inc, California, USA). As previously described [30], the *SLC6A4* 5-HTTLPR and *SLC6A4* rs25531 were divided into three groups; low (S_A/S_A), medium (SL_G, L_A/L_G, SL_A) or high (L_A/L_A) 5-HTT expression types.

Statistical analysis

Statistical analyses were conducted using SPSS Statistics version 25 (IBM, Armonk, NY). The distribution of sample characteristics and experimental pain test results were assessed in preliminary analyses by a Shapiro–Wilk test for normality and inspection of descriptive statistics, histograms, boxplots, and Q-Q plots.

Sample size calculations showed that with a two-sided significance level of 5 and 80% power, 228 subjects were needed to detect a 10% difference in pain scores between genotypes with a standard deviation of 20 cm on a 10 cm visual analogue scale (VAS, left end: 'no pain', right end: 'worst pain imaginable'), assuming a genetic variant is present in 20% of the population. When offset analgesia and secondary hyperalgesia were added to the test protocol, new sample size calculations were made based on a standard deviation of 17, resulting in 168 subjects needed to detect a difference. No difference in individual differences in experimental pain have been observed between our two samples of low back pain patients and healthy volunteers ([27] and unreported studies), so to increase our sample size we chose to combine low back pain patients and healthy volunteers in the association analysis. To ensure that findings was due to associations between genetic variance and individual differences in experimental pain, low back pain patients and healthy volunteers were tested for systematic differences in sample characteristics, individual differences in experimental pain and genotype distributions. Similar comparison were done between patients who had almost or fully recovered from the acute back pain (defined as <3 VAS at the six weeks follow-up) and patients still in a pain state when the experimental tests were performed (defined as leg pain ≥3 VAS at the six weeks follow-up). Independent sample Student's t-test was used for normally-distributed variables, Mann-Whitney U-test was used for variables with non-normal distribution, and Chi-square or Fisher's exact test was used for categorical variables.

Paired sample Student's t-test was used to determine if there was a CPM effect, temporal summation, and offset analgesia. Analysis of variance (ANOVA) or the non-parametric alternative Kruskal-Wallis test was used to determine the association between the selected genetic variants and individual differences in experimental pain.

Since earlier studies have shown that *OPRM1* A118G may be sex-specific [31–33], a multivariate ANOVA was performed to investigate interactions between *OPRM1* A118G and sex. Findings with p-values<0.01 were regarded as significant for all statistical analyses due to multiple testing.

Results

Sample characteristics

In total 356 subjects (232 back pain patients and 124 healthy volunteers) were genotyped and pain tested (Figure 1). Sample characteristics and gene frequencies are presented in Table 1, with details in Supplementary Table 3. Low back pain patients and healthy volunteers did not differ with regards to sample characteristics or distribution of genotypes, except for age (p<0.001), body-mass index (BMI) (p<0.001) and diastolic blood pressure (p<0.001) (Supporting information TableS3). No differences were found in sample characteristics and distribution of genotypes between patients with leg pain VAS <3 or VAS ≥3, except for age (p=0.002) and education (p=0.002) (Supporting information TableS3).

