Supporting Information

Reimann et al. 10.1073/pnas.0913181107

SI Results

Osteoarthritis Cohort Results. A total of 578 subjects with osteoarthritis (OA) from four different studies were included in the final analysis, all of whom had a WOMAC pain score, genotype data, and nonmissing covariate information. Allele frequencies for each single nucleotide polymorphism (SNP) were calculated. All SNPs were in Hardy–Weinburg equilibrium except rs4286289 and rs12620053. These two SNPs were out of Hardy–Weinburg with borderline P values ($P = 0.01$–0.05). Neither was associated with the pain phenotypes examined. The unadjusted mean pain scores were tabulated for each genotype of each SNP (Table S2). Potential genetic effects were evaluated using linear regression, which allowed adjustment for covariates and an estimate of the slope. In the linear regression analysis for pain score (trend effect per rare allele and gender interaction), adjustments were made for age, gender, BMI, and age–gender interaction.

Chronic Lumbar Root Pain Cohort Results. Single-marker analysis was conducted for the association between rs6746030 and the seven-point pain score using a linear regression model. There were 129 (72%) individuals with a G/G genotype; they had an adjusted pain score of 0.471. Forty-five (25%) individuals had a A/G genotype and a pain score of 0.651. And five (3%) individuals had an A/A genotype and a pain score of 0.971. The P value (additive model) for pain association with allele was 0.0882.

Pancreatitis Results. We included 373 subjects. There were 205 chronic pancreatitis (CP) patients and 168 controls. Genomic DNA from all participants was analyzed for *SCN9A* SNP rs6746030 alleles.

We first employed a classic case control approach. This showed no difference in the proportion of controls versus patients with CP who carried the A allele: for the Fisher's exact test, the two-sided P value is 0.55, odds ratio (OR) 0.8526 (95% CI 0.54–1.35). There were 168 controls; 121 had no A allele and 47 had an A allele. There were 205 patients; 154 had no A allele and 51 had an A allele. In total there were 373 subjects; 275 had no A allele and 98 had an A allele. Of the 168 control haplotypes, 121 were G/G, 41 G/A, and 6 A/A; of the 205 CP patients, 154 were G/G, 44 G/A, and 7 A/A.

We then adopted a nested approach to assess whether patients with a rs6746030 A allele had more or fewer surgical procedures for the chronic pancreatitis. Data were available for 195 patients. No association was found: for the Fisher's exact test, the two-sided P value is 0.21, OR 1.425 (95% CI 0.7–2.9). For the 128 that had surgery, 35 had no A allele and 93 did. For the 67 that did not have surgery, 14 had no A allele and 53 did. In total there were 195 assessed subjects; 49 had no A allele and 146 had an A allele.

Pain questionnaire data were available for 109 chronic pancreatitis patients. We compared the median scores for those who carried the rs6746030 A allele with those who did not by using a Mann–Whitney test. No association between pain score and rs6746030 alleles was found. The mean score in the mutation-positive CP patients was 18.165 (SD = 15.1) compared with 18.5 (SD = 15.9) for the mutation-negative patients; $P = 0.276$.

Finally, we calculated the score for the composite pain score for the same 109 chronic pancreatitis patients. We compared the median scores of those who carried the rs6746030 A allele with those who did not by using a Mann–Whitney test. No association was found. The mean score in the mutation-positive CP patients was 2.6 (SD = 1.7) compared with 2.7 (SD = 1.57) for the mutation-negative patients; $P = 0.7316$.

Detailed Electrophysiology Results. To measure the voltage dependence of activation, cells were first depolarized to different potentials for 50 ms from a holding potential of −100 mV. This triggered similar-sized currents in cells expressing either $Na_V1.7$–1150R or $Na_V1.7$–1150W and revealed no differences in the voltage dependence of channel activation, with $V_{1/2}$ from a Boltzmann fit of -29 ± 1 mV ($n = 10$) and -26 ± 2 mV ($n = 17$), respectively (Fig. 2). Using a classical m^3h Hodgkin–Huxley model for voltage gated sodium channels to assess the kinetic parameters underlying these currents, we also found no significant differences between the two channel types in either the activation or the inactivation time constants.

