

Supporting Information:

Study population

Subjects for the baseline analysis were 202 healthy pain-free females aged 18-34 years who provided a blood sample and consent for genotyping from among a larger cohort of 244 females who volunteered for a research project. Volunteers were recruited between April 1998 and March 2000 using advertisements placed in local newspapers in the Raleigh-Durham-Chapel Hill area of central North Carolina (Table S1). The advertisements explained that healthy females were sought for an ongoing prospective study designed to examine and identify factors that influence sensory perception. No treatment was offered.

Females who responded to advertisements completed a comprehensive medical history to rule out any previous or current history of pain-related disorder or other medical related conditions that might alter their pain perception. They also attended the UNC School of Dentistry where they completed a series of pain perception assessments and underwent a physical examination. All subjects underwent pain perception assessments as described below.

The study was conducted with both written and verbal informed consent using protocols reviewed and approved by the UNC School of Dentistry's Committee on Investigations Involving Human Subjects. Subjects were paid up to \$300 for their participation on a sliding scale depending on the number of visits they completed.

Baseline characteristics of subjects and consent for genotyping: At baseline, 244 females volunteered to take part in the study and completed baseline assessments. 202 (83%) of them also provided a blood sample and written consent for genotyping. The percentage genotyped did not differ among sociodemographic subgroups of age, race, education or marital status (Table S1). There were marginal differences ($P=0.07$) in the percent genotyped among tertiles of baseline summary pain zscore (see below), although the trend was uniform: those who were least pain responsive were most likely to be genotyped (90% of subjects in the lowest quartile) followed by the most pain responsive (81% of subject in the highest quartile) while those with the mid-tertile of pain responsiveness were least likely to be genotyped (77%).

Pain Perception Assessments

All pain measurements, with the exception of pressure pain measures, were performed during the follicular phase of the subject's menstrual cycle between days 3-10 where day 0 represents the onset of menstruation. The reason for this was to control for the modest effects of menstrual cycle on pain sensitivity(1). Pressure pain thresholds were taken at a separate session approximately 1 week prior to thermal and ischemic pain assessments. All subjects were asked to refrain from consuming over-the-counter pain relieving medications for at least 48 hours before visiting the laboratory and all subjects were free of prescription pain medications for at least two weeks prior to sensory testing.

Pressure pain thresholds: Pressure pain thresholds were assessed over the right and left temporalis muscles, masseter muscles, temporomandibular joints, and ventral surfaces of the wrists with a hand-held pressure algometer (Pain Diagnosis and Treatment, Great Neck, NY) using methods similar to those described by Jaeger and Reeves(2). The algometer's tip consisted of a flat 10 mm diameter rubber pad. Pressure stimuli were delivered at an approximate rate of 1 kg/sec. Participants were instructed to signal either verbally or by a hand movement when the pressure sensation first became painful. When this occurred, the stimulus was removed. The pressure pain threshold was defined as the amount of pressure (kg) at which the subjects first

perceived to be painful. The pressure application was not allowed to exceed 6 kg for the wrists and 4 kg for other sites. When those values were attained, the trials were terminated and these values were entered into the calculation for the subject's pressure pain thresholds. One pre-trial assessment was performed at each site followed by two additional assessments. The two values from the right and left sides were then averaged to obtain one pressure pain threshold value per test site, yielding a total of four measures.

Assessment of thermal pain thresholds and tolerances: A modified "Marstock" procedure(6,7) was used to measure thermal pain thresholds and tolerances with a 10 mm diameter computer (486 DOS-based PC) controlled contact thermal stimulator. Thermal stimuli were applied to the skin overlying the right masseter muscle, the skin overlying the right hairy forearm, and the skin overlying the dorsal surface of the right foot. Thermal pain threshold was defined as the temperature (°C) at which the subjects perceived the thermal stimuli as painful, whereas thermal pain tolerance was defined as the temperature (°C) at which the subjects can no longer tolerate the thermal stimulus.

