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Association between genetic polymorphisms and pain sensitivity in patients with hip osteoarthritis

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ABSTRACT

Background

Factors such as age, gender and genetic polymorphisms may explain individual difference in pain phenotype. Genetic associations to pain sensitivity have previously been investigated in osteoarthritis patients with focus on the *P2X7*, *TRPV1* and *TACR1* genes. However, other genes may play a role as well. Osteoarthritis is a common joint disease and many patients suffering from this disease are thought to have increased sensitivity to noxious stimuli resulting from sensitization in the nociceptive system. The aim of the study was to investigate if genetic variants of mu, kappa, and delta opioid receptor genes (*OPRM1, OPRK1,* and *OPRD1*), and the catechol-O-methyltransferase gene (*COMT*) influenced the pain phenotype in patients with osteoarthritis.

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Methods

The frequencies of seventeen polymorphisms were examined. Pain sensitivity was assessed pre-operatively by: 1) hip rotation; 2) contact heat stimulation; 3) conditioned pain modulation effect and 4) pressure stimulation at the tibia in both the affected and the unaffected leg.

Results

Ninety-two patients (mean age 66 years) with unilateral hip osteoarthritis were included in the study. Carriage of the *OPRM1* rs589046T allele was found to be associated with increased pain rating during hip rotation (P=0.04) and increased conditioned pain modulation (P=0.049). Carriage of the *OPRD1* rs2234918C allele was found to be associated with increased pain detection threshold to contact heat stimulation (P=0.001). No other associations were found (all $P > 0.05$).

Conclusion

Results from the present study suggest that, in patients with hip osteoarthritis, genetic variants in *OPRM1* and *OPRD1* may contribute to the pain phenotype.

INTRODUCTION

Osteoarthritis (OA) is the most common joint disease worldwide [1]. Many patients with OA are thought to have increased sensitivity to noxious stimuli resulting from sensitization in the nociceptive system [2]. Age, gender, height and genetic polymorphisms are some factors that may explain individual difference in pain sensitivity [3–6].

Human genetic association studies have provided insight into the inter-individual variability in pain sensitivity [7–10]. For example, *P2X7*, *TRPV1* and *TACR1* genes have been studied in OA patients [11–13]. However, other genes may also play a role, for example, rs4680A/G and

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rs6269A/G in the *COMT* gene [14,15]. Additionally, single nucleotide polymorphisms (SNPs) in the mu, kappa and delta opioid receptor genes (*OPRM1*, *OPRK1*, *OPRD1*) have shown to alter pain sensitivity through variations in receptor function [8,16,17]. However, findings are still inconclusive.

Inconclusive results may be explained by different methodologies used [7]. Moreover, socio-psychological confounders may blur the findings in the clinical setting. Such confounders can be avoided in studies using experimentally induced pain in healthy volunteers [18]. On the other hand, it is not possible to reproduce the full complexity of clinical pain and external validity may be limited [19]. Thus, using a combination where experimentally noxious stimuli are induced in patients suffering from pain might be advantageous, when trying to elucidate the genetic factors influencing pain sensitivity. In patients with OA the inflammation process causes peripheral sensitization (the primary afferent neurons in the joint are sensitized) and central sensitization (neurons in the central nervous system receiving the input from the inflamed joint are hyperexcitable and undergo neuroplastic changes) [20]. The influence of inflammation on pain perception can therefore be investigated by applying experimentally induced pain in these patients. Additionally, it is possible to induce an acute clinical pain by for example rotating the hip.

We hypothesized that application of experimental pain models in a clinical setting would elucidate if, carriers of the minor alleles of *COMT* or *OPRM1* or *OPRK1 or OPRD1* have altered pain sensitivity as compared to non-carriers. Thus, the aims were to compare the following variables in the two groups (carriers versus non-carriers): 1) clinical pain rating during hip rotation; 2) pain reported during contact heat stimulation of the forearm; 3)

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conditioned pain modulating (CPM) effect; 4) pain pressure threshold at tibia distal to the affected and unaffected hip for each polymorphism separately.

METHODS

The trial was conducted by the Department of Pain Medicine, Clinic for Anesthesiology, University Hospital, Georg-August-university of Göttingen, Germany, and the Department of Anesthesiology and Intensive Care, Annastift, Hannover, between April and August 2013. The study was approved by the Ethical committees of the University Hospital of Göttingen (No 5/4/12) and the Medical School Hannover (No. 1483-2012). Written informed consent was obtained from the patients before the study procedures. The study was carried out according to the recommendations of the Helsinki declaration (2013). Patient recruitment and pain assessments were performed at the Annastift, Hannover. The results regarding the primary endpoints from the study has previously been published (Prediction of postoperative opioid analgesia using machine learning and encephalography) [21]. This manuscript concerns the secondary endpoint of the protocol.

Patients

The included patients suffered from hip OA and were scheduled to undergo total hip replacement surgery. A total of 175 patients were screened for participation. Inclusion criteria were: 1) older than 18 years old, 2) capacity to give the consent on his/her own, and 3) had sufficient knowledge of the German language to understand the study information and the required questionnaires. Exclusion criteria were: 1) severe neurological disease; 2) severe psychiatric disease like major depression or schizophrenia or active drug abuse; 3) a high dose of opioid therapy (> 30 mg oral morphine equivalent dose per day); and 4) participation in other studies conducted at the same time.

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Procedure

Patients were usually admitted to hospital on the day before surgery. Thus, patients were recruited in the morning of the day prior to surgery. After consent was obtained the pain history was registered, followed by completion of questionnaires (in waiting time between the hospital procedures). The functional testing and pain assessments were conducted in the afternoon of the pre-operative day (figure 1).