We next investigated the voltage dependence of channel inactivation by clamping cells for 500 ms at a potential at which inactivation could occur and then measuring the remaining current triggered by a test pulse to −10 mV. The voltage dependence of inactivation was similar for $Na_V1.7$–1150R and $Na_V1.7$–1150W channels with $V_{1/2}$ values of -73 ± 2 mV in both cases ($n = 10$ and 17, respectively). Inactivation of voltage-gated sodium channels, however, has been shown to involve at least two different inactivated states, one of which can be entered relatively rapidly, whereas the other is evident only after prolonged depolarizations. To assess accumulation in the slow inactivated states, we depolarized cells for 10 s at different voltages, hyperpolarized them for 100 ms at −120 mV to recover only the fast inactivating states, and then recorded the response to a test pulse to −10 mV. Accumulation in slow inactivated states occurred at more positive potentials than those triggering fast inactivation and reached only~80%, and many of the parameters describing slow inactivation were similar for both channel variants [$V_{1/2} = -37 \pm 5$ mV and A2 = 18 ± 3% ($n = 7$) for $Na_V1.7$–1150R and $V_{1/2} = -35 \pm 3$ mV and A2 = 15 ± 2% for $Na_V1.7$–1150W]. However, we observed that the voltage dependence of slow inactivation was significantly steeper for $Na_V1.7$–1150W than for $Na_V1.7$–1150R currents ($k = 10.7 \pm 0.4$ mV and 13.7 ± 1.2 mV, respectively; $n = 7$ each; $P = 0.042$; Fig. 2*G*).

Pain-Free Female Cohort Results. The minor A allele was significantly associated with a reduced C-fiber–mediated heat pain threshold (Fig. S3, $P = 0.035$). As observed in the OA cohort, the A allele showed additive characteristics in that individuals carrying two copies of the pain-prone A allele were the most sensitive to C-fiber–mediated heat stimulation, whereas heterozygotes exhibited an intermediate sensitivity (Fig. S2). A similar significant association was observed when repetitive suprathreshold heat stimuli were applied to the forearm (Fig. S3). In this test, the minor A allele was associated with an increased pain response both to the first heat pulse ($P = 0.02$) and across a train of 15 pulses ($P = 0.03$), an effect largely mediated by C-fiber activation. We did not observe a significant association between the minor A allele and measures of C-fiber–mediated heat pain tolerance, Aδ-mediated thermal pain sensitivity, muscle ischemia, or pressure pain, although in all cases the trend was for the A allele to be associated with increased pain perception (Fig. S2, Table S5). Collectively, these psychophysical findings suggest that the minor A allele of rs6746030 increases the coding of C-fiber–mediated thermal pain perception. Although unlikely, a central affect of the minor allele on "central sensitization," which would produce similar psychophysical responses, cannot be totally excluded. The association analysis between rs6746030 with combined Z-score for all pain measures was not significant ($P = 0.221$). However, when added to the meta-analysis with all other pain cohorts analyzed in the cohort, it further strengthened the combined P value to 5.8E-05.

These results suggest that the minor A allele enhances pain perception by increasing the activity of nociceptive C-fibers.

SI Subjects and Methods

Electrophysiology Methods. The rs6746030 alleles encode either an arginine (the more frequent G allele) or a tryptophan (A allele) at position 1150 of the reference $Na_v1.7$ protein sequence NP_002968. A cDNA clone encoding the most common *SCN9A* splice variant found in dorsal root ganglia (NM_002977) was used to generate both rs6746030 alleles using the QuikChange XL site-directed mutagenesis kit (Stratagene) according to the manufacturer's instructions. HEK293A cells (QBiogene), cultured in DMEM supplemented with 5% FCS, were transiently transfected with plasmids expressing either $Na_V1.7$–1150R or $Na_V1.7$–1150W + DsRed2 and SCN1B + SCN2B + EGFP using lipofectamine 2000, as previously described (11). Experiments were performed 2–3 days after transfection on cells positive for red and green fluorescence, which was detected with excitation at 550 ± 7 nm and 488 ± 5 nm, respectively, using appropriate emission filters and MetaMorph (Molecular Devices) software controlling a monochromator (Cairn) and a CCD camera (Orca ER; Hamamatsu) mounted on an Olympus IX71 microscope with a ×40 objective. Microelectrodes were pulled from borosilicate glass (GC150T; Harvard Apparatus) and the tips were coated with melted beeswax. Electrodes were fire-polished using a microforge (Narishige) and had resistances of 2.5–3 MΩ when filled with pipette solution. Standard whole-cell currents were filtered at 10 kHz and recorded at 20 kHz at 22 °–24 °C using an EPC10 amplifier controlled by patchmaster software (HEKA Electronic). The holding potential was −100 mV, 70% series resistance compensation was used throughout, and currents were zero and leak subtracted using a p/4 protocol. Analysis was performed with pulsefit (HEKA Electronic) and origin software (OriginLab). The bath solution contained (mM): 3 KCl, 140 NaCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 Hepes, and 1 glucose (pH 7.4 with NaOH). The patch pipette solution contained (mM): 107 CsF, 10 NaCl, 1 $CaCl_2$, 2 $MgCl_2$, 10 Hepes, 10 TEACl, and 10 EGTA (pH 7.2 with CsOH).