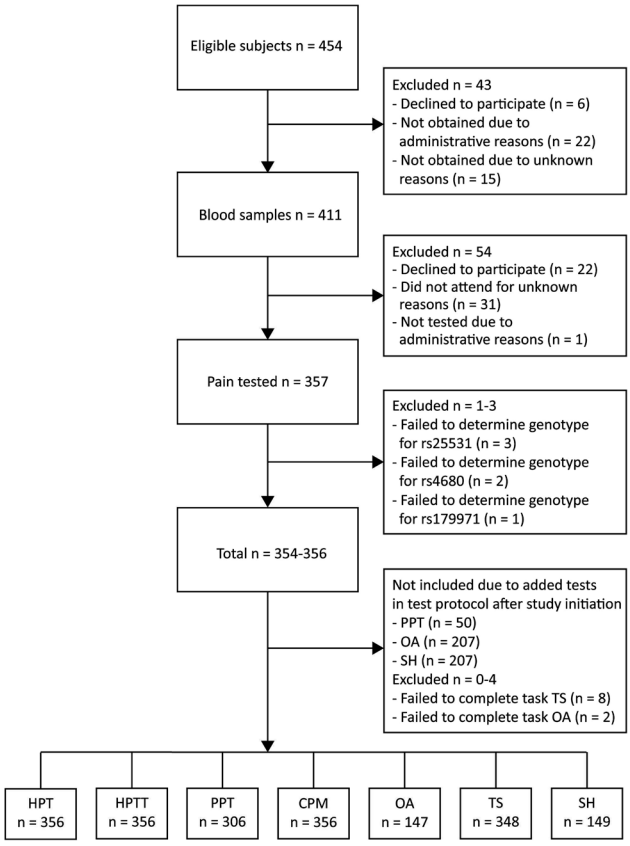

Figure 1: Flowchart.
PPT, pressure pain threshold; HPT, heat pain threshold; HPTT, heat pain tolerance; CPM, conditioned pain modulation; OA, offset analgesia; TS, temporal summation; SH, secondary hyperalgesia.

Table 1: Sample characteristics.

Variable	n	Value
Sex (males), n (%)	357	208 (58.3)
Age (years), median (IQR)	357	35 (26–45)
Education (>12 years), n (%)	357	321 (89.9)
Left handed, n (%)	346	38 (10.6)
BMI (kg/m2) mean (SD)	351	25.2 (3.6)
Systolic blood pressure (mmHg), mean (SD)	357	123.3 (13.3)
Diastolic blood pressure (mmHg), mean (SD)	357	74.7 (9.7)
Current smoker, n (%)	354	44 (12.3)
5HTTLPR/rs25531 *(SLC6A4)*, MAF	354	0.3
Val158Met *(COMT)*, MAF	355	0.4
A118G *(OPRM1)*, MAF	356	0.1

IQR, inter quartile range; SD, standard deviation; MAF, minor allele frequency.

Experimental pain tests

Results from the assessments of pain modulation are presented in Figure 2A–D. There was a significant difference between pain ratings during baseline test-stimulus

and pain ratings during test-stimulus in parallel with the conditioning stimulus (effect size=−2.5, SD=1.7, p<0.001), representing a CPM effect of −48.9%. In the offset analgesia paradigm, there was a significant difference between pain ratings during T3-T2 in the constant stimulation and pain ratings during T3-T2 in the three-temperature paradigm (effect size=−0.5, SD=1.8, p<0.001). Temporal summation of pain during constant heat stimulation was found, as there was a significant difference between pain ratings at the start and at the end of the constant heat stimulation (effect size=0.6, SD=2.1, p<0.001). For none of the experimental pain tests did test results differ between patients and healthy volunteers, or between patients with leg pain VAS<3 or VAS≥3 (Supplementary Table 3).

Genetic associations

There were no significant associations between any of the selected genetic variants, *SLC6A4* 5-HTTLPR/rs25531 A>G, *COMT* rs4680 Val158Met and *OPRM1* rs1799971 A118G and individual differences in experimental pain assessed with pressure pain threshold, heat pain thresholds, heat pain tolerance, CPM, offset analgesia, temporal summation or secondary hyperalgesia (Figure 3 and Table 2). No significant interaction was found between *OPRM1* A118G and sex in regards to individual differences in experimental pain (p=0.575).

Discussion

In the present study, we found no association between the selected genetic variants, *SLC6A4* 5-HTTLP/ rs25331 A>G, *COMT* Val158Met or *OPRM1* A118G and individual differences in pressure pain threshold, heat pain threshold, heat pain tolerance, CPM, offset analgesia, temporal summation or secondary hyperalgesia. To our knowledge, the present study is one of the largest candidate gene study investigating associations between the selected genetic variants and individual differences in experimental pain. The present study is also the first to explore the association between the selected genetic variants and offset analgesia and secondary hyperalgesia.