Two separate procedures were used to assess thermal pain thresholds and a third procedure assessed thermal pain tolerance, each at three anatomical sites. *The first set of thermal stimuli* was delivered from a neutral adapting temperature of 32 °C at a rate of 3 °C/sec which has been proposed to produce a relatively selective activation of A δ -fibers (9,10). During this procedure, subjects were instructed to depress a mouse key when they first perceived thermal pain. This caused the thermode to return to the baseline temperature and the reversal temperature was defined as the A δ mediated thermal pain threshold temperature. This procedure was repeated six times and the values from these six trials were averaged to obtain the temperature value of A δ mediated thermal pain threshold. The same procedure was repeated with *a second set of thermal stimuli* delivered at a rate of 0.5 °C/sec. This procedure has been proposed to produce a relatively selective activation of C-fibers (9,10). Finally, C-fiber thermal pain tolerance was determined by using *a third set of thermal stimuli* delivered at the rate of 0.5 °C/sec. Subjects were instructed to depress the mouse key when the probe temperature achieved a level that they could no longer tolerate. The probe temperature was not allowed to exceed 53 °C. When value approximating 53 °C was attained, the trial was terminated and this value was entered into the calculation for the subject's tolerance value. The values obtained from six repeated thermal trials were averaged to obtain a subject's C-fiber thermal pain tolerance value. This yielded nine measures: two threshold measures and one tolerance measure, each at three anatomical sites.

Assessment of temporal summation of C fiber mediated thermal pain: A procedure similar to that described(11) was used to examine the temporal summation of C fiber mediated thermal pain. A total of fifteen 53 °C heat pulses were applied to skin overlying the thenar region of the right hand. Each heat pulse was 1.5 sec in duration and was delivered at a rate of 10 °C/sec from a 40 °C base temperature with an inter-trial interval of 1.5 sec. In effect, this produced a transient 53 °C heat pulse with a peak-to-peak inter-pulse interval of 3 seconds. Subjects were instructed to verbally rate the intensity of each thermal pulse using a 0 to 100 numerical scale with '0' representing 'no sensation', '20' representing 'just painful', and '100' representing 'the most intense pain imaginable'. Subjects were informed that the procedure would be terminated when they reported a value of '100' or when 15 trials had elapsed. For subjects who terminated the procedure prior to the completion of 15 trials, a value of 100 was assigned to the subsequent missing trials. Each subject's ability to summate C-fiber pain was quantified by adding values of all 15 verbal responses. This value was used as a single measurement of the temporal summation of C fiber mediated thermal pain.

Assessment of ischemic pain threshold and tolerance: A modified submaximal effort tourniquet procedure(12) was used to evoke ischemic pain. The subject's right arm was elevated and supported in a vertical position for 30 sec to promote venous drainage. Then, a blood pressure arm cuff positioned above the elbow was inflated to 220 mmHg to abolish arterial blood supply and to render the arm hypoxic. A stopwatch was started at the time of cuff inflation and the subject's arm was then lowered to a horizontal position. Immediately afterwards, the subject started squeezing a handgrip dynamometer at 30% of maximum force of grip for 20 repetitions. Prior to the procedure, the subject's maximum grip strength was determined by having each subject squeeze the dynamometer with 'as much force as possible'. The onset, duration, and magnitude of each handgrip squeeze were signaled by computer-controlled signal lights to ensure standardized compression and relaxation periods. Ischemic pain threshold was determined by recording the time (seconds) when subjects first reported hand or forearm discomfort. Ischemic pain tolerance was determined by recording the time (seconds) when subjects could no longer endure their ischemic arm pain. The tourniquet remained in place for 25 minutes or until pain tolerance had been achieved. This procedure yielded two measures: ischemic pain threshold and ischemic pain tolerance.

Genotyping

Genomic DNA was purified from 202 subjects using QIAampTM 96 DNA Blood Kit (Qiagen, Valencia, CA, USA) and used for either 5' exonuclease or duplex-specific nuclease assays.

5' exonuclease assay: Five COMT SNPs (rs2097903, rs6269, rs4633; rs4818; rs4680 (*val158met*)) were genotyped using the 5' exonuclease assay. Probes and primers were chosen using ProbeITY (Celadon Laboratories, College Park, MD, USA) (Table S2) and were synthesized by Applied Biosystems (Foster City, CA, USA). The PCR reaction mixture consisted of 2.5 µl Master Mixture (Applied Biosystems), 100 nM detection probe for each allele, 900 nM forward and 900 nM reverse amplification primers, and 20 ng genomic DNA in a total reaction volume of 25 µl. Amplification and detection were performed with an ABI Prism 7700 Sequence Detection System. General conditions for TaqManTM PCR were as described(13), the optimized temperatures varied from 60⁰C to 63.5⁰C. The primers and detection probes for each locus are listed in Table S2. Genotype determination was conducted manually using the Sequence Detector 7700 (Applied Biosystems). A verification plate consisting of 17% of the AN probands and control group samples was genotyped in order to assess the reproducibility of the assay. Genotyping error rate was directly determined and was <0.005. Genotype completion rate was 95%.