Voltage dependence of activation was fitted to the Boltzmann equation: $I = (A2 + (A1 - A2)/(1 + \exp((V0.5 - x)/k))) \times (x - Vrev)$. Voltage dependence of inactivation was fitted to the Boltzmann equation: $I = (A1 - A2)/(1 + \exp((x - V0.5)/k)) + A2$.

Cohort Data. ***Osteoarthritis cohort.*** Clinical information and DNA samples were available from 578 individuals from 4 osteoarthritis clinical trials (064340, A4141004, A3171005, and A4201008). All studies were internal Pfizer-sponsored studies and appropriate informed consent was obtained. The baseline characteristics of the cohort were the following: 226 were male (36%) and the mean age was 61.4 (standard deviation ± 8.9) with a range of 37–91 years. The mean height was 168.1 cm (standard deviation ± 10.2) with a range of 138–198 cm. The mean weight was 90.5 kg (standard deviation ± 19.6) with a range of 28–181 kg. The mean BMI was 32.1 kg/m^2 (standard deviation ± 6.6) with a range of 11.7–67.9 kg/m^2. The mean pain score (WOMAC scale) was 9.4 (standard deviation ± 3.8) with a range of 0–20. The distribution of pain severity across the four study groups is shown in Fig. S1.

Osteoarthritis cohort statistical analysis. For OA cohort 1, an initial analysis was undertaken to establish the importance of covariates in the absence of any genotype information. Linear regression models were used with pain score as the dependent variable, applying a statistical transformation if indicated by inspection of normality and heteroscedasticity (if the random variables in the sequence had different variances). A base model was built starting with gender and age terms and adding further covariates and nonlinear terms until a suitable parsimonious model was established. We sought differences between the four OA studies using regression model analysis with and without the study term. Despite the minor differences in the distribution of pain scores between the four studies, adjusting for this potential study effect made no difference to the results. Therefore, a study term was not included in the final regression model.

To evaluate SNP associations, separate models were run with each SNP term added to the base model. Genotype was initially modeled as a linear variable corresponding to a test for trend in genotype frequencies (i.e., a codominant genetic model). To check the assumption of codominance, the fit was compared with models that include a term for deviation from additivity due to either a dominant or a recessive mechanism. A likelihood ratio test, which compared the model with and without the SNP, was used to assess the strength of association between genotype and dependent variable. Results of the pain severity analysis are reported as a unit incremental pain score per rare allele with corresponding 95% confidence intervals. All gender–SNP interactions were assessed to seek evidence of differential pain score distributions according to gender. No formal correction for multiple testing was applied in this exploratory study.

Finnish sciatica pain cohort. In brief, single-marker analysis was conducted for the association between rs6746030 and Visual Analog Pain Scale (VAS) scores using a linear regression model. This group consisted of 195 patients referred to the Oulu University Hospital (Finland) due to sciatica symptoms. Criteria for inclusion in the study were unilateral pain radiating from the lower back down to below the knee. All patients had MRI-based confirmation of having a lumbar disk herniation concordant with sciatica pain. The primary outcome for the association analysis was leg pain intensity at baseline, which was determined with a visual analog pain scale, using a 10-cm horizontal line having the anchor "no pain" associated with the left end of the line and the anchor "the highest imaginable pain" with the right end. This outcome was adjusted for the covariates of age, sex, and work compensation. Informed consent was collected from all participants. The research protocol was approved by the Ethics Committee of the University Hospital of Oulu, Finland.