The serotonin transporter (5-HTT), encoded by the *SLC6A4* gene, plays a central role in the uptake of serotonin in the synaptic cleft. A length polymorphism (5-HTTLPR) in the promoter region of *SLC6A4* results in two common variants; a short (S) and a long (L) allele [34–36]. The S allele leads to reduced 5-HTT expression, which may influence 5-HT signaling [37, 38]. In addition, a single nucleotide polymorphism (SNP) rs25531 A>G in the same promoter region is also associated with reduced 5-HTT expression [39]. Previous studies have shown a relationship between *SLC6A4* 5-HTTLPR/rs25531 A>G and individual differences in experimental pain, where the low 5-HTT expression type typically is associated with lower heat pain threshold [23, 40], and impaired CPM [24], but one study

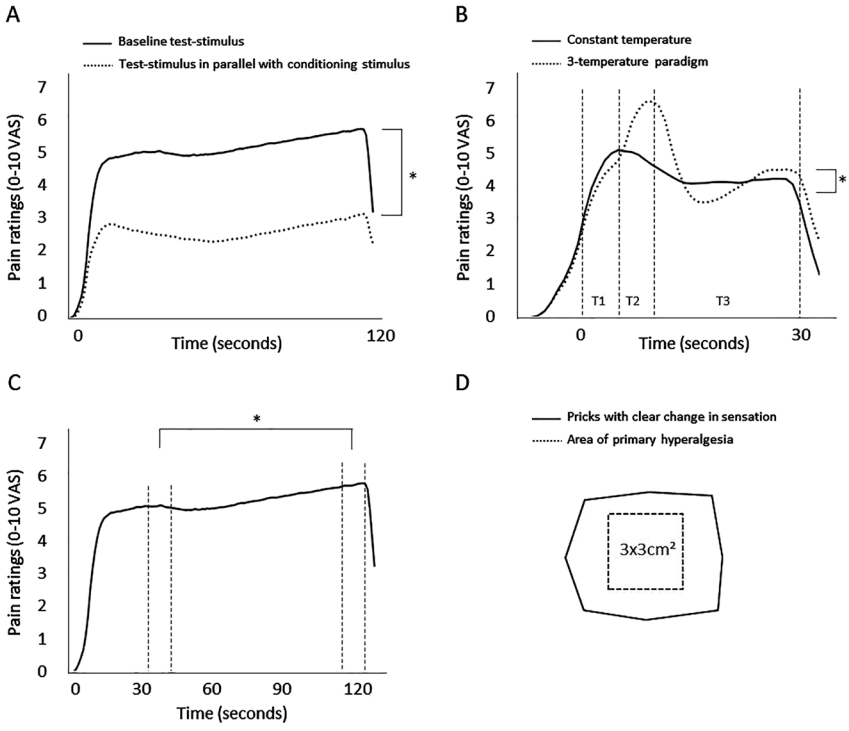

Figure 2: Results from the experimental pain assessments of pain modulation.
(A) A conditioned pain modulation (CPM) effect was present, with a decrease in pain ratings of the test-stimulus during conditioning stimulus (p<0.001), (B) Offset analgesia was present with a larger decrease in pain ratings in the three-temperature paradigm than in the constant paradigm (p<0.001), (C) Temporal summation of pain was observed with a significant increase in mean pain ratings during the continuous heat pain stimulation (p<0.001). The vertical lines marks the time periods that was compared, (D) Illustration of the area of secondary hyperalgesia for 97 of 149 subjects. In 52 of the subjects the direction of the transparency film, which the markings were transferred to, was unknown and could not be used in the illustration.
VAS, visual analog scale.

