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Multiple chronic pain states are associated with a common amino acid–changing allele in KCNS1

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Not all patients with nerve injury develop neuropathic pain. The extent of nerve damage and age at the time of injury are two of the few risk factors identified to date. In addition, preclinical studies show that neuropathic pain variance is heritable. To define such factors further, we performed a large-scale gene profiling experiment which plotted global expression changes in the rat dorsal root ganglion in three peripheral neuropathic pain models. This resulted in the discovery that the potassium channel alpha subunit KCNS1, involved in neuronal excitability, is constitutively expressed in sensory neurons and markedly downregulated following nerve injury. KCNS1 was then characterized by an unbiased network analysis as a putative pain gene, a result confirmed by single nucleotide polymorphism association studies in humans. A common amino acid changing allele, the 'valine risk allele', was significantly associated with higher pain scores in five of six independent patient cohorts assayed (total of 1359 subjects). Risk allele prevalence is high, with 18–22% of the population homozygous, and an additional 50% heterozygous.

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At lower levels of nerve damage (lumbar back pain with disc herniation) association with greater pain outcome in homozygote patients is $P = 0.003$, increasing to $P = 0.0001$ for higher levels of nerve injury (limb amputation). The combined *P*-value for pain association in all six cohorts tested is 1.14 E−08. The risk profile of this marker is additive: two copies confer the most, one intermediate and none the least risk. Relative degrees of enhanced risk vary between cohorts, but for patients with lumbar back pain, they range between 2- and 3-fold. Although work still remains to define the potential role of this protein in the pathogenic process, here we present the KCNS1 allele rs734784 as one of the first prognostic indicators of chronic pain risk. Screening for this allele could help define those individuals prone to a transition to persistent pain, and thus requiring therapeutic strategies or lifestyle changes that minimize nerve injury.

Keywords: neuropathic pain; phenotype; molecular genetics; axonal injury; gene expression

Abbreviations: DRG = dorsal root ganglia; SNP = single nucleotide polymorphism

Introduction

Not all individuals with nerve injury develop neuropathic pain. Neuropathic pain is the consequence of maladaptive changes in the nervous system that lead to spontaneous pain and pain hypersensitivity (Costigan *et al*., 2009*b*). Although the risk is higher with more extensive injuries (Kehlet *et al*., 2006) and with increased age at the time of injury (Kristensen *et al*., 2009)—which we have recently suggested is connected to control of the immune response (Costigan *et al*., 2009*a*)—it is not possible to predict who is more or less susceptible among those with a similar risk exposure and age. This hinders development, investigation and application of therapies to prevent the establishment of persistent pain. Because inbred mouse strain studies indicate a large (50%) heritable component of neuropathic pain sensitivity (Mogil *et al*., 1999), it is likely that genetic risk factors are important. This cannot be teased out by traditional genetic family history studies due to the rarity of neuropathic pain-inducing events.

An alternative approach is to look for associations between allelic variations in genes and the degree of pain experienced by cohorts of patients with neuropathic pain versus controls, using single nucleotide polymorphism (SNP) association. As yet, no successful genome-wide association studies have been performed because of the complexity of the pain phenotype. However, a strategy that uses preclinical studies to identify gene candidates and then tests these for SNP associations in patients has proved effective. GTP cyclohydrolase 1, the rate limiting enzyme in the tetrahydrobiopterin synthetic pathway, was identified in injured rat dorsal root ganglia (DRG) neurons by expression profiling, followed by identification of a loss-of-function common haplotype of GTP cyclohydrolase 1 in humans associated with reduced post-surgical chronic low back pain (Tegeder *et al*., 2006), as well as lowered experimental pain sensitivity in at least three healthy volunteer cohorts (Tegeder *et al*., 2006; Naylor *et al*., 2010).

We have now used the same approach, first identifying a novel pain-related gene by mining expression profiling data in rodent neuropathic pain models, and then searching for associations between polymorphisms in the gene and pain phenotypes in human cohorts. From an analysis of global gene expression profiles in the rat DRG, across three distinct neuropathic pain models over five time points, we have identified KCNS1, a potassium channel modulatory subunit (also called Kv9.1) as a gene regulated in all neuropathic pain models tested. KCNS1, by an unbiased network analysis of the expression profiles, defines a group of genes that are co-regulated in a number of pain models, many of which are related to sensory neuron signalling and pain. We then found an association between a common amino acid–altering KCNS1 polymorphism and pain phenotype in five of six independent cohorts. The combination of a bioinformatic analysis of transcriptional changes in rodent models and human gene polymorphism association studies provides, therefore, a useful strategy to identify putative pain modulating genes that influence the risk of developing neuropathic pain.

Materials and methods

Microarray analysis

Array methods, including details of producing and phenotyping the pain in the rat models of neuropathy, tissue preparation, RNA extraction and chip hybridization, have been described previously (Griffin *et al*., 2007). Spared nerve injury, chronic constriction injury and spinal nerve ligation injury were each carried out on three separate groups of rats in accordance with the Massachusetts General Hospital/Childrens Hospital Boston animal care regulations. L4 and L5 DRGs ipsilateral to the nerve injury were dissected. Each cRNA probe was prepared using pooled tissues from five rats; for each time point three biologically independent hybridizations were performed (Costigan *et al*., 2002).