Patients refrained from smoking as well as drinking coffee or other caffeine-containing beverages 1-2 hours prior to the assessment of pain sensitivity. Additionally, the patients were familiarized with the various procedures and given standardized test instructions. To avoid investigator emerging biases, all psychophysiological measurements were done by the same researcher (MR).

Opioid consumption (substance and dose) was registered for each patient.

Passive hip rotation

The perceived intensity of evoked pain with passive rotation of the hip scheduled for replacement was assesed on an 11-point numerical rating scale (NRS), where 0 was "no pain" and 10 "worst imaginable pain". First, the hip was brought into a 90° flexed position, with the knee flexed at 90° at the same time, and then slowly rotated interiorly until the patient reported the first onset of pain, the pain detection threshold (PDT). Then, the passive rotation was continued until the pain threshold was reached and this position was maintained for 30 seconds. Afterwards the patients were asked to rate the evoked pain on a NRS and this value was used for further analysis. In patients who could not tolerate this choreography (i.e. with severe pain already with hip flexion), hip flexion was used as the stimulus.

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Quantitative sensory testing (QST)

Contact heat stimulation

Contact heat stimulation was determined by a computer driven heat pain device (TSA-II NeuroSensory Analyzer, Medoc Ltd, Ramat Yishai, Israel). A thermode was applied to the volar surface of the dominant forearm at 10 cm from the elbow. The temperature increased gradually from 32°C to a maximum of 52°C at a rate of 1°C/s until the patient reached PDT. The stimulation was stopped, when the patient clicked a button. Four consecutive temperature measurements were performed and the average of the last three PDTs was computed and retained for further analysis.

Conditioned pain modulation effect (CMP)

To assess descending inhibitory pain modulation a CPM paradigm was carried out. CPM was measured as the change in pain sensitivity to a test stimulus before (pre-CPM) and during (peri-CPM) a conditioned pain stimulus [22]. The conditioned pain stimulus was performed the following way: The patient's hand was immersed in cooled water (around 8 °C) for 2 minutes or less if the pain was intolerable for the patient. Contact heat PDTs were repeated at 60, 90 and 120 seconds after immersion of the hand in cold water. CPM effect was calculated for the PDT to contact heat stimulation as the percentage change from baseline [e.g.(T120min-baseline)/baseline*100%]. The time point with the highest CPM effect was used for further analysis.

Pressure pain detection threshold

Pressure pain threshold was assessed at the tibial bone, 15 cm below the patella. A handheld electronic pressure algometer (Somedic AB, Stockholm, Sweden) was used. The pressure rate was gradually increased with 30 kPa/sec until the patients reported PDT. Maximum pressure

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intensity applied was 1000 kPa. Altogether four measurements were recorded within close proximity. The mean pressure intensity (kPa) at PDT was calculated as the mean of the last three measures and was used for further analysis.

DNA extraction and genotyping

Genomic DNA was extracted from an aliquot of venous blood with the QIAsymphony DSP DNA Midi kit (Qiagen, Copenhagen, Denmark) using the QIAsymphony^{TM} SP instrument (Qiagen, Copenhagen, Denmark). Selected SNPs in *COMT* (rs4680A/G), *OPRM1* (rs1799971A/G, rs589046C/T, rs563649C/T, rs9479757G/A, rs533586C/T), *OPRK1* (rs16918875C/T, rs7016778T/A, rs10504151T/C, rs7836120A/G, rs6473799T/C, rs1365098G/T, rs7824175G/C, rs963549G/A), and *OPRD1* (rs533123G/A, rs2236857T/C, rs2234918C/T) were genotyped using predesigned TaqMan SNP genotyping assays on a StepOne Plus real-time instrument (Applied Biosystems, Foster City, California, USA). Genotyping was conducted in accordance with the manufacturer's protocol. The choice of candidate SNPs was based on location along the gene (i.e. SNPs in regions most likely to have an impact on gene function were selected), and the existence of published data for the specific SNPs. Thus, within each selected gene, SNPs were selected in attempt to cover the allelic diversity.

Statistical analysis and considerations

The Chi-square goodness-of-fit test (Haploview version 4.2, Broad Institute, Cambridge, USA) was used to determine if all genotype frequencies were in Hardy-Weinberg equilibrium. The demographic characteristics for all patients are presented as medians with 25^{th} to 75^{th} percentile. The demographic characteristics for carriers and non-carriers of the minor alleles were both visually inspected as histograms with corresponding normal curve

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and analyzed by Shapiro-Wilk test to test for normality (P<0.05 indicate non-normally distributed data). Age, weight, and body mass index (BMI) were non-normally distributed and Man-Whitney U-test was used as non-parametric test. Height and duration of hip pain were normally distributed, and analyzed using parametric t-tests. Categorical variable such as gender were analyzed by Fisher's exact test. All tested genetic variants were bi-allelic SNPs, and it was unknown whether the variant allele could be a risk-enhancing allele (increases the likelihood of a poorer outcome) or a protective allele (increase the likelihood of improving the outcome). Thus, genotype distributions were calculated for subjects homozygous (AA) and heterozygous (AB) for the A allele (minor allele), and for subjects homozygous (BB) for the B allele (major allele). The genetic association analysis included two groups: Carriers of the minor allele (AA+AB) and non-carriers of the minor allele (BB). Correction for confounders, such as age, BMI and duration of hip pain, were not conducted as no difference was seen between carriers and non-carriers of the minor alleles in any genes. However, parametric multiple regressions adjusted for "gender" and "opioid treatment versus no opioid treatment" was used in the evaluation of the association of genetic variations in *OPRM1*, *OPRD1*, *OPRK1* and *COMT* with the investigated assessments of pain sensitivity. No adjustment for multiple testing was performed as the genetic variations are known to be associated to the pain sensitivity [23]. All statistical analyses were conducted in STATA 12.0 (StataCorp, Texas, US), and the results are presented as mean with corresponding 95% confidence interval. A *P*-value smaller than 0.05 was considered significant, and effect sizes are presented as the differences between the means of the two groups.