Danish phantom pain cohort. In brief, single-marker analysis was conducted for the association between rs6746030 and phantom pain experience using a proportional hazard model. Saliva for analysis of DNA and pain data was collected from 100 amputees (66 males and 34 females; mean age 59 years) following the approval of the Central Denmark Region Committee on Biomedical Research Ethics. Of the amputees, 43 had suffered traumatic amputations and 57 had amputations for medical reasons, mostly vascular insufficiency and cancer. Nineteen were upper-limb amputees, 80 were lower-limb amputees, and one had undergone amputation of both an upper and a lower limb. The primary phenotype was pain severity at the typical phantom pain episode determined with the VAS. This outcome was adjusted for gender and age.

Chronic lumbar root pain cohort. We collected DNA from peripheral blood samples of 179 Caucasian adults who had participated in a prospective observational study of surgical discectomy for persistent lumbar root pain caused by intervertebral disk herniation (1, 2). The phenotyping methods and sociodemographic details of this cohort have been previously described (3). Briefly, before data analyses we specified the following single primary endpoint: persistent leg pain over the first postoperative year, as a reflection of ongoing neuropathic pain. We designated this the pain phenotype for genetic association analysis. Leg pain was assessed on 13 time points (at baseline and at 3, 6, and 12 months post surgery and then annually through year 10), using the following four items: frequency of "leg pain" and of "leg pain after walking" in the week preceding data collection, as well as improvements in "leg pain" or in "leg pain after walking" since surgery (3). For each patient, we calculated an area-under-the-curve score for every pain variable in the first year and converted it to a Z-score by comparing the patient to the rest of the cohort. The primary pain outcome variable for association analysis was the mean of these four Z-scores per patient. Genotype–phenotype analysis was done using a prespecified regression equation, incorporating our assumption that one or two copies of the rare allele would affect the pain score in an additive model, and was adjusted by the following covariates: sex, age, worker's compensation status, delay in surgery after

enrollment, and the Short Form-36 General Health subscale. The study had been approved by the Institutional Review Board of the National Institute of Dental and Craniofacial Research (National Institutes of Health).

Pancreatitis cohort. In brief, analysis of the pancreatitis cohort was done by a classic case control approach, comparing the genotypes of the controls with those with pancreatitis by using the Fisher's exact test. Data were further analyzed according to whether possessing the A allele was associated with more or fewer surgical approaches for pancreatic pain, pain questionnaire scores, and composite pain scores.

The subjects that we included were patients diagnosed with CP between 1980 and 2006 who visited the outpatient Department of Gastroenterology and Hepatology, Radhoud University Nijmegen. All patients had to be at least 18 years old. Patients received an invitation to participate in the study. Information from the patient files about the first attack, the cause, number of hospitalizations, pain pattern, complications, operations, and medication use was collected into a database. The clinical diagnosis of CP was based on one or more of the following criteria: presence of typical complaints (recurrent upper abdominal pain, radiating to the back, relieved by leaning forward or sitting upright and increased after eating); suggestive radiological findings, such as pancreatic calcifications or pseudocysts; and pathological findings (pancreatic ductal irregularities and dilatations) revealed by endoscopic retrograde pancreaticography or magnetic resonance imaging of the pancreas before and after stimulation with secretin. For comparison, we collected a control group consisting of 108 adult healthy subjects, recruited by advertisement in either a local paper or on the Web.

We recorded the following pancreatic surgical procedures: thoracoscopical splanchnicus denervation and surgical procedures according to Puestow, Beger, Whipple, and Roux-Y (end-to-side anastomosis). Procedures had been performed in our patient population for pain management only.

Pain was measured by using a slightly modified version of the Gastrointestinal Symptoms Questionnaire (Table S4) (4). This questionnaire includes a pain score assessed by a 100-mm, non-hatched VAS scale marked at one end as "no pain" and at the other as "worst pain imaginable," and we measured the pain experienced by the patient in the past 4 weeks. The questionnaire also includes a numerical rating scale, scoring the pain in the upper abdomen (0 = absent to 6 = very severe). Furthermore, the pain perception during the last pancreatitis attack, the use of pain medication in the past 4 weeks, and the pain pattern was completed on a separate form. This questionnaire yields reproducible results and has been validated as an instrument to monitor gastrointestinal symptoms (4).

