Effect of Catechol-o-methyltransferase-gene (*COMT*) Variants on Experimental and Acute Postoperative Pain in 1,000 Women undergoing Surgery for Breast Cancer

Oleg Kambur, M.Sc.(Pharm.),* Mari A. Kaunisto, Ph.D.,† Emmi Tikkanen, M.Sc.,‡ Suzanne M. Leal, Ph.D.,§ Samuli Ripatti, Ph.D.,‖ Eija A. Kalso, M.D., Ph.D.#

ABSTRACT

Background: Catechol-o-methyltransferase (COMT) metabolizes catecholamines in different tissues. Polymorphisms in *COMT* gene can attenuate COMT activity and increase sensitivity to pain. Human studies exploring the effect of *COMT* polymorphisms on pain sensitivity have mostly included small, heterogeneous samples and have ignored several important single nucleotide polymorphisms (SNPs). This study examines the effect of *COMT* polymorphisms on experimental and postoperative pain phenotypes in a large ethnically homogeneous female patient cohort.

Methods: Intensity of cold (+2-4°C) and heat (+48°C) pain and tolerance to cold pain were assessed in 1,000 patients scheduled for breast cancer surgery. Acute postoperative pain and oxycodone requirements were recorded. Twenty-two *COMT* SNPs were genotyped and their association with six pain phenotypes analyzed with linear regression.

Results: There was no association between any of the tested pain phenotypes and SNP rs4680. The strongest association signals were seen between rs165774 and heat pain intensity as well as rs887200 and cold pain intensity. In both cases, minor allele carriers reported less pain. Neither of these results remained significant after strict multiple testing corrections. When analyzed further, the effect of rs887200 was, however, shown to be significant and consistent throughout the cold pressure test. No evidence of association between the SNPs and postoperative oxycodone consumption was found.

Conclusions: SNPs rs887200 and rs165774 located in the untranslated regions of the gene had the strongest effects on pain sensitivity. Their effect on pain is described here for the first time. These results should be confirmed in further studies and the potential functional mechanisms of the variants studied.

What We Already Know about This Topic

- Variants of the gene for the catecholamine-metabolizing enzyme, COMT, are associated in some small studies with differences in sensitivity to pain

What This Article Tells Us That Is New

- In a more complete assessment of variants in the *COMT* gene in 1,000 women undergoing surgery for breast cancer, there were weak associations with sensitivity to heat or cold pain
- The lack of association of COMT variants with acute postoperative opioid requirement raises questions regarding the clinical relevance of this gene in acute postoperative pain

CATECHOL-o-METHYLTRANSFERASE (COMT) metabolizes catecholamines in glial cells and postsynaptic

◇ This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 1A.

◆ This article is accompanied by an Editorial View. Please see: McLean SA: The scientific journey to predicting and preventing postoperative pain: Recalling Dr. Wall's stories along the way. ANESTHESIOLOGY 2013; 119:1244–6.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org).

* Student, Department of Anaesthesia, Intensive Care Medicine, Emergency Medicine and Pain Medicine, Helsinki University Central Hospital, Helsinki, Finland, and Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland. † Senior Researcher, Institute for Molecular Medicine Finland (FIMM), University of Helsinki, and Folkhälsan Institute of Genetics, Folkhälsan Research Center. ‡ Student, ‖ Professor, Institute for Molecular Medicine Finland (FIMM), University of Helsinki, and Department of Chronic Disease Prevention, National Institute for Health and Welfare. § Professor, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas. # Professor, Department of Anaesthesia, Intensive Care Medicine, Emergency Medicine and Pain Medicine, Helsinki University Central Hospital.

Received from the Department of Anaesthesia, Intensive Care Medicine, Emergency Medicine and Pain Medicine, Helsinki University Central Hospital, Helsinki, Finland, and Institute of Clinical Medicine, University of Helsinki, Helsinki, Finland. Submitted for publication April 17, 2013. Accepted for publication July 16, 2013. Funding was received from the Academy of Finland (grant numbers 110489 and 217028), and the Research Funds of the Helsinki and Uusimaa Hospital District (grant numbers TYH2008225 and TYH2010210), Helsinki, Finland. Dr. Kambur received grants from The Paulo Foundation, Helsinki, Finland, and from Finnish Cultural Foundation, Helsinki, Finland. The authors declare no competing interests. The first two authors contributed equally to this article.

Address correspondence to Dr. Kalso: Department of Anaesthesia and Intensive Care Medicine, Helsinki University Central Hospital, Haartmaninkatu 2A, POB 140, 00029 HUS, Helsinki, Finland. eija.kalso@helsinki.fi. Anesthesiology 2013; 119:1422-33

neurons in the central nervous system and other tissues.[1] Metabolic activity of COMT is modulated by genetic variation which can lead to significant differences in COMT activity in humans.[2,3] The role of *COMT* has previously been studied in pain sensitivity and opioid analgesia.[4–9] However, most of the studies have focused on a few polymorphisms only.

The *COMT* gene is located in the long arm of chromosome 22. It consists of six exons covering more than 27 kb. Four of the exons are translated into protein, and promoters located in exon 3 regulate the synthesis of distinct types of messenger RNAs that code for the membrane-bound and soluble forms of the enzyme. Within the 27-kb genomic area, more than 900 genetic variants have been identified, the vast majority being located within the introns. The only common *COMT* single nucleotide polymorphism (SNP) that is nonsynonymous is rs4680 (Val$^{108/158}$Met). The Met allele has been shown to reduce COMT activity three- to four-fold.[10] In addition, several other polymorphisms have been suggested to modulate expression or activity of COMT. However, their effects on COMT activity *in vivo* and on pain phenotypes have not been characterized.[11–16]

COMT polymorphisms, SNP rs4680 in particular, have been studied in several painful conditions such as temporomandibular dysfunction, fibromyalgia, migraine, and headache. Low COMT activity has been suggested to increase the intensity of pain in these conditions (see study by Kambur and Männistö[17] for references). In osteoarthritis, for example, the Val$^{108/158}$Met variant has been associated with more intense pain with similar tissue destruction.[18] Low COMT activity has also been related to increased pain sensitivity in experimental thermal and mechanical pain tests.[19,20] The relevance of *COMT* polymorphism in acute postsurgery pain has been assessed in only two reports. One of them found an association between *COMT* SNP rs740603 and maximum postoperative pain ratings,[6] and the other study reported associations between SNPs rs4818 and rs6269 and intensity of postoperative pain.[21]

Previous studies exploring the effect of *COMT* on pain have mostly been of small size and they have included both men and women of different ethnic origins. Most studies have analyzed only SNP rs4680 (Val$^{108/158}$Met). Recent studies have suggested that other nearby SNPs (*e.g.*, rs6269 and rs4818) and haplotypes containing these SNPs could be as important.[19,20] There is much genetic variation within and around the *COMT* gene that has been overlooked in previous studies. Especially, synonymous and 3′- and 5′-untranslated region (UTR) SNPs remain largely uninvestigated even though they can also significantly affect the gene expression and thus have a phenotypic effect as well. In this study, our aim was to test the association between all common variation within and around the *COMT* gene and pain phenotypes in by far the largest homogenous dataset consisting of 1,000 Finnish female breast cancer patients. Detailed measures of experimental pain as well as acute postsurgery pain and oxycodone consumption were analyzed. Our study combines dense, high-quality genotype data, which allows a more comprehensive evaluation of *COMT* variants, and a large patient sample with rich phenotypic data.

Materials and Methods

Ethics

Written informed consent was obtained from each subject participating in the study by either a research nurse or a physician. Surgery and all other treatments were part of the patients' normal clinical care under the supervision of the physician in charge. The research protocol had been approved by the coordinating ethics committee (136/E0/2006) and the ethics committee of the Department of Surgery (Dnro 148/E6/05) of the Hospital District of Helsinki and Uusimaa.

Subjects and Initial Clinical Assessment

This study reports the data of 1,000 patients who were operated for breast cancer at the Unit for Breast Surgery at the Women's Hospital, Helsinki University Central Hospital, Helsinki, Finland, between August 2006 and December 2010. The study cohort is described in detail in the accompanying article.[22] In brief, either mastectomy or breast conserving surgery, with sentinel node biopsy, axillary clearance, or both, was performed. Before surgery, the patients filled in questionnaires on depression (Beck Depression Inventory)[23] and anxiety (State and Trait Anxiety Inventory),[24] and underwent experimental pain tests.

Experimental Pain

Contact heat pain and cold pain were assessed as described in the study by Kaunisto *et al.*[22] For the heat pain, the volar surface of the forearm was first exposed to 43°C for 5 s and then 48°C also for 5 s. The patients assessed the intensity and unpleasantness of pain with a 0–10 numerical rating scale (NRS) at the end of each test; zero represented "no pain," and 10 "worst imaginable pain." All 1,000 women were evaluated for heat pain sensitivity.