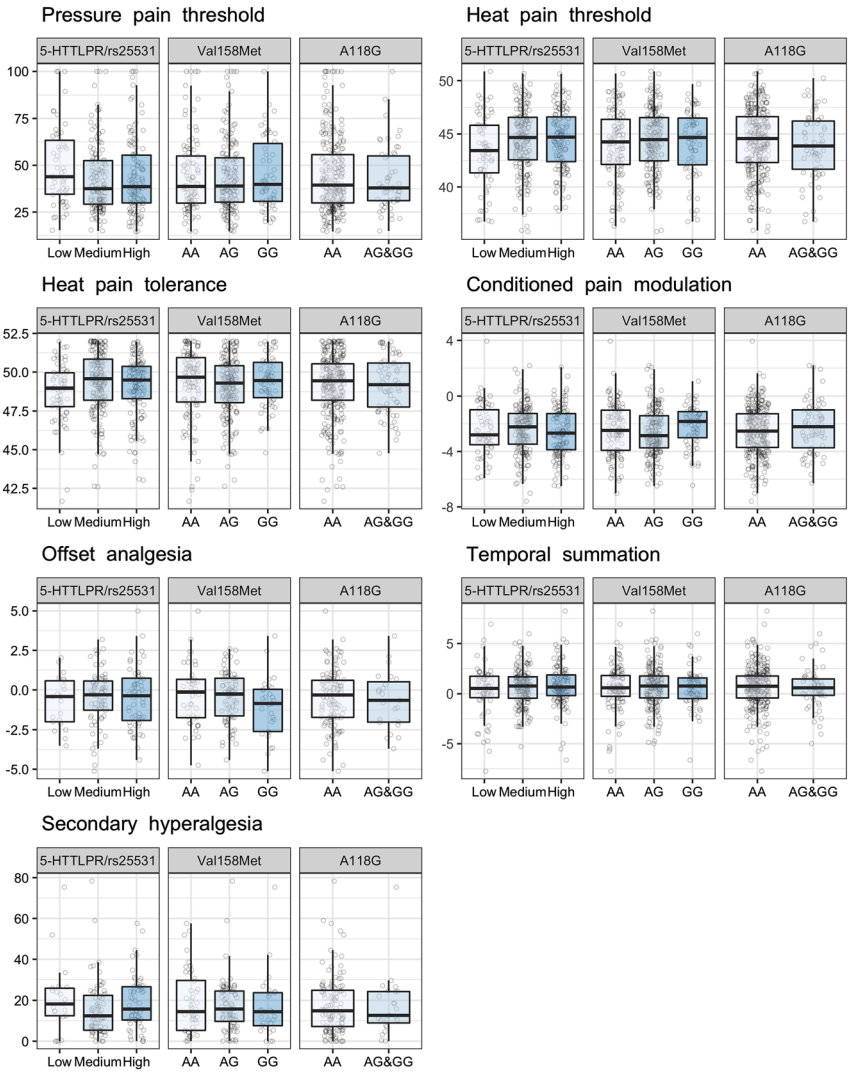

Figure 3: Associations between the selected genetic variants and individual differences in experimental pain. Findings with p-values≤0.01 were regarded as significant. There was no significant association between the selected genetic variants and individual differences in experimental pain.

observed higher heat pain thresholds for the low expression type [41]. The present study did not show significant evidence to support these studies, but may point in the direction of a possible association between low expression type and lower heat pain threshold (p=0.03) and heat pain tolerance (p=0.04) due to the observed trend. Consistent with our results, some studies have shown no relationship between the *SLC6A4* 5-HTTLPR/rs25531 A>G and individual differences in experimental pain [25, 42–44]. A possible explanation for the diverse findings in the literature can be use of different test parameters when assessing experimental pain. Another explanation for conflicting results could be related to the complexity of the serotonergic system. A high concentration of serotonin in the synaptic cleft may impact the nearby postsynaptic 5-HT receptors, which results in increased signaling, or it may impact the presynaptic autoreceptors, which results in an increase of negative feedback and thereby decrease signaling [35]. However, this may depend on the location of localization of the 5-HTT relative to the 5-HT autoreceptors [45, 46]. Serotonin is also regulated by the seven different groups of 5HT-receptors, which mediate both excitatory and inhibitory neurotransmission.

Catechol-O-methyltransferase (COMT) encoded by the *COMT* gene, is an enzyme that promote degradation of catecholamines (dopamine, epinephrine, and norepinephrine). The SNP rs4680 G>A causes a substitution of the amino acid valine (Val) to methionine (Met) at codon 158, and reduces enzyme activity which results in higher levels of catecholamines [47]. The relationship between *COMT* Val158Met and individual differences in experimental pain has been studied in numerous animal and human pain models. Results are somewhat conflicting, with some studies reporting that the Met allele is associated with lower pressure pain thresholds, heat pain threshold, and temporal summation [21, 48–51], while other studies find

Table 2: Associations between the selected genetic variants and individual differences in experimental pain. Findings with p-values<0.01 were regarded as significant.