A composite pain score was developed to identify two groups of patients: patients with low/moderate pain and those with severe pain. Therefore, all subjects were scored on five items as shown in Table S4. The pain types as distinguished by Ammann and Muellhaupt (5) were used to distinguish between the two groups. Type A pain was indicated as patients with low/moderate pain because of long pain-free intervals and short-lived pain episodes. The performance of specific pancreatic operations and the use of morphinomimetics in the past were also used to make this distinction. The most common indication for surgical intervention is refractory pain, which adversely affects quality of life (6, 7). Furthermore, opioids are the next step, when nonopioid analgesics do not yield sufficient pain relief (8). The group with a score of 0–3 points is considered as patients with low/moderate pain, whereas the group with a score of 4–6 is considered as patients with severe pain.

Pain-free female cohort methods. A total of 186 healthy European–American pain-free females were phenotyped and genotyped. We restrict our analysis to Europeans to avoid the possible effect of population stratification (9). Each enrollee in the analyzed cohort donated a DNA sample and was quantified for responsiveness to a set of 13 noxious stimuli applied to various anatomical sites (10, 11).

SNP rs6746030 was analyzed by the 5′ nuclease method using TaqMan assay (Applied Biosystems). Linear regression was used to find the additive effect of each A allele on multiple pain phenotypes. All pain scores were normalized to Z-scores before this analysis. Age was incorporated as a covariable into the model.

1. Atlas SJ, et al. (1996) The Maine Lumbar Spine Study, Part II. 1-year outcomes of surgical and nonsurgical management of sciatica. *Spine (Phila Pa 1976)* 21: 1777–1786.
2. Atlas SJ, Keller RB, Chang Y, Deyo RA, Singer DE (2001) Surgical and nonsurgical management of sciatica secondary to a lumbar disc herniation: Five-year outcomes from the Maine Lumbar Spine Study. *Spine (Phila Pa 1976)* 26:1179–1187.
3. Tegeder I, et al. (2006) GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence. *Nat Med* 12:1269–1277.
4. Bovenschen HJ, et al. (2006) Evaluation of a gastrointestinal symptoms questionnaire. *Dig Dis Sci* 51:1509–1515.
5. Ammann RW, Muellhaupt B (1999) The natural history of pain in alcoholic chronic pancreatitis. *Gastroenterology* 116:1132–1140.
6. Sohn TA, et al. (2000) Quality of life and long-term survival after surgery for chronic pancreatitis. *J Gastrointest Surg* 4:355–364; discussion 364–365.
7. Gupta V, Toskes PP (2005) Diagnosis and management of chronic pancreatitis. *Postgrad Med J* 81:491–497.
8. Cepeda MS, Camargo F, Zea C, Valencia L (2007) Tramadol for osteoarthritis: A systematic review and metaanalysis. *J Rheumatol* 34:543–555.
9. Diatchenko L, Nackley AG, Tchivileva IE, Shabalina SA, Maixner W (2007) Genetic architecture of human pain perception. *Trends Genet* 23:605–613.
10. Diatchenko L, et al. (2005) Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Hum Mol Genet* 14:135–143.
11. Diatchenko L, et al. (2006) Catechol-O-methyltransferase gene polymorphisms are associated with multiple pain-evoking stimuli. *Pain* 125:216–224.