Cold pain was measured by immersing the hand to a cold water (2-4°C) bath for the maximum time tolerated by the patients but not longer than 90 s. Time to withdrawal of the hand and pain intensity (NRS) and unpleasantness were measured every 15 s during the test and at withdrawal. The cold pain test was not performed on the first 100 patients studied (n = 900).

Anesthesia, Postsurgical Pain Assessment, and Administration of Oxycodone

The patients received standardized anesthesia during which a blood specimen was drawn for DNA isolation and banking.[22] In the postanesthesia care unit, the patients were asked about the pain intensity at rest and during motion. Motion pain was assessed by asking the patient to raise the arm ipsilateral to the surgical intervention up to 90°. Patients were titrated with *i.v.* oxycodone by a research nurse who

asked about the pain intensity every 5 min and administered oxycodone in doses of 1–3 mg until adequate analgesia (NRS < 4/10) was achieved. After this, pain intensity was recorded every 15 min until the patient needed the next dose of oxycodone. Time to the first dose of oxycodone and time needed to achieve satisfactory pain relief were documented. For the ward, the patients were provided with a patient-controlled analgesia device for up to 20 h except for 70 day-case surgery patients. The amount of oxycodone needed to achieve satisfactory pain relief, oxycodone consumption in the postanesthesia care unit, and the total amount of oxycodone consumed during 20 h after the operation were recorded.

SNP Selection and Genotyping

A total of 22 SNPs were genotyped from a 47 kb region (chr22: 18,299–18,346 kb, NCBI36/hg18 assembly) covering the *COMT* gene as well as 10 kb of 5′ and 3′ flanking regions (table 1). Information about the linkage disequilibrium (LD) within this area was used to select an optimal set of SNPs capturing most of the genetic information. The SNP selection was based on phase 3 data of the HapMap database and performed using the Tagger program included in the Haploview 4.1 software.**[25] The HapMap database†† contained 30 SNPs having a minor allele frequency of greater than 10% in the European population (Utah residents with North-Western European ancestry from the Centre d'Etude du Polymorphisme Humain collection and Toscans in Italy). The aim was to capture all these SNPs by setting the limit for the pair-wise r^2 to 0.9 or greater. Nineteen SNPs were chosen to be genotyped, and 17 of these were successfully included in the genotyping multiplexes. Thus, 28 of the 30 HapMap SNPs were captured with a mean r^2 of 0.994. In addition to these 17 genotyped SNPs, two additional SNPs were selected based on their position in the exonic areas (rs4818 and rs16599) and three because they were known to be common based on HapMap phase 2 data but not available on phase 3 data (rs74063, rs165774, and rs2518824).

DNA was extracted from peripheral blood using the Autopure LS automated DNA purification instrument (Gentra Systems, Inc., Minneapolis, MN). The 22 SNPs were genotyped using the Sequenom MassARRAY system and the iPLEX Gold Single Base Extension chemistry (Sequenom, San Diego, CA) in a multiplex format.[26] This method has excellent success (>95%) and accuracy (100%) rates.[27] Both duplicate, Centre d'Etude du Polymorphisme Humain control and water control samples were included in each DNA plate to confirm the accuracy of the genotyping results. Genotyping was performed blind to phenotypic information.

Statistical Analysis

SNPs. The entire sample was tested to determine whether the null hypothesis of Hardy–Weinberg equilibrium could be rejected by applying the chi-square method. The Haploview 4.1 program was used to determine the pair-wise LD between the SNPs (r^2 value) and to identify haploblock structures using the CI algorithm.[25]

Power Calculations. The theoretical effect size needed for our study sample to have power to detect association with the previously pain-associated variant, rs4680, was calculated using R software environment.‡‡ The minor allele frequency was set to be the same as detected in our sample, 0.457. Additive inheritance model (each copy of the minor allele having an effect) was assumed. The results showed that in order to have a power greater than 0.9 to reach a *P* value of 0.05 (considered to be an appropriate level of evidence when replicating a previous association), the effect size should be 15% of the SD for the phenotype per allele (additive model). In our case, the minimum effect size of 0.36 NRS units on a scale 0–10 for heat pain and 0.39 NRS units for cold pain would produce a *P* value less than 0.05 with 90% certainty. Thus, our sample size provides enough power to detect meaningful effect sizes for rs4680.

Association Analyses. Association between the *COMT* SNPs and pain phenotypes was tested using linear regression analysis. Additive, dominant, and recessive models were all considered. Analyses were performed with PLINK software.§§[28]

The experimental pain variables that were analyzed include intensity of thermal pain produced by heat stimulation (48°C, NRS) and two different cold pain–related variables: time to withdrawal and pain intensity (NRS) at 15 s. Those patients who withdrew their hand before the analyzed time point were coded as having the maximum NRS value (n = 118 at 15 s).

The postoperative pain-related variables that were studied include pain intensity during motion (NRS) when needing the first dose of oxycodone, the amount of oxycodone needed to achieve adequate analgesia (NRS <4/10), and total oxycodone consumption ($mg \cdot kg^{-1} \cdot 20\,h^{-1}$).

All association analyses were performed using variables adjusted for potential confounding factors, that is, on residual phenotypic scores after regressing out covariate effects. The covariates used for experimental pain phenotypes include age, body mass index, presence of any preoperative chronic pain condition (yes/no), and anxiety (State and Trait Anxiety Inventory score). For postoperative variables, the residual phenotypes were also adjusted for the effect of the type of surgery performed. When analyzing the association with total oxycodone consumption, the 70 day-case surgery patients who did not use the patient-controlled analgesia device were excluded.

Follow-up Analyses for SNP rs887200. SNP rs887200 was explored further using parametric tests where homozygous

** Haploview. Available at: http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview. Accessed June 14, 2012.

†† HapMap database. Available at: http://www.hapmap.org/. Accessed February 25, 2010.

‡‡ Available at: http://www.r-project.org/. Accessed March 25, 2013.

§§ Available at: http://pngu.mgh.harvard.edu/~purcell/plink/. Accessed May 17, 2013.

Table 1. List of Genotyped SNPs

SNP	Position (dbSNP Build 130)	Obs HET	HWE *P* Value	Success Rate	MAF	Alleles Minor/Major	Genotype Counts
rs6518591	chr22:18304021	0.262	0.449	1.000	0.162	G/A	32/268/695
rs737866	chr22:18310109	0.327	0.903	0.998	0.208	G/A	38/322/634
rs737865	chr22:18310121	0.328	0.911	0.997	0.209	C/T	38/323/632
rs1544325	chr22:18311668	0.524	0.260	1.000	0.492	A/G	228/523/244
rs8185002	chr22:18313048	0.330	0.995	1.000	0.206	G/T	35/323/636
rs174675	chr22:18314051	0.417	0.802	0.998	0.302	T/C	92/433/469
rs5993882	chr22:18317533	0.299	0.459	1.000	0.191	G/T	40/310/645
rs740603	chr22:18325177	0.488	0.731	0.998	0.398	G/A	151/486/356
rs4646312	chr22:18328337	0.424	0.788	0.995	0.313	C/T	96/427/469
rs4633	chr22:18330235	0.504	0.772	1.000	0.457	C/T	205/502/284
rs2239393	chr22:18330428	0.430	0.902	0.997	0.317	G/A	91/356/356
rs4818	chr22:18331207	0.425	0.836	1.000	0.313	G/C	96/427/471
rs4680	chr22:18331271	0.505	0.715	0.994	0.457	G/A	204/501/286
rs4646316	chr22:18332132	0.313	0.261	1.000	0.207	T/C	50/302/643
rs165774	chr22:18332561	0.378	0.658	1.000	0.244	A/G	51/380/564
rs174696	chr22:18333176	0.439	0.401	1.000	0.351	C/T	129/450/416
rs9306235	chr22:18335157	0.114	1.000	0.998	0.060	A/G	4/115/874
rs9332377	chr22:18335692	0.217	0.974	1.000	0.122	T/C	14/229/752
rs165599	chr22:18336781	0.457	0.408	0.995	0.327	G/A	99/459/433
rs887199	chr22:18341955	0.319	0.930	0.998	0.201	A/G	41/318/635
rs2518824	chr22:18342963	0.076	0.780	1.000	0.038	G/T	0/76/919
rs887200	chr22:18343666	0.270	0.746	0.998	0.164	C/T	27/273/694

Success rates, HWE values, minor allele frequencies, and genotype counts are presented.