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RESULTS

For this study, 112 patients signed the informed consent. Data from twenty of these patients were not included in the final analysis for different reasons as described in figure 2. Thus, data from 92 patients (50 females and 42 males) were included in the analysis. The median age was 66 years, with a range from 58 to 74 years. The with a body mass index of 27 kg/m^2 (25-31 kg/m^2). Median height and weight were 171 cm (164-176 cm; n = 90) and 83 kg (68-90 kg; n = 91), respectively.

All investigated SNPs were in Hardy-Weinberg equilibrium. The genotype distribution and corresponding demographic characteristics are presented in Table 1. The allele frequencies for the SNPs in *OPRM1*, *OPRK1*, *OPRD1*, and *COMT* were similar to the allele frequencies of the HapMap-CEU project (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_viewTable.cgi?pop=1409). With regard to *OPRM1* rs533586 SNP, the allele frequency was comparable to allele frequency of the CEU_GENO_PANEL project (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_viewTable.cgi?pop=4720). Furthermore, in this cohort we found that the *OPRM1* rs9479757G allele was more frequent than the rs9479757A allele, which was in agreement with results published by Beer et al. (2013) [24]. Nonetheless, we have previously found the reverse trend in another cohorte in rs9479757 allele frequency, showing that rs9479757A allele was the most frequent [25].

Pain sensitivity and gender

Results for pain sensitivity in males and females are shown in Table 2. There was a significant association between gender and pressure pain threshold in the unaffected leg as males could tolerate higher pressure than females (P=0.05). No other associations between gender and pain sensitivity were found (All P>0.05)

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Genetic associations with demographic characteristics

Non-carriers of the *OPRM1* rs589046T, *OPRM1* rs563649T or *OPRK1* rs1365098T alleles were taller than carriers (172.2 cm vs. 168.3 cm; *P*=0.04; 171.3 cm vs. 163.3 cm; *P*=0.01 and 172.2 cm vs. 168.3 cm; *P*=0.04) (Table 1). In addition, non-carriers of the *OPRM1* rs9479757A allele weighed more than carriers (84 kg vs. 70 kg; *P*=0.04). No other SNPs were associated with height or weight (P>0.05).

Genetic associations with pain sensitivity

Clinical pain rating during hip rotation

A higher rating of pain intensity (15.6%) was seen in carriers of the *OPRM1* rs589046T allele (mean of 5.9 (4.5 to 7.4); n=38) compared with non-carriers (mean of 5.1 (4.5 to 5.7); n=47; effect size: 0.8; *P*=0.04) (figure 3, Table 3), indicating a higher sensitivity to pain in the patients with the T allele. No other SNPs were associated with clinical pain ratings (*P*>0.05) Additionally, an explorative analysis showed that a higher rating of pain intensity was seen in patients in opioid treatment compared with patients in no opioid treatment (*P*=0.02).

Heat PDTs

A genetic association was seen between the *OPRD1* SNP rs2234918 and PDT to contact heat stimulation (*P*=0.001) (figure 2, Table 3). A higher PDT (5.1%) was seen in carriers of the rs2234918C allele (mean of 47.5°C (46.9 to 48.1°C); n=60) compared with non-carriers (mean of 45.2°C (43.7 to 46.7°C); n=29; effect size: 2.3°C) (figure 4), indicating lower sensitivity to pain in the patients with the C allele. No other SNPs were associated with contact heat PDT (*P*>0.05)

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CPM effect

The most distinct CPM effect was seen 120 seconds after initiation of the cold pressor test. Thus, values at this time point was used for further analysis. A more evident CPM effect (94.7%) was seen in carriers of the *OPRM1* rs589046T allele (pre-CPM heat PDT of 46.4°C; peri-CPM heat PDT of 47.1°C; CPM mean of 3.7% (2.5 to 5.0%); n=41) compared with non-carriers (pre-CPM heat PDT of 47.9°C; peri-CPM heat PDT of 48.0°C mean of 1.9% (0.8 to 3.2%); n=44; effect size: 1.8; *P*=0.05) (figure 5, Table 3), indicating a more pronounced pain modulation in patients with the T allele.

Pressure pain threshold

No SNPs were associated with pressure pain threshold at tibia in either the affected or unaffected hip (*P*>0.05) (Table 3).

DISCUSSION

The presented results support the hypothesis that carriage of the minor allele of *ORPM1* (rs589046) or *OPRD1* (rs2234918) was associated with pain sensitivity in patients with osteoarthritis of the hip.

Interactions with demographics

Males tolerated higher pressure in the unaffected leg than females. This confirms results from a previous study, the results of which showed that males could tolerate higher muscle pressure than females [16]. Additionally, sensitivity to pressure has been postulated to be genetically associated with an *OPRM1* rs1799971 SNP, however, only in males [26]. Recently, height has been suggested to be a classical polygenetic trait associated with newly discovered genetic factors [27]. Additionally, some investigators have suggested that gender differences in

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body height may be associated with differences in pain sensitivity based on the belief that the body height would be inversely related to the density of peripheral nociceptors [28]. For example, results from a previous study has shown that height was negatively associated with cold pressor-induced pain thresholds in females [4]. In the present study, we found a difference in height between the carriers and non-carriers group for the *OPRM1* rs589046, *OPRM1* rs563649 and *OPRK1* rs1365098 SNPs. Thus, for further investigation of inter-individual variability in pain sensitivity, height might be included as a co-factor in the analysis.

Association between genetic factors and pain

No associations were found between COMT and pain sensitivity. However, results from other studies have demonstrated association between COMT and pain phenotypes [7,14,29]. Only one polymorphism of the COMT gene was investigated in the present study and this may explain the discrepancy, as it has been demonstrated that COMT contains at least five functional polymorphisms that impact its biological activity and associated phenotypes[29]. Thus, it cannot be excluded that other COMT polymorphisms are associated with pain phenotype in OA.