Duplex-specific nuclease (DSN) assay: SNP rs165599 was genotyped using DSN technique(14). PCR was carry out in 96 well plates using HYBAID termocycler. PCR reactions were performed with the AdvantageTM 2 PCR Kit (Clontech, Palo Alto, CA, USA). Each PCR reaction (25 µl) contained 1 x Advantage 2 Polymerize mix (Clontech), 1 x reaction buffer, 200 µM dNTPs, 0.3 µM each gene-specific primer (Table S2) and 10 ng of genomic DNA. The following PCR conditions and gene-specific primers were employed: 30 PCR cycles (95⁰C for 7 s; 65⁰C for 20 s; 72⁰C for 30 s). A 7 µl aliquot of PCR products containing about 150 ng DNA was mixed with 1.5 µl 10 x DSN buffer (500mM Tris-HCl, pH8.0, 50 mM MgCl₂, 10 mM DTT), probes (to a final concentration of 0.3 µM) (Table S2), 0.75 Kunitz unit DSN nuclease, and milliQ water (to a final volume of 15 µl), and incubated for 20 min at 65⁰C, 40 min 35⁰C. Normalized emission spectra of these samples were obtained on a photofluorometer FFM-01

(Kortek, Moscow, Russia) at 538 nm for green fluorescence, *FAM* (with excitation at 482 nm) and 607 nm for red fluorescence, *TAMRA* (with excitation at 546 nm). Genotyping results for rs165599 were confirmed by restriction analysis. Restriction analysis was carried out on the 500 ng of PCR products using 10 units of *Nae I* restriction enzyme (New England Biolabs, Beverly, MA, USA). The restricted products were analyzed on 2.5% agarose/ethidium bromide gel. One DNA fragment (150 bp) was observed for the homozygous for the alleles *A*, two DNA fragments (76 and 74 bp) – for the homozygous for allele *G* and all three fragments for the heterozygous.

Assessment of COMT activity in different haplotypes.

Transient transfection of COMT cDNA clones: Full-length S-COMT cDNA clones corresponding to HPS, APS and LPS haplotypes were obtained from the IMAGE clone collection (Open Biosystems, Huntsville, AL, USA). Clones BG290167 and BG818517 represented LPS haplotype, clones BI821094 and F037202 represented APS haplotype, and clones BI759217 and BF035214 represented HPS haplotype. All clones contained the first ATG codon and were available in the mammalian expression vector pCMV-SPORT6. Plasmid DNA was purified using the EndoFree Plasmid Maxi purification kit (Qiagen, Germantown, MD, USA). Once plasmids were isolated, DNA sequences were confirmed by double sequencing at the UNC Core Sequencing Facility.

Human embryonic kidney cells (*HEK 293*) were transiently transfected into six-well plates using SuperFect Reagent (Qiagen) in accordance with the manufacture's recommendations. The amount of IMAGE clones was kept at 2 µg/well and to control for the efficiency of transfection pSV-βGalactosidase vector (Promega, Madison, WI, USA) was kept at 0.1 µg. Transfection with a vector with no insert was done for each experiment. Cells lysates were collected approximately 24 hours post-transfection. After removing the media, cells were washed twice with 0.9% saline solution (1 ml/35 mm well) and then covered with deionized water containing 10 mM CDTA (500 µl/35 mm well). The wells were frozen at -80°C overnight. In order to complete the lysing process, HEK 293 cells were pulled into a syringe and passed through a 30 ½ gauge needle into 1.5 ml tubes. The tubes were centrifuged at 2000 g for 10 min and filtrate removed.