HWE = Hardy–Weinberg equilibrium; MAF = minor allele frequency; Obs HET = observed heterozygosity; SNP = single nucleotide polymorphism.

carriers of risk allele were compared with other patients. Cold pain intensities across different time points were analyzed with two-way ANOVAs for repeated measurements using time and genotype as independent variables. Time to withdrawal was analyzed with Student unpaired *t* test.

Permutations and Correcting for Multiple Testing. Due to the fact that the distributions of the studied quantitative traits were not normal, permutation procedures were used to obtain empirical *P* values. The associations were calculated using the max(T) permutation option of the PLINK software performing 10,000 permutations for each SNP. This option not only obtains empirical *P* values for each SNP but also produces *P* values adjusted for performing multiple tests in order to control for the family-wise error rate. Because this method takes into account the correlation due to LD between the studied SNPs, it provides a less stringent correction than using a Bonferroni correction to control for multiple testing. These *P* values were further multiplied by 18, to correct for the number of tested phenotypes (six) and statistical models (three). The level of significance was set at a corrected *P* value of 0.05.

Haplotype Analyses. In addition to single-SNP analyses, haplotypes were also tested for association. The PLINK software was used for calculating the most likely haplotype combinations for each patient using the haploblock structure identified with the Haploview program as a guideline (fig. 1).[25,28] Three different haplotype combinations, corresponding with the three identified haploblocks, were determined for each patient. Furthermore, we constructed the haplotypes previously associated with pain sensitivity phenotypes in a study by Diatchenko *et al.*[19,20] who classified them as low pain sensitivity (LPS), average pain sensitivity, and high pain sensitivity. SNPs rs4633, rs4818, and rs4680 were used for this purpose. Haplotype-association analyses were performed using linear regression with an additive model, where all haplotypes having a frequency above 1% within a haploblock were tested one by one against all the others. Permutations were performed in a similar way as to the single-SNP analyses.

In Silico *eQTL Database Analyses*

The expression Quantitative Trait Loci-association patterns between COMT mRNA levels and genotypes of SNPs rs165744 and rs887200 were investigated using GENEVAR (GENe Expression VARiation) software‖‖ developed at Wellcome Trust Sanger Institute (Hinxton, Cambridge, United Kingdom). This interactive Java interface was used to perform searches in three available gene expression–profiling datasets containing data based on HapMap lymphoblastoid cell lines,[29] three tissue types derived from female twins of the MuTHER study,[30] and three cell types derived from umbilical cords of GenCord individuals.[31]

‖‖ Available at: http://www.sanger.ac.uk/resources/software/genevar/. Accessed May 24, 2012.

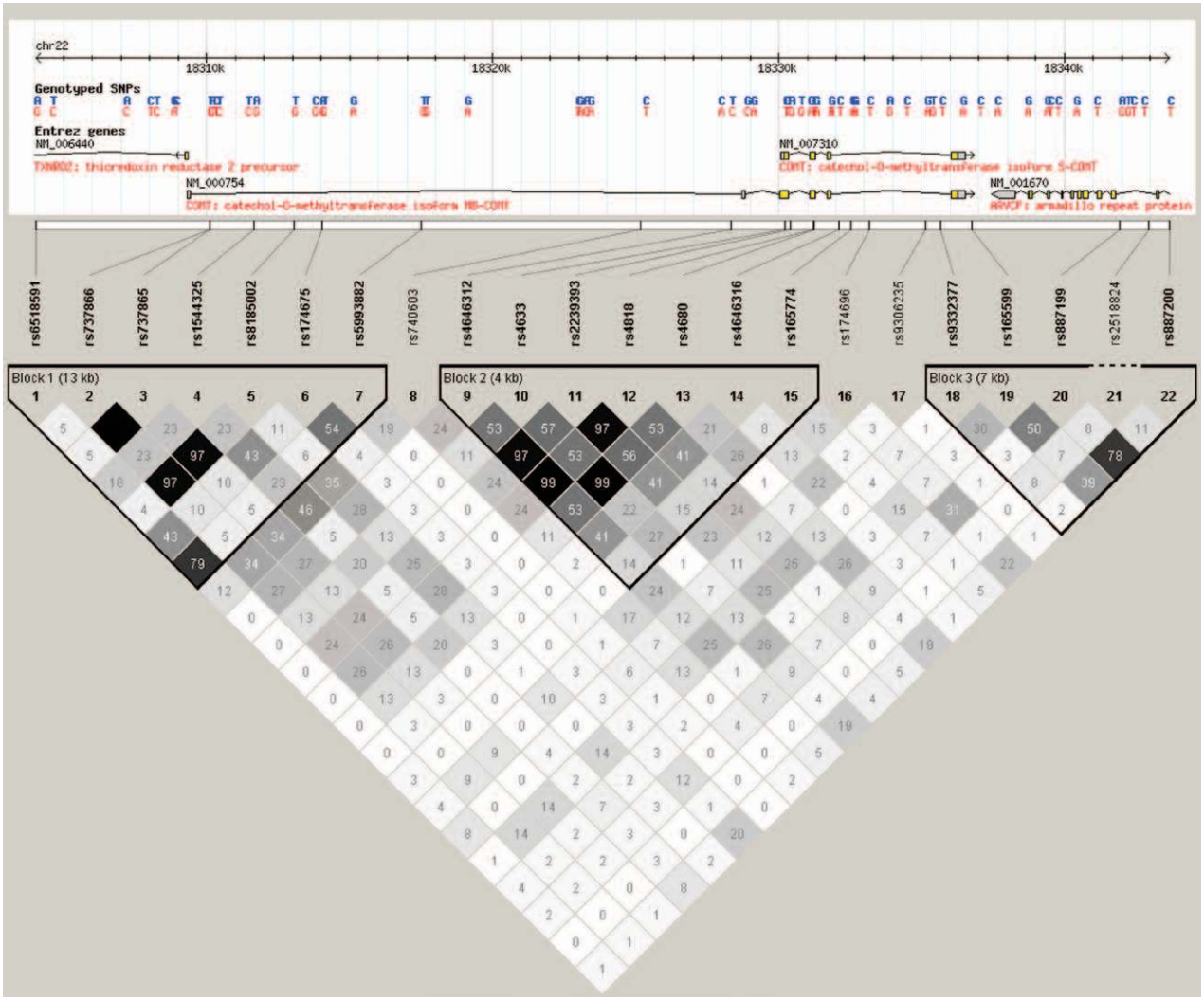

Fig. 1. The catechol-o-methyltransferase (*COMT*) gene single nucleotide polymorphisms analyzed in this study, their location and the haploblock structure of the area based on our samples. The haploblocks have been constructed using the CI algorithm implemented in the Haploview program.

Results

Genetic Association Studies

Twenty-two SNPs within or nearby the *COMT* gene were genotyped. The success rates, minor allele frequencies, and Hardy–Weinberg equilibrium values of the genotyped SNPs are presented in table 1. The average success rate of genotyping was 99.0%. None of the SNPs showed deviation from Hardy–Weinberg equilibrium ($P < 0.05$). Figure 1 presents the location of the studied *COMT* SNPs and the CI method–based LD block structures in the study subjects.

The clinical characteristics of the patients are shown in table 2. The results of the analyses testing association between *COMT* and experimental heat pain intensity are shown in table 3 and figure 2. Of the SNPs studied, rs165774 showed the strongest association with the intensity of heat pain at 48°C. The smallest uncorrected empirical *P* value of 0.003 was obtained when the dominant model was used. This *P* value remained less than 0.05 after correcting for the number of SNPs analyzed but not when the more stringent corrections were performed ($P = 1$). Regression coefficient (β) that represents the effect size and direction (using the major allele as the reference allele) was −0.46. The

Table 2. Clinical Characteristics of the Studied Patients

N	1,000
Age (yr)	57.0 ± 9.3
Weight (kg)	69.4 ± 4.3
BMI (kg/m^2)	25.4 ± 4.3
Anxiety score	38 (20–78)
Type of surgery (BCS/mastectomy)	62.6%/37.4%
Sentinel biopsy and/or axillary clearance (yes/no)	44.0%/56.0%
Preoperative chronic pain condition (yes/no)	24%/76%

Data are summarized as mean ± SD or median (range).
BCS = breast conserving surgery; BMI = body mass index.