Carriage of the widely studied *OPRM1* rs1799971G allele has been found to be associated with higher thresholds to pressure pain, which indicates a lower pain sensitivity to pressure in participants carrying the G allele [26]. However, despite several reports of positive associations between the rs1799971 SNP of *OPRM1* and altered pain sensitivity, based on the results of a meta-analysis of published genetic association studies it was concluded that the rs1799971 SNP is inconsistently associated with altered pain-related phenotypes [30]. The results from the present study and from another study in patients with knee OA [6] also failed to confirm association between this specific allele and pain sensitivity. However, in the present study,

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other *OPRM1* SNPs were explored and it was found that the *OPRM1* rs589046T allele was associated with increased perceived pain intensity during hip rotation and also with a more pronounced CPM effect. These findings are contradictory as a high pain rating is normally associated with a poor pain modulation (reduced CPM effect). In summary, the well-studied *OPRM1* rs1799971 SNP could not be associated with pain sensitivity in the OA patient population. However, less-studied *OPRM1* SNPs were found to be associated with pain sensitivity and therefore, for future studies we recommend including several SNPs of the same gene.

In healthy volunteers, it has been demonstrated that carriers of *OPRK1* rs6473799C allele had higher pain tolerance threshold to mechanical visceral stimulation [31]. Additionally, it has been demonstrated that two other *OPRK1* SNPs (rs7016778 and rs7824175) are associated with the pain tolerance threshold to muscle pressure stimulation [16]. However, in the present study no associations to *OPRK1* SNPs were found. This may be explained by the use of other pain models as specific experimental-pain paradigms are controlled by partially distinct nociceptive mechanisms based on the pain-evoking stimuli. It has been suggested that these distinct mechanisms may be influenced by different sets of genes [32].

Results from experimental pain studies in healthy volunteers have shown no differences in pain thresholds to contact heat stimulations between carriers and non-carriers of different *OPRD1* SNPs [16,31,33,34]. However, in this study in patients with OA, the carriers of *OPRD1* rs2234918C allele reported higher PDT to contact heat stimulation compared with non-carriers, indicating a lower pain sensitivity in patients with the C allele. *OPRD1* is thought to be involved in neuropathic and inflammatory pain perception [35,36], hence, investigating the influence of *OPRD1* SNPs on these pain types in clinical studies is recommended.

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Gene selection

The investigated candidate genes were selected based on previous work[16,25,31,37], although they are not specific for pain, they may have an impact on the pain sensitivity as endogenous opioid-like ligands, enkephalins, interact with opioid receptors, and thereby may modulate the pain system [38]. Catechol-O-methyltransferase has an indirect effect on the mu opioid receptor by changing its activity through enkephalin's [39]. In the present study, associations between altered pain sensitivity and variants of the opioid receptor genes (but not for *COMT* variants) were identified. It has been shown that other SNPs than the seventeen in the four candidate genes included in this study are associated with pain sensitivity. For example, cold pressor-induced pain sensitivity and osteoarthritis pain have previously shown to be associated with a SNP in the P2RX7 receptor gene [3,11]. Additionally, if epistasis, which is the interaction between genes, is present, the effect of one SNP may be altered or masked by the effect of another SNP and thereby, reduce the power to detect genetic associations. Thus, it is difficult to exclude that other non-significant SNPs are associated with the assessment of pain sensitivity. Thus, in future studies with higher sample size epistatic effects should be considered. The sample size of this study was relatively small for a genetic association study, but the use of experimental measurements has the potential to minimize factors that may affect detection of true associations [18]. Finally, each SNP was analyzed separately for each investigated assessment of pain sensitivity and therefore, the potential for an additive effect in patients with multiple genetic associations may exist. However, the present study was not designed and powered for a multipoint SNP analysis, as the aim was to investigate separate candidate polymorphisms. As increased sensitivity to noxious stimuli has been postulated as a risk factor for the development of chronic pain conditions [14,40], following surgery it may be important to investigate if genetic polymorphisms can be used to predict the patients at high risk.

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External validity

We showed that using experimentally induced pain stimuli in patients suffering from clinical pain offers a new platform to explore the inter-individual difference in pain sensitivity to the actual pain states of interest, relatively free of confounders seen in traditional clinical studies. The investigation of genetic association with variability in pain sensitivity may be of particular relevance for patients with inflammatory pain disorders, such as osteoarthritis, as these disorders are characterized by an increased pain sensitivity [20].

CONCLUSION

Results from the present study suggest that, in patients with hip osteoarthritis, genetic variants in OPRM1 and OPRD1 may contribute to the variability in pain sensitivity. Clinical relevance cannot be confirmed from the present study as many other factors as e.g. psychological status may also play a role in individual sensitivity to noxious stimuli. However, the results adds information on opioid receptor polymorphisms and indicates that other polymorphisms than the most studied OPRM1 rs1799971 may play an important role.