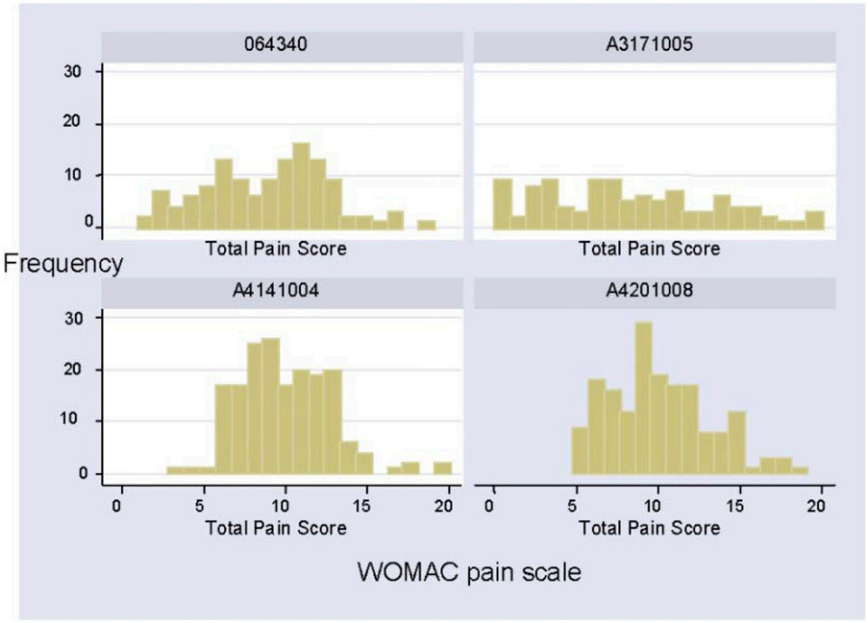

Fig. S1. Osteoarthritis cohort: The distribution of pain scores in the four clinical trials from which data were drawn to assess the contribution of *SCN9A* SNPs to pain score.

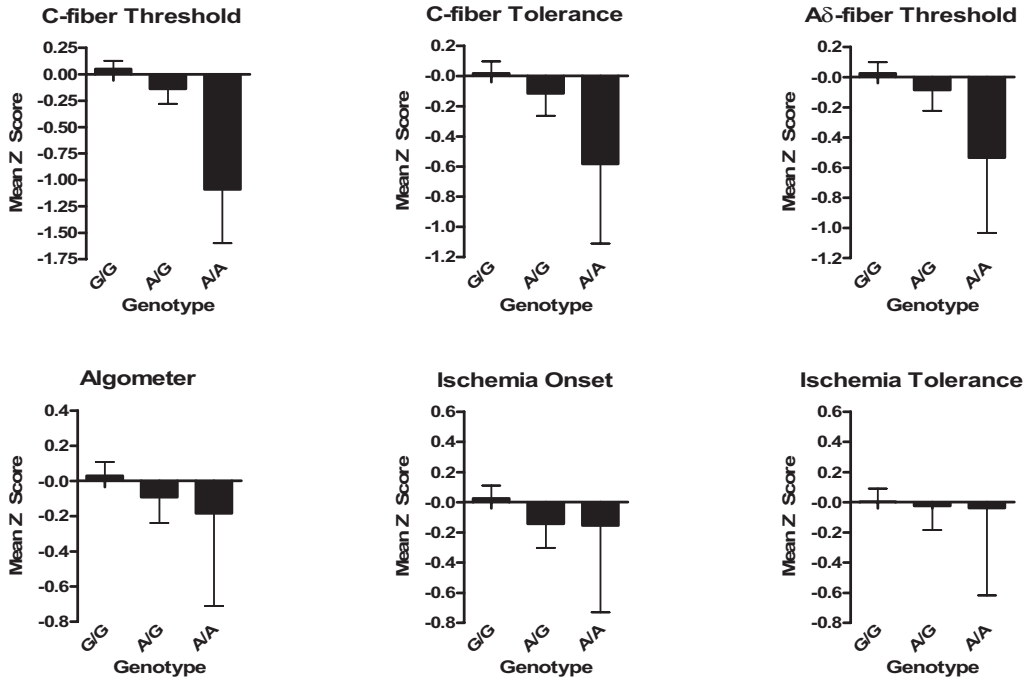

Fig. S2. Pain-free female cohort: Effects of the rs6746030 genotype on three experimental nociceptive modalities. The algometer (pressure pain) score consists of the average value of normalized algometer readings obtained from four distinct body sites. Thermal measurements of C- and Aδ-fiber sensitivity were taken at three sites and likewise transformed to Z-scores before averaging. For ischemic pain, the latency to pain onset and tolerance were not transformed. The figures show the unadjusted mean pain scores for each allele.