Table 3. Association between *COMT* SNPs and Experimental Pain

	Heat Pain Intensity, 48°C					Cold Pain Intensity, NRS at 15 s					Cold Withdrawal Time				
SNP	Naive *P* Value	β	Perm *P* Value	SNP Cor *P* Value	Cor *P* Value	Naive *P* Value	β	Perm *P* Value	SNP Cor *P* Value	Cor *P* Value	Naive *P* Value	β	Perm *P* Value	SNP Cor *P* Value	Cor *P* Value
Dominant model															
rs6518591	0.835	0.035	0.842	1.000	1.000	0.834	0.043	0.836	1.000	1.000	0.399	−1.788	0.402	0.998	1.000
rs737866	0.059	−0.300	0.055	0.554	1.000	0.378	−0.174	0.373	0.997	1.000	0.055	3.890	0.060	0.504	1.000
rs737865	0.056	−0.303	0.054	0.534	1.000	0.308	−0.201	0.300	0.989	1.000	**0.046**	4.038	0.051	0.450	1.000
rs1544325	0.634	0.084	0.637	1.000	1.000	**0.031**	0.473	**0.035**	0.352	1.000	**0.007**	−6.106	**0.007**	0.095	1.000
rs8185002	0.047	−0.315	0.044	0.474	1.000	0.402	−0.165	0.396	0.998	1.000	0.061	3.796	0.068	0.543	1.000
rs174675	0.410	0.126	0.418	0.998	1.000	0.479	0.135	0.471	1.000	1.000	0.309	−1.987	0.310	0.988	1.000
rs5993882	0.339	0.153	0.345	0.992	1.000	0.344	0.188	0.339	0.995	1.000	0.088	−3.478	0.097	0.674	1.000
rs740603	0.330	−0.155	0.335	0.990	1.000	0.440	0.153	0.438	0.999	1.000	0.989	0.029	0.986	1.000	1.000
rs4646312	0.772	0.044	0.783	1.000	1.000	0.142	0.279	0.138	0.836	1.000	0.982	0.044	0.982	1.000	1.000
rs4633	0.290	0.179	0.287	0.980	1.000	0.393	0.179	0.397	0.998	1.000	0.457	1.597	0.461	0.999	1.000
rs2239393	0.454	−0.127	0.462	0.998	1.000	0.135	0.317	0.136	0.819	1.000	0.744	0.730	0.742	1.000	1.000
rs4818	0.733	0.052	0.747	1.000	1.000	0.126	0.290	0.123	0.796	1.000	0.972	0.070	0.971	1.000	1.000
rs4680	0.230	0.203	0.234	0.952	1.000	0.341	0.198	0.341	0.994	1.000	0.447	1.627	0.446	0.999	1.000
rs4646316	0.597	0.084	0.607	1.000	1.000	0.222	0.243	0.223	0.950	1.000	0.496	−1.394	0.502	1.000	1.000
rs165774	**0.003**	−0.464	**0.003**	**0.040**	1.000	0.170	−0.262	0.167	0.886	1.000	0.139	2.909	0.134	0.824	1.000
rs174696	0.234	0.184	0.222	0.954	1.000	0.752	−0.061	0.755	1.000	1.000	0.688	0.797	0.667	1.000	1.000
rs9306235	0.442	0.182	0.450	0.998	1.000	**0.026**	0.656	**0.027**	0.306	1.000	0.240	−3.567	0.241	0.962	1.000
rs9332377	0.932	−0.015	0.932	1.000	1.000	0.398	0.187	0.399	0.998	1.000	0.475	−1.625	0.470	1.000	1.000
rs165599	0.780	0.043	0.775	1.000	1.000	0.738	−0.064	0.738	1.000	1.000	0.304	2.028	0.296	0.987	1.000
rs887199	0.191	0.208	0.192	0.920	1.000	0.783	−0.054	0.783	1.000	1.000	0.342	1.921	0.346	0.993	1.000
rs2518824	0.203	0.368	0.205	0.930	1.000	0.670	−0.155	0.674	1.000	1.000	0.936	−0.300	0.937	1.000	1.000
rs887200	0.151	0.239	0.156	0.866	1.000	0.994	0.002	0.992	1.000	1.000	0.303	2.181	0.298	0.987	1.000
Additive model															
rs6518591	0.815	0.033	0.818	1.000	1.000	0.751	−0.056	0.747	1.000	1.000	0.800	−0.463	0.801	1.000	1.000
rs737866	0.092	−0.228	0.090	0.650	1.000	0.372	−0.150	0.369	0.992	1.000	**0.044**	3.462	**0.043**	0.724	1.000
rs737865	0.088	−0.230	0.086	0.638	1.000	0.316	−0.168	0.313	0.981	1.000	**0.039**	3.563	**0.037**	0.692	1.000
rs1544325	0.547	0.067	0.543	1.000	1.000	0.554	0.082	0.550	1.000	1.000	0.138	−2.107	0.135	0.657	1.000
rs8185002	0.071	−0.246	0.069	0.558	1.000	0.447	−0.129	0.443	0.998	1.000	0.058	3.302	0.057	0.779	1.000
rs174675	0.351	0.109	0.353	0.989	1.000	0.970	−0.005	0.972	1.000	1.000	0.955	−0.085	0.954	1.000	1.000
rs5993882	0.421	0.108	0.422	0.998	1.000	0.827	0.037	0.821	1.000	1.000	0.308	−1.772	0.305	1.000	1.000
rs740603	0.288	−0.119	0.284	0.971	1.000	0.736	0.047	0.732	1.000	1.000	0.917	0.151	0.916	1.000	1.000
rs4646312	0.561	0.068	0.549	1.000	1.000	**0.036**	0.307	**0.040**	0.352	1.000	0.297	−1.571	0.293	0.523	1.000
rs4633	0.300	0.114	0.308	0.977	1.000	0.156	0.193	0.154	0.830	1.000	0.943	−0.099	0.941	0.997	1.000
rs2239393	0.813	−0.030	0.811	1.000	1.000	**0.026**	0.354	**0.029**	0.271	1.000	0.305	−1.716	0.305	0.442	1.000
rs4818	0.542	0.072	0.532	1.000	1.000	**0.033**	0.312	**0.037**	0.327	1.000	0.305	−1.545	0.302	0.525	1.000
rs4680	0.257	0.125	0.263	0.954	1.000	0.127	0.207	0.125	0.762	1.000	0.981	−0.033	0.980	0.997	1.000
rs4646316	0.549	0.079	0.543	1.000	1.000	0.078	0.288	0.078	0.582	1.000	0.156	−2.380	0.157	0.382	1.000
rs165774	0.015	−0.312	0.013	0.176	1.000	0.117	−0.251	0.120	0.734	1.000	0.185	2.178	0.184	0.734	1.000
rs174696	0.057	0.213	0.058	0.486	1.000	0.976	−0.004	0.975	1.000	1.000	0.927	0.131	0.926	1.000	1.000
rs9306235	0.441	0.173	0.446	0.998	1.000	**0.034**	0.609	**0.035**	0.335	1.000	0.228	−3.553	0.227	0.160	1.000
rs9332377	0.977	0.005	0.975	1.000	1.000	0.370	0.184	0.370	0.992	1.000	0.358	−1.933	0.363	0.997	1.000
rs165599	0.345	0.111	0.353	0.988	1.000	0.780	0.041	0.776	1.000	1.000	0.614	0.760	0.614	0.994	1.000
rs887199	0.414	0.109	0.422	0.997	1.000	0.590	−0.089	0.585	1.000	1.000	0.163	2.372	0.163	0.762	1.000
rs2518824	0.203	0.368	0.213	0.906	1.000	0.670	−0.155	0.667	1.000	1.000	0.936	−0.300	0.937	0.996	1.000
rs887200	0.410	0.120	0.419	0.996	1.000	0.376	−0.159	0.372	0.993	1.000	0.094	3.094	0.092	0.513	1.000
Recessive model															
rs6518591	0.867	0.070	0.872	1.000	1.000	0.124	−0.848	0.124	0.828	1.000	0.141	8.320	0.137	0.872	1.000
rs737866	0.824	−0.090	0.830	1.000	1.000	0.678	−0.202	0.675	1.000	1.000	0.274	5.457	0.266	0.986	1.000
rs737865	0.835	−0.080	0.839	1.000	1.000	0.695	−0.191	0.694	1.000	1.000	0.280	5.398	0.269	0.988	1.000
rs1544325	0.618	0.090	0.609	1.000	1.000	0.211	−0.284	0.212	0.960	1.000	0.723	0.827	0.721	1.000	1.000

(*Continued*)

Table 3. (*Continued*)