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TABLES

Table 1. Genotype distribution and corresponding demographic characteristics of the study population (n=92)

Gene	dbSNP	Genotype	N	D	Gender (female:male)	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Duration of hip pain (years)
μ-opioid receptor (*OPRM*)	rs1799971	AA	73	0.80	39:34	66 (58-74)	170 (164-176)	83 (68-90)	27 (25-31)	4 (3-5)
		AG	17	0.18	11:8	66 (60-73)	172 (165-175)	78 (68-88)	26 (24-31)	5 (4-6)
		GG	2	0.02						
	rs589046	CC	47	0.51	24:24	66 (56-74)	**172 (167-177)**	83 (68-94)	27 (24-31)	4 (3-5)
		CT	35	0.38	26:18	68 (58-74)	**168 (161-176)**	82 (69-87)	27 (25-31)	4.5 (3-5.5)
		TT	10	0.11						
	rs563649	CC	81	0.88	42:39	66 (56-74)	**172 (165-177)**	83 (69-90)	27 (24-30)	4 (3-5)
		CT	10	0.11	8:3	66 (60-73)	**163 (160-168)**	72 (65-93)	28 (25-32)	5 (4-6)
		TT	1	0.01						
	rs9479757	GG	71	0.77	36:35	66 (58-74)	171 (166-177)	**84 (70-90)**	27 (25-31)	4 (3-5)

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		AG	18	0.20	14:7	66 (58-73)	165 (162-175)	**70 (64-85)**	26 (24-28)	5 (4-5)
		AA	3	0.03						
	rs533586	TT	33	0.36	17:16	64 (58-72)	172 (164-177)	83 (71-88)	27 (25-32	5 (4-6)
		TC	45	0.49	33:26	67 (58-74)	170 (164-176)	83 (66-90)	27 (25-30)	4 (3-5)
		CC	14	0.15						
κ-opioid receptor (*OPRK*)	rs16918875	CC	88	0.96	47:41	67 (58-74)	171 (164-176)	83 (67-90)	27 (24-31)	4 (3-5)
		CT	4	0.04	3:1	58 (54-66)	168 (166-173)	85 (76-94)	29 (28-32)	3 (2-5)
		TT	0	0						
	rs7016778	TT	76	0.83	42:34	66 (58-74)	172 (163-178)	84 (67-90)	27 (24-31)	4 (3-5)
		TA	15	0.16	8:8	67 (58-72)	169 (166-172)	79 (71-89)	28 (25-31)	5 (3.5-6)
		AA	1	0.01						
	rs10504151	TT	84	0.91	45:39	67 (58-74)	171 (164-177)	82 (67-89)	27 (24-30)	4 (3-5)
		TC	8	0.09	5:3	59 (54-67)	168 (166-171)	87 (76-92)	29 (27-33)	4.5 (3-6)
		CC	0	0						
	rs7836120	AA	73	0.79	40:33	66 (58-74)	172 (164-178)	84 (67-90)	27 (24-31)	4 (3-5)

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	AG	17	0.19						
				10:9	66 (54-72)	169 (166-172)	80 (71-90)	28 (26-31)	5 (4-6)
	GG	2	0.02						
rs6473799	TT	47	0.51	27:20	66 (58-74)	172 (165-176)	85 (69-90)	27 (24-31)	4 (3-5)
	TC	36	0.39						
				23:22	66 (58-73)	169 (162-176)	80 (67-88)	27 (25-31)	5 (4-6)
	CC	9	0.10						
rs1365098	GG	47	0.51	25:22	66 (58-74)	**174 (165-179)**	85 (69-90)	27 (24-31	4 (3-5)
	GT	34	0.37						
				25:20	67 (58-73)	**168 (162-175)**	80 (67-87)	27 (25-31	5 (4-6)
	TT	11	0.12						
rs7824175	GG	72	0.78	40:32	66 (58-74)	171 (165-176)	84 (70-90)	27 (25-31	4 (3-5.5)
	GC	17	0.19						
				10:10	71 (59-76)	170 (159-178)	76 (66-86)	27 (23-29)	4.5 (3-5)
	CC	3	0.03						
rs963549	GG	68	0.74	37:31	66 (58-74)	171 (165-176)	84 (69-90)	27 (25-31	4 (3-5.5)
	GA	21	0.23						
				13:11	68 (56-74)	169 (161-177)	81 (67-87)	27 (24-29)	4.5 (3.5)
	AA	3	0.03						
δ-opioid	rs533123	AA	54	0.59	28:26	69 (59-74)	172 (165-176)	84 (70-90)	28 (26-31)	4 (3-6)

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receptor (*OPRD*)		AG	34	0.37	22:16	64 (54-74)	170 (163-177)	80 (64-90)	26 (24-31)	5 (3-5)
		GG	4	0.04						
	rs2236857	TT	55	0.60	31:24	65 (56-73)	170 (163-176)	80 (68-89)	26 (24-31)	5 (3-6)
		TC	30	0.33	19:18	69 (59-74)	172 (164-176)	85 (69-90)	27 (25-30)	4 (3-5)
		CC	7	0.08						
	rs2234918	TT	29	0.32	17:12	68 (58-74)	168 (163-175)	83 (70-90)	27 (24-34)	4 (3-6)
		TC	50	0.54	33:30	66 (58-74)	171 (165-179)	83 (67-90)	27 (25-30)	4 (3-5)
		CC	13	0.14						
COMT	rs4680	AA	31	0.34	14:17	63 (54-73)	171 (164-178)	85 (67-94)	27 (25-33)	4 (3-6)
		AG	45	0.49	36:25	69 (58-74)	170 (164-176)	81 (69-88)	27 (24-30)	4 (3-5)
		GG	16	0.17						

dbSNP = single nucleotide polymorphism database identification; D = distribution; demographic characteristics are for the carriers of the minor allele vs. for the non-carriers of the minor allele; age, height, weight and BMI are represented as median (25^{th} -75^{th} percentile); BMI = body mass index; Bold typing indicates that there is a significant difference between carriers of the minor allele and non-carriers of the minor allele.