Expression profiling

An iteratively re-weighted least squares outlier-resistant regression method was used to estimate gene expression levels across each time point within each nerve injury model (Griffin *et al*., 2007). Sammon's nonlinear mapping was done to display the Euclidean distance matrix between pairs of conditions in a 2D space. Bootstrap *P*-values were calculated. The threshold *P*-value consistent with a false discovery rate near 5% was identified as 0.01 (Storey and Tibshirani, 2003). This yielded a false discovery rate for the spared nerve injury of 3.7, 7.2% for the chronic constriction injury, and 1.5% for the spinal nerve ligation. A threshold fold change of 1.25 was imposed for the expression levels averaged across all post-operative time points, relative to naïve rat expression levels.

Weighted gene coexpression network analysis

The weighted gene co-expression network analysis was performed as described (Oldham *et al*., 2006, 2008). Briefly, after selecting genes present in at least five samples, the absolute Pearson correlation coefficients between one gene and every other screened gene were computed, weighted and used to determine the topological overlap, a measure of connection strength, or 'neighbourhood sharing' in the network. A pair of nodes in a network is said to have high topological overlap if they are both strongly connected to the same group of nodes. In gene networks, genes with high topological overlap have been found to have an increased chance of being part of the same tissue, cell type or biological pathway. Network Neighbourhood Analysis provides a set neighbourhood for an initial seed or node. Using KCNS1 (Affymetrix probe Y17606) as the chosen seed, the top 30 nearest neighbours were selected using topological overlap as a measure of connection strength. We also included the microglial marker MHC class II alpha (U31598_at) as a control seed. Visual C++ implementation of the multinodeTOM software can be found at http://www.genetics.ucla.edu/labs/horvath/MTOM/.

Population stratification

Evidence of population stratification was assayed for in the Maine lumbar root pain cohort and the Israeli post-amputation stump and phantom limb pain cohort by Pritchard's Structure 2.1 using 178 ancestry informative markers (AIMs) (Pritchard *et al*., 2000; Enoch *et al*., 2006).

Maine chronic lumbar root pain cohort

We collected DNA from peripheral blood samples of 151 Caucasian adults who had participated in a prospective observational study of surgical discectomy for persistent lumbar root pain caused by intervertebral disc herniation (Atlas *et al*., 1996, 2001). For phenotyping methods and socio-demographic details of this cohort see Tegeder *et al*. (2006). Briefly, we specified the following single primary endpoint: persistent leg pain over the first postoperative year, as a reflection of ongoing neuropathic pain and designated it the pain phenotype for genetic association analysis. Leg pain was assessed on 13 occasions (at baseline, followed by 3, 6 and 12 months post-surgery, and then annually through to Year 10), using the following: 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 (Tegeder *et al*., 2006). For each patient, we calculated an area-under-the-curve score for every pain variable in the first year, and converted these to a *z*-score by comparing the patient with 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 adjusted by the following covariates: sex, age, worker's compensation status, delay in surgery after enrolment and the Short Form-36 General Health subscale. This study, Institutional Review Board (Atlas *et al*., 1996), has been approved by the National Institute of Dental and Craniofacial Research (NIH, Bethesda, MD).

Israel limb amputation pain cohort

The study was approved by the Institutional Review Boards at Sheba Medical Centre (Ramat Gan, Israel) and Beit Levinshtein Hospital (Tel Aviv, Israel) and the Ministry of Health, Israel. DNA and chronic pain data were collected from 199 Israelis of Jewish origin who had undergone limb amputations. Of the amputees, 79 had suffered traumatic battle-related amputations 10–35 years prior to joining the study and 120 had a leg amputated for medical reasons, mostly vascular insufficiency and cancer, between 1 and 5 years before the study. Each subject was asked to rate the typical intensity of their phantom limb pain and stump pain episodes and these values were used for genotype–phenotype association analyses. Israeli Jews originate from two major ethnicities: 'Ashkenazi' (i.e. North- and Eastern-European) and non-Ashkenazi ('Sephardi': North African, South European and Middle Eastern). The participants of this cohort were of one ethnicity or the other, none were of mixed origin. To minimize a possible effect that genetic differences among these ethnicities could introduce into the association analysis, we modelled the ethnicity as a covariate. Informed consent was collected from all participants.

Finland sciatica pain cohort

This group consisted of 195 patients referred to the Oulu University Hospital (Finland) due to sciatica symptoms (Virtanen *et al*., 2007). Inclusion criteria to 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 disc herniation concordant with sciatica pain. The primary outcome for the association analysis was leg pain intensity at baseline, determined with a visual analogue scale, using a 10 cm horizontal line and 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 following covariates: 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.

Denmark phantom limb pain cohort

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, Denmark. 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. Visual analogue scale scores of phantom pain intensity during the typical episode was the primary outcome for the association analysis, adjusted for covariates (age and sex).