	Heat Pain Intensity, 48°C					Cold Pain Intensity, NRS at 15 s					Cold Withdrawal Time				
SNP	Naive *P* Value	β	Perm *P* Value	SNP Cor *P* Value	Cor *P* Value	Naive *P* Value	β	Perm *P* Value	SNP Cor *P* Value	Cor *P* Value	Naive *P* Value	β	Perm *P* Value	SNP Cor *P* Value	Cor *P* Value
rs8185002	0.772	–0.120	0.787	1.000	1.000	0.907	–0.059	0.909	1.000	1.000	0.397	4.414	0.395	0.999	1.000
rs174675	0.502	0.180	0.496	1.000	1.000	0.194	–0.425	0.199	0.946	1.000	0.104	5.446	0.103	0.777	1.000
rs5993882	0.997	0.000	0.996	1.000	1.000	0.087	–0.843	0.090	0.714	1.000	0.212	6.313	0.214	0.960	1.000
rs740603	0.470	–0.150	0.460	1.000	1.000	0.688	–0.108	0.698	1.000	1.000	0.856	0.502	0.852	1.000	1.000
rs4646312	0.424	0.210	0.434	0.999	1.000	**0.030**	0.716	**0.028**	0.355	1.000	**0.017**	–8.114	**0.018**	0.203	1.000
rs4633	0.547	0.110	0.556	1.000	1.000	0.135	0.354	0.133	0.854	1.000	0.335	–2.345	0.333	0.996	1.000
rs2239393	0.500	0.180	0.503	1.000	1.000	**0.018**	0.802	**0.018**	0.230	1.000	**0.007**	–9.693	**0.006**	0.082	1.000
rs4818	0.438	0.200	0.447	0.999	1.000	**0.032**	0.709	**0.029**	0.369	1.000	**0.017**	–8.068	**0.019**	0.209	1.000
rs4680	0.543	0.120	0.548	1.000	1.000	0.115	0.373	0.113	0.803	1.000	0.364	–2.213	0.360	0.998	1.000
rs4646316	0.657	0.160	0.657	1.000	1.000	**0.040**	0.903	**0.046**	0.446	1.000	**0.021**	–10.470	**0.020**	0.240	1.000
rs165774	0.816	0.080	0.811	1.000	1.000	0.254	–0.491	0.256	0.980	1.000	0.808	1.075	0.808	1.000	1.000
rs174696	0.034	0.480	0.036	0.380	1.000	0.689	0.113	0.691	1.000	1.000	0.688	–1.162	0.689	1.000	1.000
rs9306235	0.832	0.250	0.826	1.000	1.000	0.809	–0.482	0.810	1.000	1.000	0.654	–9.193	0.686	1.000	1.000
rs9332377	0.663	0.290	0.661	1.000	1.000	0.628	0.414	0.632	1.000	1.000	0.280	–9.484	0.285	0.988	1.000
rs165599	0.112	0.410	0.110	0.789	1.000	0.245	0.370	0.236	0.977	1.000	0.541	–2.003	0.552	1.000	1.000
rs887199	0.421	–0.310	0.421	0.999	1.000	0.386	–0.406	0.396	0.999	1.000	0.093	8.049	0.093	0.734	1.000
rs2518824	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
rs887200	0.170	–0.640	0.171	0.912	1.000	**0.004**	–1.624	**0.004**	0.067	1.000	**0.013**	14.550	**0.015**	0.163	1.000

Naive *P* values are based on linear regression analysis. Variables have been adjusted for age, BMI, anxiety (STAI score), and presence of preoperative chronic pain condition. Bold figures indicate *P* value < 0.05.

BMI = body mass index; *COMT* = catechol-o-methyltransferase; NA = not available; NRS = numerical rating scale; SNP = single nucleotide polymorphism; STAI = state and trait anxiety inventory.

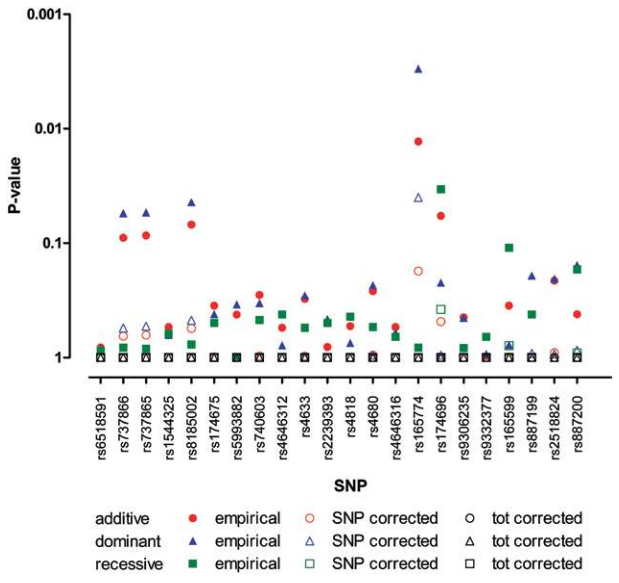

Fig. 2. Association results between the studied catechol-o-methyltransferase (*COMT*) gene single nucleotide polymorphisms (SNPs) and heat pain intensity (48°C). Additive, dominant, and recessive models were used, and both uncorrected (empirical) and experimental-wise corrected (by the number of SNPs tested) *P* values as well as *P* values corrected with all performed tests (tot corrected) are shown. The analyses were performed using variables adjusted for potential confounding factors (age, body mass index, presence of any preoperative chronic pain condition, and anxiety score).

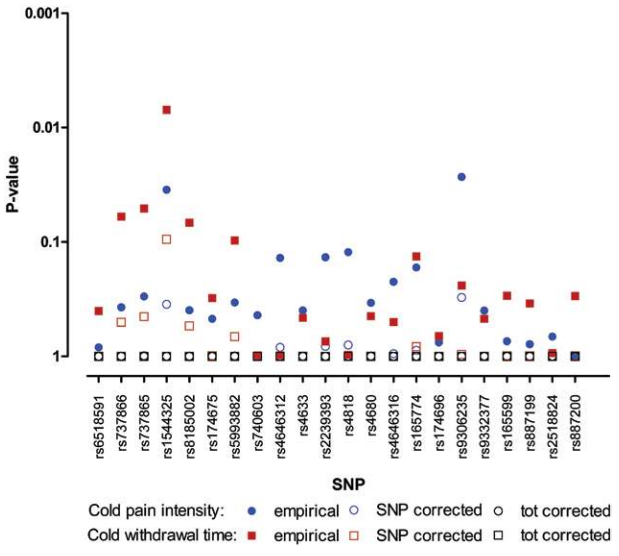

Fig. 3. Association results between the studied catechol-o-methyltransferase (*COMT*) gene single nucleotide polymorphisms (SNPs) and two cold pressure pain phenotypes (pain intensity at 15 s and cold withdrawal time). Results are based on the dominant model, and both uncorrected (empirical) and experimental-wise corrected (by the number of SNPs tested) *P* values as well as *P* values corrected with all performed tests (tot corrected) are shown. The analyses were performed using variables adjusted for potential confounding factors (age, body mass index, presence of any preoperative chronic pain condition, and anxiety score).

mean NRS of the patients homozygous for the major allele (G) of this SNP (56%) was 3.72 and the mean NRS of the heterozygous individuals (38%) was 3.20 whereas that of the individuals homozygous for the minor allele (5%) was 3.48.

Four to five SNPs, depending on the model used, showed nominal association with cold pain (table 3 and figs. 3 and 4). The strongest signal was seen between SNP rs887200 located within the 3′ prime region of the gene and the NRS score at 15 s using a recessive model. The uncorrected empirical *P* value was 0.004, but the association, however, did not sustain stringent multiple testing corrections (table 3). This SNP was relatively rare, only 25 of the 900 patients tested for the cold pain were homozygous for the minor allele (C). The regression coefficient for this SNP was −1.62 meaning that the homozygous individuals reported in average 1.6 points lower NRS values compared with the other groups. In a follow-up analysis, C/C-carriers reported lower pain intensity scores than other patients (two-way ANOVA for repeated measurements, effect of genotype: $P < 0.0001$), and the effect of rs887200 was consistent across all measured time points from 15 to 90 s as there was no time–genotype interaction ($P > 0.05$; fig. 4). Furthermore, C/C-carriers tolerated cold stimulation longer as compared with noncarriers ($P = 0.005$; fig. 5).