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Table 2. Measurements for pain sensitivity in males and females

Parameters	Males		Females	
	N	Median (25^{th}-75^{th})	N	Median (25^{th}-75^{th})
Measurements for pain sensitivity:				
Clinical pain rating during hip rotation (NRS)	41	5 (4-6)	44	6 (5-7)
Heat PDTs (°C)	41	47.8 (45.9-49.1)	48	47.8 (45.5-48.7)
CPM effect (%)	38	1.5 (0.6-4.7)	47	2.4 (0.7-5.2)
Pressure pain thresholds in the affected hip (kPa)	42	71 (54.5-83.5)	50	64.8 (53.5-77.8)
Pressure pain thresholds in the unaffected hip (kPa)	42	**73.4 (60.8-90.8)**	50	**57.7 (47-69.8)**

N = number of patients; BMI = body mass index; NRS = numeric rating scale; PDTs = pain detection thresholds; 25^{th}-75^{th} percentiles; Bold parameter indicates that there is a significant difference between males and females

Assessment of pain sensitivity \ **Candidate genes**	*COMT*	*OPRM1*	*OPRK1*	*OPRD1*
Clinical pain rating during hip rotation	-	rs589046*	-	-
Heat pain detection threshold	-	-	-	rs2234918
CPM effect	-	rs589046*	-	-
Pressure pain threshold in the affected hip	-	-	-	-
Pressure pain threshold in the unaffected hip	-	-	-	-

Table 3. Summary of the genetic associations with the assessment of pain sensitivity

* indicates that the specific SNP was associated with more than one assessment, - indicates that no significant association could be demonstrated.

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FIGURE LEGENDS

Figure 1. Flow diagram of the procedures

Figure 2. A flow diagram showing the process from patient screening to inclusion.

Figure 3. Clinical pain rating during hip rotation and the *OPRM1* rs589046T SNP (univariate analysis)

A higher clinical pain rating was seen in carriers of the *OPRM1* rs589046T allele (n=38) compared with non-carriers (n=47; effect size: 0.8; *P*=0.04), NRS = numerical pain rating from zero to ten, horizontal line represents the mean

Figure 4. Pain detection thresholds to contact heat stimulation and the *OPRD1* rs2234918 SNP (univariate analysis)

A higher heat PDT was seen in carriers of the *OPRD1* rs2234918C allele (n=60) compared with non-carriers (n=29; effect size: 2.3 °C; *P*=0.001), PDTs = heat pain detection thresholds

Figure 5. CPM effect and the *OPRM1* rs589046 SNP (univariate analysis)

A higher CPM effect was seen in carriers of the *OPRM1* rs589046T allele (n=41) compared with non-carriers (n=44; effect size: 1.8; *P*=0.05)

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FIGURES

Figure 1.

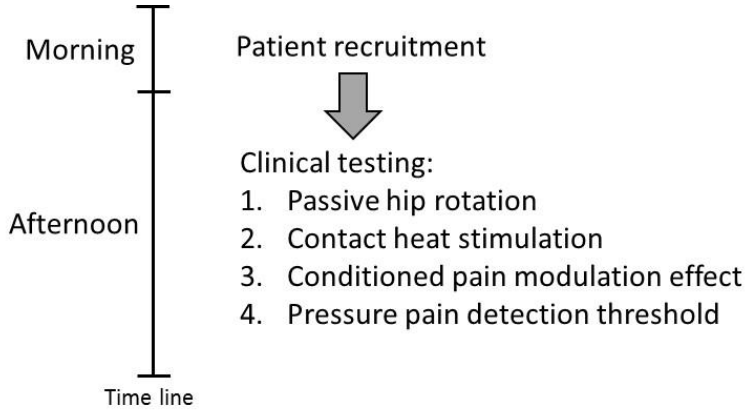

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Figure 2.