Enzymatic assay. The enzymatic COMT assay was based on the method described by Masuda et al(15) . Purified lysates (8 µl) were incubated with 200 µM Sadenosyl-L-methionine (SAME; ICN Chemicals (Aurora OH, USA)), 7.5 mM L-norepinephrine (NE, Sigma Chemical Co. (St. Louis MO, USA) and 2mM MgCl₂ in 50 mM phosphate buffered saline for 60 min in the final volume of 22 µl. The reaction was terminated using 20 µl of 0.4 M hydrochloric acid and 1 µl of 330 mM EDTA. The same reaction in the presence of 15 mM EDTA was carried out in parallel for each lysate to bind Mg⁺² ions that are required for COMT activity. COMT activity was measured with a Normetanephrine (NMN) ELISA kit (IBL, Hamburg, Germany; distributed by IBL-America, Minneapolis, MN) in accordance with the manufacture's recommendations using 10 µl of the above reaction mixture. COMT activity was determined after subtracting the amount of NMN produced by endogenous enzymatic activity (transfection with empty vector) as well as the amount of NMN in transfected cells produced by exogenous COMT activity (enzymatic reaction in the presence of EDTA). COMT activity was then normalized for transfection efficiency by measuring the β-galactosidase activity for each lysate. β-galactosidase activity was determined using a β-galactosidase enzyme systems (Promega), according to the supplier's protocol.

RT-PCR: Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The isolated RNA was treated with RNase free-DNase I (Promega) and reverse transcribed by M⁻MLV reverse transcriptase (Invitrogen). As a control for transfection efficiency, 200 ng of pSEAP-control plasmid (CLONTECH) was co-transfected with each COMT clones. The cDNA was amplified with DyNAmo-SYBRGreen qPCR kit (MJ Research, Reno, NV, USA) using forward and reverse PCR primers, specific for COMT cDNA (TGAACGTGGGCGACAAGAAAGGCAAGAT and TGACCTTGTCCTTCACGCCAGCGAAAT, respectively) or for SEAP cDNA (GCCGACCACTCCCACGTCTT and CCCGCTCTCGCTCTCGGTAA, respectively) Opticon-2 Real Time Fluorescence Detection System (MJ Research) was used for measuring fluorescence.

Animal Behavior.

Subjects. Sixteen adult male Sprague-Dawley rats (285-325g; Charles River Laboratories, Wilmington, MA) were used in these experiments. All procedures were approved by the University of North Carolina Animal Care and Use Committee and followed the guidelines for the treatment of animals of the International Association for the Study of Pain (Zimmermann, 1983).

Drugs and Chemicals. OR486, a potent peripheral and central COMT inhibitor, and lambda carrageenan were obtained from Sigma Aldrich (St. Louis, MO). OR486 was dissolved in dimethylsulfoxide and saline (3:2 ratio) for systemic administration. Carrageenan (3%) was dissolved in saline and administered in a volume of 100 μ l.

Assessment of Responsiveness to Mechanical and Thermal Stimulation. Rats were placed in plexiglass cages positioned over an elevated perforated stainless steel platform and habituated to the environment for 15 - 25 min prior to testing. Paw withdrawal threshold to punctate mechanical stimulation was assessed using the up-down method of Chaplan (16). A series of nine calibrated filaments (with bending forces of 0.40, 0.68, 1.1, 2.1, 3.4, 5.7, 8.4, 13.2, and 25.0 g; Sammons Preston Rolyan) with approximately equal logarithmic spacing between stimuli (Mean \pm SEM: 0.232 \pm 0.04 units) were presented to the hind paw in successive order, whether ascending or descending. Filaments were positioned in contact with the hindpaw for a duration of 3 s or until a withdrawal response occurred. Testing was initiated with the middle hair of the series (3.4 g). In the absence of a paw withdrawal response, an incrementally stronger filament was presented and in the event of a paw withdrawal, an incrementally weaker filament was presented. After the initial response threshold was crossed, this procedure was repeated four times in order to obtain a total of six responses in the immediate vicinity of the threshold. The presence of paw withdrawal (X) and absence of withdrawal (O) was noted together with the terminal filament used in the series of six responses. The 50% g threshold = $(10^{[X_f + kd]})/10,000$, where X_f = value (in log units) of the final von Frey hair used; k = tabular value of pattern of positive (X) and negative (O) responses, and d = mean difference (in log units) between stimuli. Immediately following determination of the response threshold, paw withdrawal frequency (%) to punctate mechanical stimulation was assessed. A von Frey monofilament with a calibrated bending force of 25 g was presented to the hind paw 10 times for a duration of 1 s with an interstimulus interval of approximately 1 s. Mechanical hyperalgesia was defined as an increase in the percentage frequency ($[\# \text{ of paw withdrawals}/10] \times 100$) of paw withdrawal evoked by stimulation with von Frey monofilaments.