Variant (gene)	Genotype	n	Mean (SD)/median (IQR)	r^2/χ^2	p-Value	n	Mean (SD)/median (IQR)	r^2/χ^2	p-Value	n	Mean (SD)/median (IQR)	r^2/χ^2	p-Value
			Pressure pain threshold[a]				Heat pain threshold[b]				Heat pain tolerance[a]		
5-HTTLPR/rs25531 *(SLC6A4)*	Low[c]	51	43.9 (34.3–65.5)	4.3	0.12	63	43.3 (3.4)	33.7	0.03	63	49.0 (47.7–50.0)	6.7	0.04
	Medium[d]	141	37.5 (29.2–52.5)			169	44.4 (3.1)			169	49.6 (48.2–50.9)		
	High[e]	114	38.6 (29.8–55.7)			124	44.5 (2.9)			124	49.5 (48.3–50.4)		
Val158Met *(COMT)*	AA	98	38.7 (29.7–55.4)	1.0	0.79	114	44.1 (3.2)	2.9	0.82	114	49.7 (48.0–51.0)	2.2	0.53
	AG	152	38.9 (30.1–54 0)			176	44.4 (3.0)			176	49.3 (48.0–50.4)		
	GG	54	39.8 (30.4–63.5)			64	44.2 (3.2)			64	49.5 (48.3–50.7)		
A118G *(OPRM1)*	AA	253	39.4 (29.9–56.0)	0.3	0.60	294	44.3 (3.1)	14.7	0.21	294	49.4 (48.2–50.5)	0.1	0.75
	AG + GG	53	37.9 (30.8–55.4)			62	43.8 (3.1)			62	49.2 (47.7–50.6)		
			Conditioned pain modulation[b]				Offset analgesia[b]						
5-HTTLPR/rs25531 *(SLC6A4)*	Low[c]	63	−2.4 (1.8)	1.0	0.73	21	−0.7 (1.6)	0.6	0.84				
	Medium[d]	169	−2.4 (1.7)			69	−0.5 (1.7)						
	High[e]	123	−2.6 (1.7)			56	−0.5 (1.9)						
Val158Met *(COMT)*	AA	114	−2.4 (1.9)	7.1	0.07	43	−0.3 (2.0)	4.3	0.25				
	AG	176	−2.6 (1.7)			73	−0.4 (1.6)						
	GG	63	−2.1 (1.5)			29	−1.1 (1.9)						
A118G *(OPRM1)*	AA	293	−2.5 (1.7)	4.7	0.21	123	−0.5 (1.8)	0.4	0.73				
	AG + GG	62	−2.2 (1.9)			23	−0.6 (1.8)						
			Temporal summation[b]				Secondary hyperalgesia[a]						
5-HTTLPR/rs25531 *(SLC6A4)*	Low[c]	62	0.2 (2.6)	7.8	0.16	21	18.2 (12.3–26.0)	4.2	0.12				
	Medium[d]	163	0.7 (1.8)			71	12.4 (5.3–22.6)						
	High[e]	122	0.8 (2.0)			57	15.7 (10.4–26.6)						
Val158Met *(COMT)*	AA	113	0.6 (2.2)	0.3	0.97	44	14.5 (5.2–30.3)	2.3	0.52				
	AG	169	0.7 (2.0)			75	15.7 (9.1–24.4)						
	GG	63	0.6 (1.9)			29	14.4 (7.2–23.9)						
A118G *(OPRM1)*	AA	288	0.7 (2.1)	0.8	0.67	126	14.9 (7.1–24.9)	0.1	0.75				
	AG + GG	59	0.5 (2.0)			23	12.6 (7.6–24.2)						

SD, standard deviation; IQR, inter quartile range; r^2, Analysis of variance (ANOVA) R-squared value; χ^2, Kruskal-Wallis test chi-squared value.