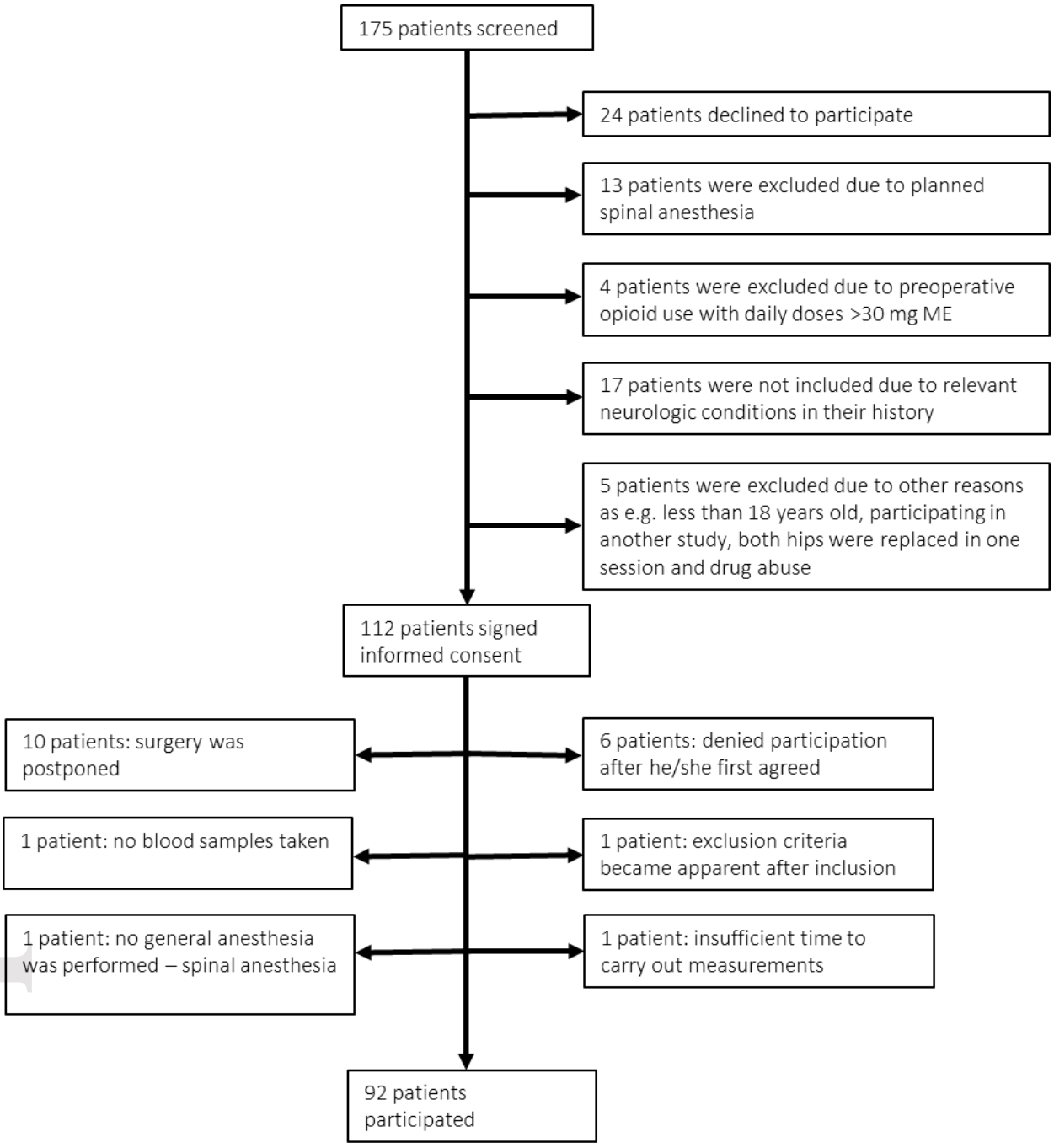

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Figure 3.

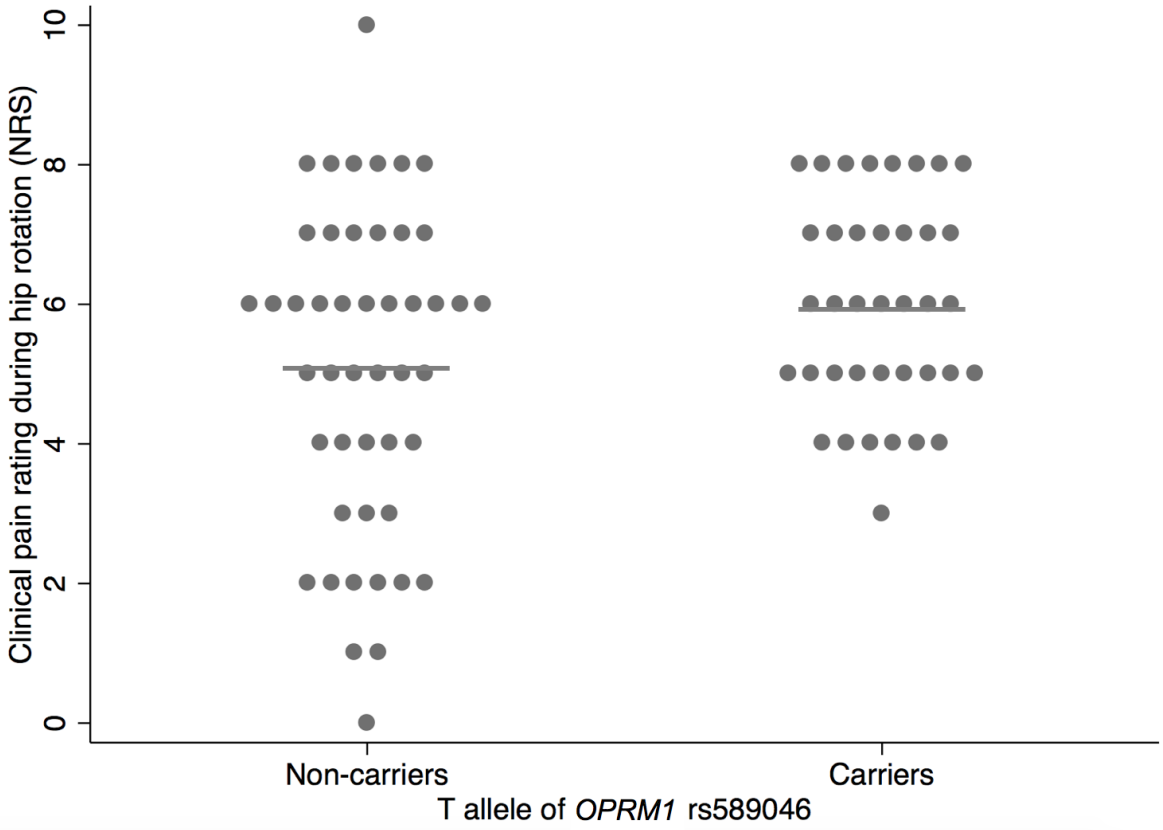

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Figure 4.