Thermal hyperalgesia was evaluated using the radiant heat method(17) in the same animals evaluated for responsiveness to von Frey monofilaments. Radiant heat was presented through

the floor of a finely perforated stainless steel platform to the midplantar region of the hind paw. Stimulation was terminated upon paw withdrawal or after 20 s if the rat failed to withdraw from the stimulus.

Assessment of effect of COMT inhibitors. After establishing stable baseline responsiveness to mechanical and thermal stimuli, separate groups of rats received intraperitoneal injections of OR486 (30 mg/kg i.p.; N=8) or vehicle (N= 8) one hour prior to behavioral testing. Responsiveness to von Frey filaments was reassessed at 30 min intervals for 2 hours. Paw withdrawal latencies to radiant heat were subsequently assessed at 2.5 hours into the testing procedure. Twenty-four hours later, baseline responsiveness to mechanical and thermal stimuli was reestablished. Animals received OR486 (30 mg/kg i.p.) or vehicle (consistent with the previous day). Thirty minutes following administration of drug or vehicle, separate groups of rats received intraplantar carrageenan (N=4 per group) or saline (N=4 per group). Responsiveness to von Frey filaments was reassessed at 30 min intervals for 2 hours following the induction of inflammation. Paw withdrawal latencies to radiant heat were subsequently assessed at 2.5 hours post carrageenan.

Statistical analysis.

Summary measure of sensitivity to experimental pain: A single measure of pain sensitivity was computed for each subject from the sixteen experimental pain procedures. We first reversed the direction of measurement (by subtracting from zero) fifteen of the measures that quantified threshold or tolerance to experimental pain: four measures of pressure pain threshold, nine measures of thermal threshold/tolerance, and two measures of ischemic threshold/tolerance. The sixteenth measure of temporal summation of C fiber mediated thermal pain was retained in its original value. All sixteen measures were standardized to unit normal deviates (z-scores) by subtracting the sample mean, then dividing by the sample standard deviation. Three of the 202 genotyped subjects had a single missing pain measure, one subject had two missing pain measure and one subject had 10 missing pain measures, and we imputed the mean value of zero in each instance. Each subject's summary z-score was then computed by adding together all sixteen unit normal deviates. This yielded an approximately normal distribution (Figure S1) which satisfied assumptions for homogeneity of variance in all subsequent t-tests and ANOVA models.

Statistical evaluation of associations between SNPs, haplotypes and pain responsiveness: Statistical evaluation began with analysis of variance (ANOVA) models for each of six SNPs and Student's *t*-test to contrast homozygotes (Fig. 3). We then evaluated the independent effects of SNPs in a multivariable generalized linear model in which each SNP was entered as a separate pair of dummy variables (with two degrees of freedom). SNPs were entered in the following sequence of steps: first, rs4680 (*met*⁵⁸*val*), second rs4818 rs4633 and rs6269, and third rs2097903 and rs165599. This sequence was nominated on the following theoretical grounds: first we controlled for the one SNP in the coding region that caused a synonymous change in COMT amino acid sequence; second we examined the effects of SNPs that are associated with a non-synonymous change in the amino acid sequence; and third we evaluated any additional effects of SNPs in the promoter and untranslated regions. SNPs entered in the third step contributed less than one percent each to the total variation (R-squared) of the model. In addition, eight subjects had missing values for the two SNPs in this third block, so those SNPs were eliminated from further analysis. The four SNPs from the coding region accounted for 10.6 percent of R-squared (Table S3).