Of the other SNPs showing nominal evidence of association with cold pain, rs1544325 is of interest since using the dominant model it was associated with both of the cold pain–related phenotypes tested. The smallest uncorrected empirical

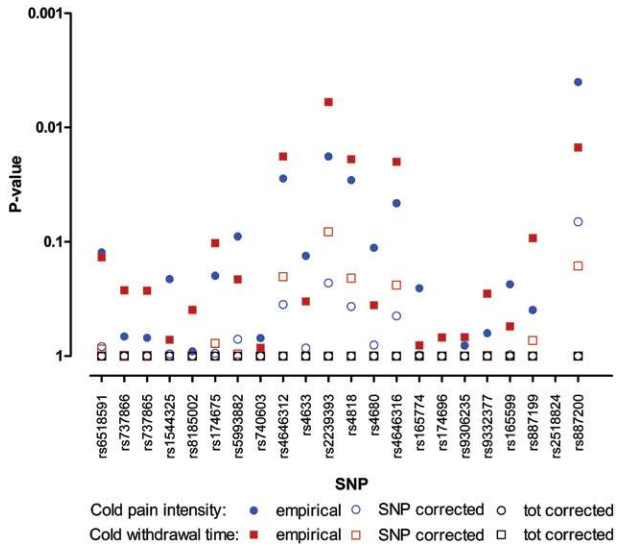

Fig. 4. Association results between the studied catechol-o-methyltransferase (*COMT*) gene single nucleotide polymorphisms (SNPs) and two cold pressure pain phenotypes (pain intensity at 15 s and cold withdrawal time). Results are based on the recessive model, and both uncorrected (empirical) and experimental-wise corrected (by the number of SNPs tested) *P* values as well as *P* values corrected with all performed tests (tot corrected) are shown. The analyses were performed using variables adjusted for potential confounding factors (age, body mass index, presence of any preoperative chronic pain condition, and anxiety score).

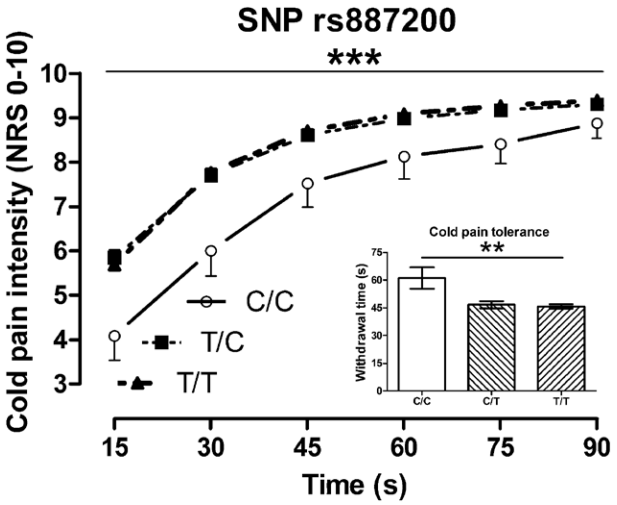

Fig. 5. The mean (± SEM) cold pain intensity ratings (numerical rating scale [NRS]) over time and the mean cold withdrawal times are shown in individuals who were homozygous for the single nucleotide polymorphism (SNP) rs887200 minor allele (C) compared with those who had one or two copies of the major allele (T). *Asterisks* indicate a difference between the genotype groups (two-way ANOVA for repeated measurements, effect of genotype, ***$P < 0.0001$; Student unpaired one-tailed *t* test, ** $P < 0.01$).

P value of 0.007 (β = 6.11) was reached when association with cold pain tolerance (time to withdrawal) was tested (table 3).

Postoperative Pain and Oxycodone Consumption

Association between the *COMT* SNPs and several postoperative pain variables was studied. Three SNPs, rs4646312, rs2239393, and rs4818, all located within the same (middle) haploblock gave uncorrected empirical *P* values in the range of 0.008–0.01 for the phenotype "pain intensity during motion when the patient needed the first dose of oxycodone" using the dominant model (table 1, see Supplemental Digital Content 1, http://links.lww.com/ALN/B3). After multiple testing corrections, no evidence of association remained. In all cases, the minor allele (C, G, and G, respectively) carriers had higher postoperative pain scores than the patients being homozygous for the major allele (β = 0.25–0.28).

None of the studied SNPs showed any evidence of association ($P < 0.05$) with the other postoperative phenotypes, that is, the amount of oxycodone needed to achieve adequate analgesia and total oxycodone consumption. Results for the total oxycodone consumption are presented in table 2, Supplemental Digital Content 1, http://links.lww.com/ALN/B3.

Haplotype Analyses

In addition to single-SNP analysis, association between the pain phenotypes and the haplotypes constructed both based on the LD structure and previous literature was studied. Of the three haploblocks formed (fig. 1), blocks 1 and 2 consisted of seven SNPs (from rs6518591 to rs5993882 and from rs4646312 to 165774) and block 3 of five SNPs (from rs9332377 to

rs887200). Within block 1, five haplotypes having a frequency above 1% could be constructed, whereas six haplotypes were identified within blocks 2 and 3 (table 2, see Supplemental Digital Content 1, http://links.lww.com/ALN/B3).

The strongest haplotype-association signal for experimental pain phenotypes was seen between heat pain intensity and haplotype TTACACA of block 2 (uncorrected *P* value 0.014). Carriers of this haplotype reported less heat pain (β = −0.31) compared with the carriers of all the other haplotypes. This haplotype has a frequency of 24% and was the only one of the six detected haplotypes containing the minor allele (A) of the SNP rs165774 associating with decreased heat pain sensitivity in single-SNP analyses as well. None of the haplotypes showed evidence of association with cold pain phenotypes. These results are presented in table 2, Supplemental Digital Content 1, http://links.lww.com/ALN/B3.

None of the haplotypes showed evidence of association with the phenotypes related to postoperative pain or oxycodone consumption.

In the studies by Diatchenko *et al.*,[19,20] haplotypes consisting of three to four SNPs all located within our haploblock 2 have been suggested to have a significant effect on pain perception. To test this specific hypothesis, we constructed these haplotypes classified in the original study as LPS, average pain sensitivity, and high pain sensitivity haplotypes and tested association. Haplotype LPS (alleles CGG) was nominally associated with cold pain measured by NRS at 15 s ($P = 0.031$) and with postoperative pain ($P = 0.045$). However, the effect was opposite to what was expected based on the previous studies because the carriers of the LPS haplotype had a higher pain score than the others (β = 0.31 and 0.14, respectively), and after experimental-wise corrections, the *P* values were not significant. None of the LPS/high pain sensitivity/average pain sensitivity haplotypes showed any evidence of association to the other studied variables.

In Silico *eQTL Analysis*

The possible functional significance of the SNPs showing statistically significant evidence of association with pain phenotypes in this study (rs165744 and rs887200) was assessed analysing the association between the genotypes of these SNPs and the *COMT*-expression levels using the GENEVAR database. These SNPs did not show any significant effect on the expression levels of *COMT* in any of the three datasets available.

Discussion

A few variants of the *COMT* gene have been much studied in relation to various aspects of pain in fairly small patient samples. The current study is unique in having analyzed 22 different SNPs in a large and homogenous patient cohort with rich phenotypic data. The main finding of our study is that there was no association between the most studied SNP rs4680 ($Val^{108/158}Met$) and any of the studied pain phenotypes, whereas the strongest association signals were seen between experimental pain and *COMT* SNPs rs887200 and rs165774. These associations have not been described before.

Experimental Pain

SNP rs4680 ($Val^{108/158}Met$), which modulates COMT activity, has been examined in most detail to date. No evidence of association between this SNP and the studied experimental pain phenotypes was seen in our study in agreement with the work by Kim *et al.*[5] Our study was well powered to detect an association for this SNP had there been any. Any effect of rs4680 has been shown mainly in settings applying nociceptive stimuli of higher intensity and longer duration.[9,19,32] It is possible that the effect is present only after a more intense stimulation than what was used in our study or in certain specific aspects of pain such as the rate of temporal summation of heat pain.[19] It has also been suggested that the effect of rs4680 may depend on the genomic environment, for example, haplotype or cooccurring rare variants.[19,20,33]

Interesting evidence has also been provided for an association between pain sensitivity and certain *COMT* haplotypes (LPS/average pain sensitivity/high pain sensitivity) containing the rs4680.[19,20] Our results, however, did not support these findings. There are several possible reasons for this lack of replication. The experimental pain phenotypes examined are rather different between the studies. In the original studies, a composite phenotype consisting of 16 parameters was analyzed.[20] The strongest associations were seen for thermal pain, which was the only modality also assessed in our study.[19] Its parameters, however, were different from those used in our study, and we did not assess heat pain threshold or tolerance. There are also other important differences as we studied breast cancer patients of whom approximately 25% had some previous chronic pain condition. Also, it should be noted that the sample sizes of the earlier studies are rather modest which could increase the risk of false-positive findings.