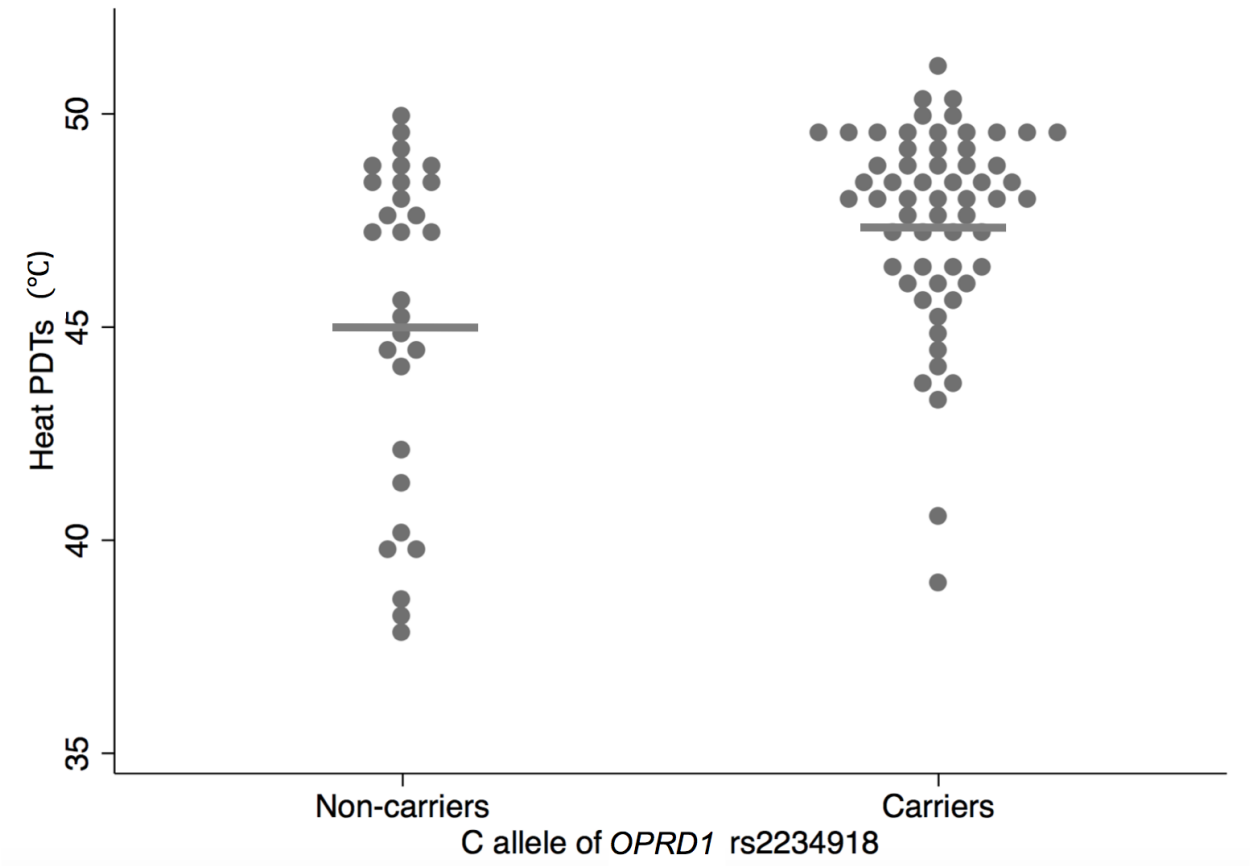

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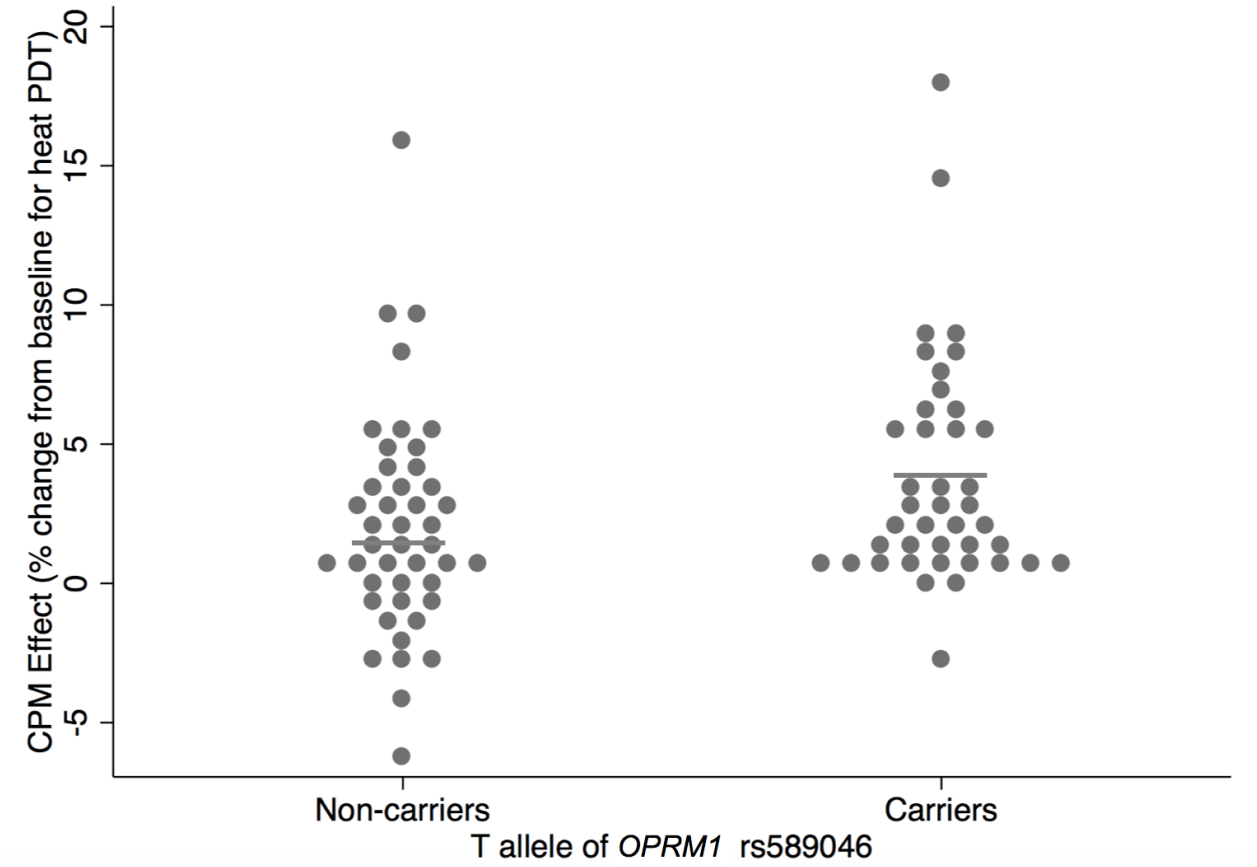
Figure 5.



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