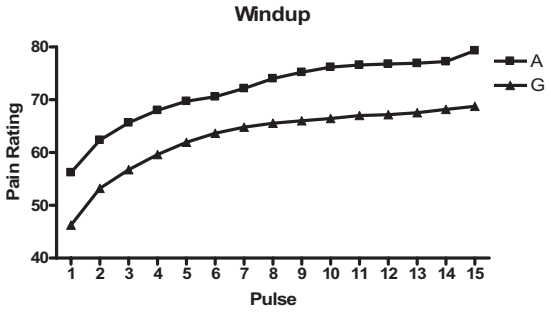

Fig. S3. Time-course curve across 15 thermal pulses showing average pain rating (100-point VAS) by allelic status. The A allele group has a higher first pulse ($P = 0.02$) and overall (by area-under-the-curve method, $P = 0.03$) thermal sensitivity. The slopes of the curves, indicating temporal summation (windup), are not significantly different ($P = 0.29$). These findings are consistent with an increased C-fiber sensitivity in the A allele group because the associated ratings are largely mediated by heat-sensitive C-fibers.

```
SCN9A human-A   GEEAEAEPMNSDEPEACFTDGCVWRFSCCQVNIESGKGKIWWNIRK
SCN9A human-G   GEEAEAEPMNSDEPEACFTDGCVRRFSCCQVNIESGKGKIWWNIRK
SCN9A chimp     GEEAEAEPMNSDEPEACFTDGCVRRFSCCQVNIESGKGKIWWNIRK
SCN9A macaque   GEEAEAEPMNSDEPEACFTDGCVRRFSCCQVNIESGKGKIWWNIRK
SCN9A rabbit    GEEAEAEPVNSDEPEACFTDGCVRRFPCCQVSIESGKGKIWWNIRK
SCN9A dog       GEEAEAEPVNSDEPEACFTDGCVRRFPCCQVDIESGKGKIWWNIRK
SCN9A rat       GEEAEAEPVNADEPEACFTDGCVRRFPCCQVNVDSGKGKVWWTIRK
SCN9A mouse     GEEAEAEPINADEPEACFTDGCVRRFPCCQVNIDSGKGKVWWTIRK
```

Fig. S4. Comparison of the protein region surrounding the amino acid encoded by the *SCN9A* SNP rs6746030 in mammals. Sequence comparisons of 46 amino acids of the $Na_v1.7$ protein, encoded by the *SCN9A* gene, surrounding the nonsynonymous SNP rs6746030. The two human $Na_v1.7$ proteins are shown first: the rs6746030 A allele (associated with a lower pain threshold) and below this the wild-type G allele. Subsequent species are shown in approximate evolutionary divergence from humans, and gray shading shows the amino acids conserved with humans. The amino acid changed by the SNP rs6746030 is in boldface. R is arginine the wild type, and W is tryptophan encoded by the rarer A allele.

Table S1. Osteoarthritis cohort: Details of the SNPs used in the study

SNP*	SNP position*	Alleles†	Minor allele frequency (%)‡	Effect§
rs4447616	Intron 1 (4604)	c/t	33	
rs10171225	Intron 3 (5618)	a/g	20	
rs4286289	Intron 4 (6556)	a/c	27	
rs4605385	Intron 4 (6669)	a/g	18	
rs6432896	Intron 4 (6847)	a/g	43	
rs12994338	Intron 5 (8238)	c/t	34	
rs13017637	Intron 5 (8321)	c/t	35	
rs12620053	Intron 6 (9981)	a/c	43	
rs12619987	Intron 6 (10294)	a/g	13	
rs11688164	Intron 6 (14478)	c/t	17	
rs13402540	Intron 8 (22911)	g/t	37%	
rs6747673	Exon 9 (23293)	a/t	45.5%	Arg/Arg
rs6432894	Intron 11 (29287)	a/c	30%	
rs4453709	Intron 16 (41813)	a/t	36%	
rs4443014	Intron 16 (44046)	a/t	19%	
rs4561679	Intron 16 (50225)	c/t	10%	
rs4371369	Intron 16 (51612)	a/g	35%	
rs7604448	Intron 16 (55623)	a/c	12.5%	
rs10930214	Intron 17 (62414)	c/g	27%	
rs6746030	Exon 18 (69109)	g/a	10%	Arg/Trp
rs12621853	Intron 18 (71703)	c/t	17%	
rs17748381	Intron 18 (71783)	a/g	11%	
rs10170041	Intron 20 (81406)	a/t	41%	
rs16851799	Intron 20 (81853)	c/t	43%	
rs7595255	Intron 23 (85289)	c/t	10%	
rs6432885	Intron 23 (95577)	g/a	48%	
rs3750904	Exon 26 (112874)	c/t	0.85%	Gly/Asp

*The SNP position in the gene is given by intron and exon location and as the genomic nucleotide position counting from the start codon of the *SCN9A* reference sequence NM_002977.
†The common allele nucleotide is shown first.
‡The minor allele frequency was obtained from dbSNP using Hapmap data.
§Effect gives the amino acid encoded by the exonic *SCN9A* SNP alleles, with the common allele effect given first.