Associations between haplotypes and pain responsiveness were assessed first by classifying the 186 subjects whose two COMT haplotypes were among the three most prevalent haplotypes: GCGG (which we label as low pain sensitive - LPS), ATCA (which we label as average pain sensitive - APS), ACCG (which we label high pain sensitive - HPS). This yielded five possible combinations that we evaluated in a factorial analysis of variance model (Table S5). We used the *F*-tests for each term in this model to first determine that each haplotype in the subjects' pairs had independent effects. We then computed least squares means to test three hypotheses about haplotypes (using Dunnett's post-hoc adjustment):

- For the first haplotype, APS had higher adjusted mean summary z-score than LPS subjects ($P=0.01$, adjusting for the other haplotype – Table S5).
- For the second haplotype, HPS had higher adjusted mean summary z-score than APS subjects ($P=0.04$, adjusting for the other haplotype) and HPS had higher adjusted mean summary zscore than LPS ($P<0.01$ – adjusting for the other haplotype – Table S5)

Stratification by race: there were too-few non-white subjects ($n=31$) genotyped from this volunteer sample to permit meaningful statistical analyses of them as separate strata. However, the percentage of subjects classified as HPS/APS was virtually identical between whites (35%) and non-whites (36% - Chi-square test, $P=0.89$) and the two race groups did not differ significantly in mean values of experimental pain zscores (t-test, $P=0.30$). Furthermore, when the preceding factorial analyses of COMT haplotypes were restricted to whites, the results remained virtually identical. Specifically, *P*-values for the effect of each haplotype were significant ($P<0.03$), and the pairwise effects were significant for ATCA vs GCGG ($P=0.03$) and for ACCG vs. GCGG ($P<0.001$).

Linkage disequilibrium (LD) analysis: LD between SNPs was estimated with SAS Proc Allele software using default parameters.

Incidence of newly-diagnosed TMD: Among the 186 subjects who had the five major haplotype combinations, 170 completed one or more follow-up evaluations when fifteen new cases of TMD were diagnosed (cumulative incidence = 8.8%). New cases were diagnosed at periods ranging from nine months to three years, and the duration of follow-up for all subjects ranged from seven months to 43 months. Because of this variation in follow-up periods, we computed the average incidence rate for TMD and contrasted the rates for two subgroups of haplotypes, the first comprising subjects with APS/HPS haplotype ($n=58$ people with A_C_C_G/A_T_C_A or A_T_C_A/A_T_C_A) and the second comprising subjects with at least one LPS haplotype ($n=112$ people with A_T_C_A/G_C_G_G, A_C_C_G/G_C_G_G or G_C_G_G/G_C_G_G). Life-table methodology was used to compute incidence rates. The numerator for the incidence rate was the number of subjects who developed TMD and the denominator was TMD-free-time-in-study, expressed in years. For subjects who developed TMD, their TMD-free-time-in-study was computed as the period from recruitment to the halfway point between their penultimate three monthly screening interviews and their diagnosis examination. For subjects who did not develop TMD but who withdrew from the study before the final annual assessment, their TMD-free-time-in-study was computed as the period from recruitment to the halfway point between their penultimate three monthly screening interview and date of withdrawal. For all remaining subjects, their TMD-free-time-in-study was computed as the period from recruitment to the final examination. Results were expressed as the rate of new TMD diagnoses per 100-person-years of follow-up, equivalent to the average number of new TMD cases that would be expected in an initially TMD-free cohort of female volunteers who were followed each for one year.

For the complete cohort of n=170 subjects, cumulative incidence was 8.8 percent and the incidence rate was 3.5 cases per 100-person-years (Table S5). Cumulative incidence was compared between the HPS/APS group and the LPS group using the Mantel-Haenszel test, yielding a relative risk of 2.2 (95% confidence interval [95%CI] = 0.8-5.8 – Table S5) Differences in incidence rates between the same two groups were determined in a Poisson regression model in which the numerator was number of TMD cases and the offset was the log of person-years of follow-up, yielding an incidence density ratio of 2.3 (95%CI = 1.1 – 4.8).

Analysis of rat behavioral data. Behavioral data were analyzed by ANOVA for repeated measures and post hoc comparisons were performed using the Bonferroni test. $P < 0.05$ was considered to be statistically significant. |

Supplementary tables

Table S1: Baseline characteristics of the cohort and percentage genotyped

Group		No. in cohort*	No. genotyped*	% genotyped	P-value†
Age:	18-22 yrs	129	109	84.5	0.46
	23-34 yrs	115	93	80.9	
Race:	White	205	171	83.4	0.55
	Other	39	31	79.5	
Highest level of education	High school	50	41	82.0	0.48
	College	134	114	85.1	
	Post-grad	89	46	78.0	
Marital status	Single	198	164	82.8	0.97
	Other	46	38	82.6	
Baseline pain z-score	< -4.5	81	73	90.1	0.07
	-4.5 – 3.0	82	63	76.8	
	>3.0	81	66	81.5	
Total		244	202	82.8	

* Numbers that do not add to total have missing values for socio-demographic variables

† Chi-square test

Table S2. Sequences for the primer and probes used in 5' exonuclease and DSN assays.