In our study, the strongest association was seen between heat pain intensity (48°C) and SNP rs165774 (uncorrected $P = 0.003$). Subjects carrying the A-allele showed lower pain intensity (mean NRS: G/A, 3.20; A/A, 3.48) than homozygous G-carriers (NRS 3.72). This result remained significant after correcting for the number of SNPs ($P < 0.05$) but did not sustain more stringent corrections. Rs165774 is located in the intronic region between exons 5 and 6 and is part of haploblock 2. Interestingly, a preliminary report showing association of rs165774 with temporomandibular dysfunction suggests that it might be important for the expression of an alternatively spliced, truncated COMT isoform showing differential expression pattern and higher efficiency in metabolizing norepinephrine.##

Regarding the cold pain phenotypes, the strongest association in our study was detected between SNP rs887200

90th General Session and Exhibition of the International Association of Dental Research, Iguaçu Falls, Brazil. Meloto C, Segall SK, Gauthier J, Rizzatti-Barbosa CM, Diatchenko L: Characterizing a novel COMT isoform: Potential implication on TMD. J Dent Res 2012; 91(Spec Iss B):abstract 968. Available at: https://iadr.confex.com/iadr/2012rio/webprogram/Paper166239.html, www.dentalresearch.org. Accessed October 29, 2013.

and cold pain intensity. Homozygous minor allele (C/C) carriers were less sensitive to pain (mean NRS, 4.1) than the subjects having other genotypes (mean NRS, 5.8 and 5.7, respectively). The magnitude of the effect of rs887200 in our study was greater than that of other *COMT* SNPs, including several SNPs which have shown associations in earlier studies. This result did not remain significant after multiple testing corrections either. However, in the follow-up analysis taking into account individual nociceptive baselines as well as responses over the whole 90 s test, the effect of rs887200 was consistent across all measured time points ($P < 0.0001$) and C/C-carriers also tolerated cold stimulation longer as compared with noncarriers ($P = 0.005$; fig. 5).

The effect of rs887200 on pain sensitivity has not been studied before. It is located within the 3′ region of the gene, but it is also intragenic to a neighboring gene, *ARVCF* (armadillo repeat protein deleted in velocardiofacial syndrome). Other *COMT* 3′-UTR SNPs have been suggested to contribute to human neuropsychiatric phenotypes.[34,35] Furthermore, in a recent animal study, eQTL analysis showed that in inbred mouse strains, COMT activity and related behavioral phenotypes are mainly regulated by a transposon located in 3′-UTR.[36] Also in humans, one 3′-UTR SNP associated with neuropsychiatric phenotypes was connected to altered *COMT* expression.[11] Regardless of the origin, the mechanisms of action of 3′-UTR variants are less evident because they do not alter the amino acid sequence of the protein. Possible mechanisms include attenuation of interaction of 3′-UTR area with negative modulators of expression such as microRNAs[37] with a consequent increase in expression. The effect of rs887200 on COMT activity remains to be studied. The *in silico* eQTL searches did not provide evidence of this SNP affecting the *COMT*-expression levels significantly in the cell types available.

Postoperative Pain and Oxycodone Consumption

None of the SNPs showed significant association with the acute postoperative pain measures. Because of previous findings, SNP rs4818 was of special interest. This SNP as well as two other SNPs within the same haploblock, rs4646312 and rs2239393, showed nominal association (uncorrected $P = 0.008–0.01$). The carriers of the rarer alleles of these SNPs (rs4646312C, rs2239393G, and rs4818G) needed the first dose of oxycodone at higher postoperative pain scores during motion than the patients being homozygous for the major allele. In earlier studies, rs4818 has been associated with decreased experimental and postoperative pain intensity.[19,21] Also, *COMT* SNP rs740603 in intron 1 has been previously suggested to be associated with maximum postoperative pain ratings.[6] However, we were unable to replicate this finding.

Both the Val$^{108/158}$Met variant and a *COMT* haplotype carrying the Met allele have been associated with variation in the efficacy of morphine in cancer pain treatment.[8,38] However, no effect of Val$^{108/158}$Met on opioid analgesia was seen in a larger but highly variable cohort of pain patients.[39] In our study, no evidence of association with postoperative oxycodone consumption was seen even after adjusting the doses with age, body mass index, type of surgery, and other potential confounding factors.

Methodological Considerations

When multiple hypotheses are being tested, the risk of type I error and false-positive associations increases. This should be addressed by correcting for the multiple tests performed as was done in this study. We not only took into account the number of independent SNPs tested but also corrected for the number of phenotypes and statistical models used. Unfortunately, these very stringent correction methods also increase the probability of type II error (failure to reject the null hypothesis when it is false). We believe that it is possible and even likely that some of the SNPs showing border-line *P* values in this study have a true effect, but our study, even though it has 1,000 patients, is still underpowered to detect a statistically significant association. It becomes practically impossible to collect any larger, homogenous, and phenotypically well-characterized samples. With this sample size, the effect of the variant should be extremely strong for the results to remain statistically significant after multiple testing. Especially, regarding rare variants, the number of carriers can be small leaving effect sizes of these rare variants insufficient to reach the level of statistical significance.

It is commonly accepted that when replicating earlier findings, the criteria needed for evidence of association can be less stringent. However, in case of true replication also the direction of the effect (the risk allele) has to be in accordance with earlier findings. None of the replication efforts done in this study met these two criteria. In general, lack of replication of association signals that are based on candidate gene studies has resulted in the current wave of interest in genome-wide association studies where no prior hypothesis is needed.

In an ideal situation, the effects of several cooccurring SNPs can be simultaneously examined. From a statistical point of view, simultaneous assessment and testing of multiple SNPs (and phenotypes) are, however, difficult. Currently, evaluating *COMT* haplotypes appears to be the best way to assess the effect of COMT on pain and to consider multiple *COMT* SNPs at the same time. This method was used in our study as well, but the association signals seen were not more significant than the ones achieved by analyzing individual SNPs.

Our study population consists of patients having breast cancer. This can be regarded as a limitation because there are also some studies reporting associations between *COMT* variants and breast cancer.[13,40,41] Theoretically, breast cancer risk variants could be overrepresented among our patients. Also, approximately 25% of the patients reported chronic pain and some high anxiety, which sensitize to acute pain. Nevertheless, this was taken into account by adjusting the data for these variables.

Conclusions

Of the 22 *COMT* SNPs studied, 3′-UTR SNP rs887200 and intronic rs165774, whose effect on pain have not been described before, showed strongest evidence of association to pain sensitivity. Their association with human pain phenotypes should be replicated in other cohorts. Our results provide basis for future studies on the mechanism of action and effects of COMT activity.

This study was initiated in collaboration with the late K. Steven LaForge, Ph.D., Senior Research Fellow, Institute of Molecular Medicine, University of Helsinki, Helsinki, Finland; the authors thank his enthusiasm and expert knowledge. The authors are indebted to Eija R. Ruoppa (R.N.) and Minna Kaiponen (R.N.), Department of Anesthesia, Helsinki University Central Hospital, Helsinki, Finland, for excellent assistance throughout the project.