Table S2. Osteoarthritis cohort: Details of the raw pain scores for each SNP in the study

		Common/ common allele		Common/rare allele		Rare/rare allele	
SNP	Total *N*	Mean	*N*	Mean	*N*	Mean	*N*
rs4447616	568	9.50	221	9.63	268	8.56	79
rs10171225	568	9.33	343	9.34	199	10.92	26
rs4286289C	565	9.30	279	9.70	240	8.28	46
rs4605385	568	9.42	366	9.39	187	9.80	15
rs6432896	567	8.94	159	9.42	279	9.94	129
rs12994338	567	9.45	297	9.23	234	10.03	36
rs13017637	567	9.47	166	9.51	296	9.02	105
rs12620053	567	9.40	174	9.28	289	9.74	104
rs12619987	556	9.35	432	9.42	115	8.11	9
rs11688164	569	9.46	420	9.33	138	8.91	11
rs13402540	568	9.11	198	9.56	275	9.47	95
rs6747673	565	9.36	133	9.44	277	9.24	155
rs6432894	569	9.38	245	9.42	260	9.23	64
rs4453709	568	9.35	226	9.50	255	9.20	87
rs4443014	562	9.24	317	9.56	215	9.63	30
rs4561679	565	9.25	436	9.79	121	10.50	8
rs4371369	567	9.22	205	9.38	268	9.78	94
rs7604448	567	9.24	407	9.67	144	11.19	16
rs10930214	562	9.15	270	9.39	234	10.34	58
rs6746030	567	9.21	413	9.83	143	11.64	11
rs12621853	569	9.41	409	9.19	145	10.47	15
rs17748381	566	9.42	460	9.68	99	6.14	7
rs10170041	566	9.34	197	9.16	273	10.18	96
rs16851799	569	9.31	207	9.60	270	9.09	92
rs7595255	567	9.19	412	9.75	144	11.64	11
rs6432885	567	9.68	171	9.29	270	9.27	126
rs3750904	566	9.39	561	10.00	5		0

Pain score increment for each additional rare allele is adjusted for age, sex, BMI, and age/sex interaction.

Table S3. Back pain cohort: Pain scores and adjusted pain scores one year after surgery for the 179 individuals from the Maine Lumbar Spine Study

			Adjusted pain score†			
Raw pain score	Did the leg feel better or worse?	No. of patients*	Mean	Minimum	Maximum	SD
	Data missing	2				
1	Completely gone	54	−0.580	−1.287	0.899	0.384
2	Much better	74	−0.159	−1.206	1.700	0.570
3	Better	21	0.483	−0.494	1.696	0.548
4	A little better	11	1.124	−0.174	1.904	0.647
5	About the same	8	0.975	0.141	2.083	0.713
6	A little worse	4	1.184	0.048	2.662	1.094
7	Much worse	5	1.745	1.345	2.529	0.489

*Total of 179 patients.

†Based on the residuals from linear regression with one-year pain score as the dependent variable and age, sex, worker's compensation, crossing over, and the Short-Form 36 General Health Scale as independent variables.

Table S4. Pancreatitis composite pain score

No. of hospitalizations	0–5 = 0
	>5 = 1
Type of pain	Type A = 0
	Type B = 1
Operation	No operation = 0
	Operation = 1
Use of morphinomimetics in the past	No use = 0
	Use = 1
VAS scoring outcomes	VAS ≤32 = 0
	VAS 32–66 = 1
	VAS ≥66 =2
Low/moderate pain	0–3 points
Severe pain	4–6 points

Table S5. Pain-free female cohort: Results of pain testing using a regression model for experimental nociceptive sensitivity

Test	Mean effect size (β)	SE	Lower 95% CI	Upper 95% CI	STAT	*P* value*
Pressure pain threshold Z-score	−0.117	0.143	−0.397	0.161	−0.826	0.409
Thermal C-fiber threshold Z-score	−0.290	0.137	−0.558	−0.022	−2.121	0.035*
Thermal C-fiber tolerance Z-score	−0.179	0.142	−0.456	0.098	−1.26	0.208
Thermal Aδ fiber threshold Z-score	−0.156	0.133	0.417	0.105	−1.173	0.242
Ischemic onset	−0.148	0.158	−0.456	0.160	−0.940	0.348
Ischemic tolerance	−0.009	0.159	−0.319	0.302	−0.054	0.957

*$P < 0.005$.