Primer or probe name	SNP	sequence
Fwd Primer	rs2097903	gcc gtg tct gga ctg tga gt
Rev Primer		ggg ttc aga atc acg gat gtg
Probe1NV (6FAM)		aac aga cag aaa ag T ttc ccc ttc cca
Probe2V (VIC)		cag aca gaa aag C tt ccc ctt ccc ata
Fwd Primer	rs6269	agg cac aag gct ggc att t
Rev Primer		cca cac gcc cct ttg ct
Probe1NV (6FAM)		tgc ccc tct gcG aac aca agg
Probe2V (VIC)		acc ttg ccc ctc tgc Aaa cac aag
Fwd Primer	rs4633	tgc tca tgg gtg aca cca a
Rev Primer		gcc tcc agc acg ctc tgt
Probe1NV (6FAM)		atc ctg aac ca T gtg ctg cag cat
Probe2V (VIC)		atc ctg aac ca C gtg ctg cag c
Fwd Primer	rs4818	ggg ggc cta ctg tgg cta ct
Rev Primer		tca ggc atg cac acc ttg tc
Probe1NV (6FAM)		cga ggc t Ca tca cca tgc aga tca
Probe2V (VIC)		cga ggc t Ga tca cca tgc aga tca
Fwd Primer	rs4680 (<i>val158met</i>)	tcg aga tca acc ccg act gt
Rev Primer		aac ggg tca ggc atg ca
Probe1NV (6FAM)		cct tgt cct tca Cgc cag cga
Probe2V (VIC)		acc ttg tcc ttc a Tg cca gcg aaa
Fwd Primer	rs165599	cagccacagtgggtgcagag
Rev Primer		gtccacctgtccccagcg
FAM-probe-DABCY		tgcc A gcctg
TAMRA-probe-DABCY		tgcc G gcctg

Table S3: Generalized linear model of effects of four SNPs on painsensitivity*

SNP locus	Degrees of freedom	Sum of squares	Sequential <i>R</i> -squared	F	<i>P</i> -value
rs4680 (<i>val/met</i>)	2	383.5	0.017	1.8	0.17
rs4818	2	1535.1	0.068	7.3	<0.01
rs4633	2	466.4	0.021	2.2	0.11
rs6269	2	18.5	<0.001	0.1	0.91
Error	193	20324.5			

*Model uses data from n=202 subjects who were genotyped. For full model $F_{8,193} = 2.8$, $P < 0.01$, R -squared=0.106

Marker 1	Marker 2	R^2	D'
rs2097903	rs6269	0.05	0.35
	rs4633	0.08	0.34
	rs4818	0.03	0.28
	rs4680	0.08	0.34
	rs165599	0.00	0.00
rs6269	rs4633	0.59	0.99
	rs4818	0.88	0.94
	rs4680	0.59	1.00
	rs165599	0.00	0.11
rs4633	rs4818	0.59	0.99
	rs4680	0.91	0.96
	rs165599	0.01	0.14
rs4818	rs4680	0.60	1.00
	rs165599	0.00	0.04
Met/val	rs165599	0.00	0.10

Table S4. Linkage disequilibrium between paired SNP markers. Data were analyzed for significance using the PHASE 2.0 program. D' is the normalized linkage disequilibrium statistic, which lies in the range from 0 to 1 with greater values indicating stronger linkage. Consistent with previously published data, four SNPs, rs6269, rs4633, rs4818 and rs4680 (*val*¹⁵⁸*met*), which occur within the coding region of the COMT gene, were found to exhibit strong LDs (3-5). This means that these SNPs are forming one haploblock within the COMT gene locus(8). In contrast, SNPs rs2097903, which is located in the 5' promoter region, and rs165599, which is a 3' UTR SNP, did not show strong LDs. These SNPs are within two haploblocks adjusted to the first one.