References

1. Männistö PT, Kaakkola S: Catechol-O-methyltransferase (COMT): Biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol Rev 1999; 51:593–628
2. Boudíková B, Szumlanski C, Maidak B, Weinshilboum R: Human liver catechol-O-methyltransferase pharmacogenetics. Clin Pharmacol Ther 1990; 48:381–9
3. Weinshilboum RM, Raymond FA: Inheritance of low erythrocyte catechol-o-methyltransferase activity in man. Am J Hum Genet 1977; 29:125–35
4. Akil M, Kolachana BS, Rothmond DA, Hyde TM, Weinberger DR, Kleinman JE: Catechol-O-methyltransferase genotype and dopamine regulation in the human brain. J Neurosci 2003; 23:2008–13
5. Kim H, Mittal DP, Iadarola MJ, Dionne RA: Genetic predictors for acute experimental cold and heat pain sensitivity in humans. J Med Genet 2006; 43:e40
6. Kim H, Lee H, Rowan J, Brahim J, Dionne RA: Genetic polymorphisms in monoamine neurotransmitter systems show only weak association with acute post-surgical pain in humans. Mol Pain 2006; 2:24
7. Oswald LM, McCaul M, Choi L, Yang X, Wand GS: Catechol-O-methyltransferase polymorphism alters hypothalamic-pituitary-adrenal axis responses to naloxone: A preliminary report. Biol Psychiatry 2004; 55:102–5
8. Rakvåg TT, Klepstad P, Baar C, Kvam TM, Dale O, Kaasa S, Krokan HE, Skorpen F: The Val158Met polymorphism of the human catechol-O-methyltransferase (COMT) gene may influence morphine requirements in cancer pain patients. Pain 2005; 116:73–8
9. Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y, Koeppe RA, Stohler CS, Goldman D: COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. Science 2003; 299:1240–3
10. Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melén K, Julkunen I, Taskinen J: Kinetics of human soluble and membrane-bound catechol-O-methyltransferase: A revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry 1995; 34:4202–10
11. Bray NJ, Buckland PR, Williams NM, Williams HJ, Norton N, Owen MJ, O'Donovan MC: A haplotype implicated in schizophrenia susceptibility is associated with reduced COMT expression in human brain. Am J Hum Genet 2003; 73:152–61
12. Dempster EL, Mill J, Craig IW, Collier DA: The quantification of COMT mRNA in post mortem cerebellum tissue: Diagnosis, genotype, methylation and expression. BMC Med Genet 2006; 7:10
13. Ji Y, Olson J, Zhang J, Hildebrandt M, Wang L, Ingle J, Fredericksen Z, Sellers T, Miller W, Dixon JM, Brauch H, Eichelbaum M, Justenhoven C, Hamann U, Ko Y, Brüning T, Chang-Claude J, Wang-Gohrke S, Schaid D, Weinshilboum R: Breast cancer risk reduction and membrane-bound catechol-O-methyltransferase genetic polymorphisms. Cancer Res 2008; 68:5997–6005
14. Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR: Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): Effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 2004; 75:807–21
15. Tsao D, Shabalina SA, Gauthier J, Dokholyan NV, Diatchenko L: Disruptive mRNA folding increases translational efficiency of catechol-O-methyltransferase variant. Nucleic Acids Res 2011; 39:6201–12
16. Lee SG, Joo Y, Kim B, Chung S, Kim HL, Lee I, Choi B, Kim C, Song K: Association of Ala72Ser polymorphism with COMT enzyme activity and the risk of schizophrenia in Koreans. Hum Genet 2005; 116:319–28
17. Kambur O, Männistö PT: Catechol-O-methyltransferase and pain. Int Rev Neurobiol 2010; 95:227–79
18. van Meurs JB, Uitterlinden AG, Stolk L, Kerkhof HJ, Hofman A, Pols HA, Bierma-Zeinstra SM: A functional polymorphism in the catechol-O-methyltransferase gene is associated with osteoarthritis-related pain. Arthritis Rheum 2009; 60:628–9
19. Diatchenko L, Nackley AG, Slade GD, Bhalang K, Belfer I, Max MB, Goldman D, Maixner W: Catechol-O-methyltransferase gene polymorphisms are associated with multiple pain-evoking stimuli. Pain 2006; 125:216–24
20. Diatchenko L, Slade GD, Nackley AG, Bhalang K, Sigurdsson A, Belfer I, Goldman D, Xu K, Shabalina SA, Shagin D, Max MB, Makarov SS, Maixner W: Genetic basis for individual variations in pain perception and the development of a chronic pain condition. Hum Mol Genet 2005; 14:135–43
21. Lee PJ, Delaney P, Keogh J, Sleeman D, Shorten GD: Catecholamine-o-methyltransferase polymorphisms are associated with postoperative pain intensity. Clin J Pain 2011; 27:93–101
22. Kaunisto MA, Jokela R, Tallgren M, Kambur O, Tikkanen E, Tasmuth T, Sipilä R, Palotie A, Estlander A-M, Leidenius M, Ripatti S, Kalso EA: Pain in 1,000 women treated for breast cancer: A prospective study of pain sensitivity and postoperative pain. Anesthesiology 2013; 119:1410–21
23. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J: An inventory for measuring depression. Arch Gen Psychiatry 1961; 4:561–71
24. Spielberger CD, Gorsuch RL, Lushane PR, Vagg PR, Jacobs AG: Manual for the State-Trait Anxiety Inventory (Form Y). Palo Alto, Consulting Psychologists Press, Inc., 1983
25. Barrett JC, Fry B, Maller J, Daly MJ: Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21:263–5
26. Jurinke C, van den Boom D, Cantor CR, Köster H: Automated genotyping using the DNA MassArray technology. Methods Mol Biol 2002; 187:179–92
27. Lahermo P, Liljedahl U, Alnaes G, Axelsson T, Brookes AJ, Ellonen P, Groop PH, Halldén C, Holmberg D, Holmberg K, Keinänen M, Kepp K, Kere J, Kiviluoma P, Kristensen V, Lindgren C, Odeberg J, Osterman P, Parkkonen M, Saarela J, Sterner M, Strömqvist L, Talas U, Wessman M, Palotie A, Syvänen AC: A quality assessment survey of SNP genotyping laboratories. Hum Mutat 2006; 27:711–4
28. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC: PLINK: A tool set for whole-genome association and

population-based linkage analyses. Am J Hum Genet 2007; 81:559–75

29. Stranger BE, Montgomery SB, Dimas AS, Parts L, Stegle O, Ingle CE, Sekowska M, Smith GD, Evans D, Gutierrez-Arcelus M, Price A, Raj T, Nisbett J, Nica AC, Beazley C, Durbin R, Deloukas P, Dermitzakis ET: Patterns of cis regulatory variation in diverse human populations. PLoS Genet 2012; 8:e1002639
30. Nica AC, Parts L, Glass D, Nisbet J, Barrett A, Sekowska M, Travers M, Potter S, Grundberg E, Small K, Hedman AK, Bataille V, Tzenova Bell J, Surdulescu G, Dimas AS, Ingle C, Nestle FO, di Meglio P, Min JL, Wilk A, Hammond CJ, Hassanali N, Yang TP, Montgomery SB, O'Rahilly S, Lindgren CM, Zondervan KT, Soranzo N, Barroso I, Durbin R, Ahmadi K, Deloukas P, McCarthy MI, Dermitzakis ET, Spector TD; MuTHER Consortium: The architecture of gene regulatory variation across multiple human tissues: The MuTHER study. PLoS Genet 2011; 7:e1002003
31. Dimas AS, Deutsch S, Stranger BE, Montgomery SB, Borel C, Attar-Cohen H, Ingle C, Beazley C, Gutierrez Arcelus M, Sekowska M, Gagnebin M, Nisbett J, Deloukas P, Dermitzakis ET, Antonarakis SE: Common regulatory variation impacts gene expression in a cell type-dependent manner. Science 2009; 325:1246–50
32. Schmahl C, Ludäscher P, Greffrath W, Kraus A, Valerius G, Schulze TG, Treutlein J, Rietschel M, Smolka MN, Bohus M: COMT val158met polymorphism and neural pain processing. PLoS One 2012; 7:e23658
33. Kim H, Neubert JK, San Miguel A, 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
34. Stein MB, Fallin MD, Schork NJ, Gelernter J: COMT polymorphisms and anxiety-related personality traits. Neuropsychopharmacology 2005; 30:2092–102
35. Shifman S, Bronstein M, Sternfeld M, Pisanté-Shalom A, Lev-Lehman E, Weizman A, Reznik I, Spivak B, Grisaru N, Karp L, Schiffer R, Kotler M, Strous RD, Swartz-Vanetik M, Knobler HY, Shinar E, Beckmann JS, Yakir B, Risch N, Zak NB, Darvasi A: A highly significant association between a COMT haplotype and schizophrenia. Am J Hum Genet 2002; 71:1296–302
36. Segall SK, Nackley AG, Diatchenko L, Lariviere WR, Lu X, Marron JS, Grabowski-Boase L, Walker JR, Slade G, Gauthier J, Bailey JS, Steffy BM, Maynard TM, Tarantino LM, Wiltshire T: Comt1 genotype and expression predicts anxiety and nociceptive sensitivity in inbred strains of mice. Genes Brain Behav 2010; 9:933–46
37. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB: Prediction of mammalian microRNA targets. Cell 2003; 115:787–98
38. Rakvåg TT, Ross JR, Sato H, Skorpen F, Kaasa S, Klepstad P: Genetic variation in the catechol-O-methyltransferase (COMT) gene and morphine requirements in cancer patients with pain. Mol Pain 2008; 4:64
39. Lötsch J, von Hentig N, Freynhagen R, Griessinger N, Zimmermann M, Doehring A, Rohrbacher M, Sittl R, Geisslinger G: Cross-sectional analysis of the influence of currently known pharmacogenetic modulators on opioid therapy in outpatient pain centers. Pharmacogenet Genomics 2009; 19:429–36
40. Gaudet MM, Chanock S, Lissowska J, Berndt SI, Peplonska B, Brinton LA, Welch R, Yeager M, Bardin-Mikolajczak A, Garcia-Closas M: Comprehensive assessment of genetic variation of catechol-O-methyltransferase and breast cancer risk. Cancer Res 2006; 66:9781–5
41. Peterson NB, Trentham-Dietz A, Garcia-Closas M, Newcomb PA, Titus-Ernstoff L, Huang Y, Chanock SJ, Haines JL, Egan KM: Association of COMT haplotypes and breast cancer risk in caucasian women. Anticancer Res 2010; 30:217–20