[a]Presented with median (IQR) and Kruskal-Wallis χ^2.

[b]Presented with mean (SD) and ANOVA r^2.

[c]Low 5-HTT expression type (S_A/S_A).

[d]Medium 5-HTT expression type (SL_G, L_A/L_G, SL_A).

[e]High 5-HTT expression type (L_A/L_A).

an opposite effect [22] or no association with individual differences in experimental pain [52], consistent with the present study's results. However, the observed trend between *COMT* Val158Met and CPM (p=0.07) in the present study, may suggest a possible association between the Met allele and impaired CPM. The inconsistencies in the literature may be due to different sample selection [51, 53] and sample sizes, different choice of experimental tests or different tests protocols [22, 54].

Opioid signaling is regulated by the μ opioid receptor encoded by the *OPRM1* gene. The SNP rs1799971 A>G causes a substitution of the amino acid asparagine to aspartic acid at codon 40, and removes a putative N-linked glycosylation site in the receptor, which may affect the function of the receptor [55, 56]. The G allele in *OPRM1* A118G has been associated with higher pressure pain thresholds [57, 58], which is in contrast with the present study. Similar to the present study, some studies found no relationship between *OPRM1* A118G and heat pain threshold and pressure pain threshold [59–62]. The conflicting results could potentially be explained by sex-differences. An asparagine to aspartic amino acid substitution in *OPRM1* A118G affects the glycosylation site of the receptor, which is important for cellular processes such as receptor folding, sorting, expression and ligand binding [63]. The level and type of glycosylation have shown to be different between female and male mice [33, 64], and some human studies have shown opposite effects of *OPRM1* A118G in men and women [31, 32, 65]. For this reason we also analyzed the interaction of *OPRM1* A118G and sex in regards to individual differences in experimental pain, but no such interactions were found.

Strength and limitations

The present study investigated pain sensitivity as well as anti- and pro-nociceptive functions of the pain system. We chose tests which have relatively large effects, with the outcome measure as a continuous endpoint, which enables differentiation between subjects and increase the power of the study. To date, there is no gold standard for assessing the dynamic function of the pain system. When using a genetic model to predict individual differences in experimental pain, one assumes that experimental pain response is a stable trait. However, results of experimental pain assessments has been shown to be influenced by psychological and environmental factors [66], and the reliability of the different tests range from poor to good depending on the methodology of the tests as well as statistics [67, 68]. Further research should establish gold standards for assessing experimental pain, which will likely lead to more consistent results between studies, and improve the chances to identify genetic risk factors.

The present study sample was heterogeneous, consisting of both healthy volunteers, patients that had recovered from acute low back pain, and patients still in pain after an acute low back pain episode six weeks earlier. Combining experimental data from patients and healthy volunteers are potentially problematic, but could be done because the groups did not differ with regards to experimental pain test results and genetic variant allele frequencies. In a genetic association study, factors such as age and sex are not considered potential confounders, since they do not affect the genetic variants, but sample heterogeneity can lead to reduced power, contributing to our negative results [69]. Although the sample size of the present study is small compared to association studies of clinical pain disorders, the sample size is relatively large compared to studies investigating association between the same selected genetic variants and individual differences in experimental pain. That the present study with 356 subjects does not find evidence to support findings from studies of smaller sample size emphasizes the limitations of experimental studies with a candidate gene approach and the importance of replication of findings before conclusions can be reached. Several experimental tests are often used in experimental pain studies, however few are adjusting for multiple testing. In the present study, a stricter significance level was used to decrease the probability of making a type I error, but remain power to detect significance for the experimental tests that typically are highly correlated.

We did only investigate the effect of three genetic variants and cannot exclude that other polymorphisms in these or other genes affect individual differences in experimental pain. However, the genetic variants studied were carefully selected based on their physiological function as well as previous research demonstrating their relationship to individual differences in experimental pain.

In conclusion, the selected genetic variants, *SLC6A4* 5-HTTLPR/rs25531 A>G, *COMT* rs4680 Val158Met and *OPRM1* rs1799971 A118G, were not associated with individual differences in experimental pain.

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