Whether there are additional SNPs that modulate COMT enzymatic activity is of importance. While there are several dozens identified SNPs within the COMT gene locus in the NCBI and CELERA databases, the majority of these SNPs are very infrequent and are unlikely to have a profound effect on individual variations in pain perception in human population. Importantly, we tested all of the known common SNPs located in the central haploblock of the COMT gene. There are several common SNPs located in the 5' and 3' parts of the gene locus that may influence COMT activity. These SNPs also form haploblocks but the observation that the 5' SNP rs2097903 and 3' SNP rs165599 do not significant correlate with pain sensitivity (Fig. S1) suggests that these regions are unlikely to contain other frequently found SNPs that impact pain sensitivity. However, further studies are required to more specifically address this issue.

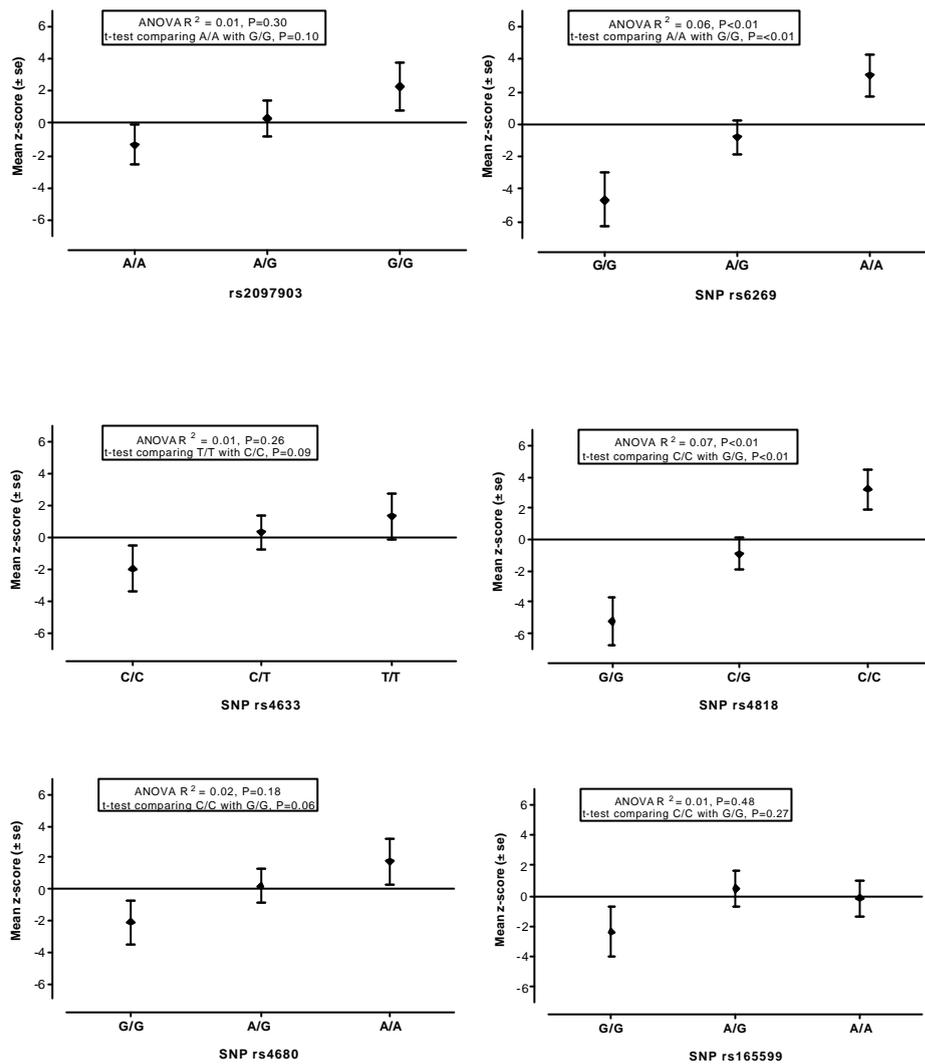


Figure S1. Variations in pain tolerance associated with individual SNPs

The first presented allele in each plot is associated with the least pain sensitivity. Differences among all three allele combinations were assessed by analysis of variance (ANOVA) using zscore as the dependent variable. The significance of the difference in the mean z-scores associated with each homozygous genotype was determined via the Students t-test. Each value represents the mean z-score with associated s.e.m.

Reference List

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