Experimental pain sensitivity in healthy volunteers

We genotyped 185 normal volunteers who had previously been phenotyped for ratings of experimental pain (Shabalina *et al*., 2009). The subjects were all pain-free Caucasian females, 18–34 years of age, taken from a larger prospective cohort study designed to examine putative risk factors for the development of temporomandibular joint disorder. All subjects gave informed consent following protocols approved by the University of North Carolina (UNC) Committee on Investigations using Human Subjects. Volunteers were phenotyped

with respect to their sensitivity to 16 experimental pain procedures corresponding to multiple pain modalities, including pressure pain, heat pain, ischaemic pain and temporal summation of heat pain (i.e. windup). To obtain a general sensitivity measure for the present analysis, we converted the raw phenotype values to *z*-scores and summed them to create a single, aggregate pain score.

Israel post-mastectomy pain cohort

This study was approved by the Institutional Review Boards at Sheba Medical Centre (Ramat Gan, Israel), Hadassah University Hospital (Ein Kerem, Jerusalem, Israel) and the Israel Ministry of Health. The study group included 529 Israeli women of Jewish origin, some of Ashkenazi and some of Sephardi ethnicities (none was of mixed ethnicity). They had unilateral breast cancer and underwent surgical removal of the malignancy by unilateral radical mastectomy (removal of the whole breast but not including the underlying chest muscles) or breast-conserving surgery (lumpectomy) at least one year prior to joining the study. This operation was accompanied, in all women, by auxiliary lymph node dissection followed by a combination of adjuvant radiotherapy, chemotherapy and hormonal therapy. Patients ranged from 22 to 80 years and were on average 52.9 years old. Breast (and auxiliary) surgery resulted in post-mastectomy pain syndrome in ~50% of the women. Similar rates of post-mastectomy pain syndrome were reported previously for other cohorts, including the higher rates of chronic pain following lumpectomy compared with mastectomy. Using a self-administered questionnaire similar to that for the leg amputees, the typical chronic pain intensity was assessed on a numerical rating scale. Informed consent was collected from all participants. Covariates used in the regression model associating genotype with phenotype included: surgery type (mastectomy versus lumpectomy), age at surgery, years since the operation, type of adjuvant treatment and ethnicity.

Combining association results

The Truncated Product Method (Zaykin *et al*., 2002) was used to combine association *P*-values for six cohorts. This method takes the product of *P*-values that are smaller than a pre-defined threshold (set to the significance level) and evaluates the distribution of the product under the null hypothesis. One-sided *P*-values for an association with the valine allele were combined, and the result was doubled (Overall and Rhoades, 1986).

Results

Oligonucleotide microarrays were used to measure changes in mRNA expression in rat DRG in three models of neuropathic pain. Global expression profiles post-nerve injury (Fig. 1A) showed that time was less important than type of nerve injury. The relative distribution of regulated genes (Supplementary Table 1) across models is shown in Fig. 1B, and their time course in Fig. 1C. Of the global total of 1238 regulated genes, 124 were co-regulated in all three pain models (Fig. 1B), and therefore may potentially contribute to the phenotype common to the three models, i.e. mechanical and cold hypersensitivity (Supplementary Fig. 1). To analyse the 124 genes, they were grouped into functional categories (Supplementary Fig. 2). Ten co-regulated genes, annotated as participating in neurotransmission and neuronal excitability, were selected for further analysis (Fig. 1D, Supplementary Fig. 2), as they may be directly involved in changes in the somatosensory pathway that result in pain.

We considered the existing literature on these 10 candidate genes. All of the six upregulated genes in this functional class have previously been shown to undergo transcriptional regulation in response to nerve injury. These include the neuropeptides NPY, GAL, ADCYAP1 and VGF, the alpha 2 delta Ca(2+) channel subunit CACNA2D1 and the GDNF receptor GFRA1, all of which are linked to the pathogenesis of neuropathic pain (Dickinson and Fleetwood-Walker, 1999; Brumovsky *et al*., 2007; Moss *et al*., 2008; Xu *et al*., 2008). Of the four downregulated genes, only a reduction in the GABA-A receptor GABRG1 is implicated in neuropathic pain hypersensitivity (Enna and McCarson, 2006). LIN7B encodes a PDZ domain protein that modulates the acid sensing ion channel ASIC3 (Hruska-Hageman *et al*., 2004), while RAB3C modulates synaptic vesicle release (Schluter *et al*., 2004). The remaining downregulated gene, which showed greatest relative decrease among co-regulated genes, is KCNS1 and has not previously been studied in pain.

KCNS1 encodes the K(+) channel subunit Kv9.1. In common with alpha subunits of the Kv5, Kv6 and Kv8 subfamilies, members of the Kv9 group are electrically silent when expressed alone but modulate channel properties when forming heteromers with other K(+) channels (Gutman *et al*., 2005). The KCNS1 transcript is expressed in naïve rats at high levels in a subset of DRG neurons, most of which are neurofilament 200 positive, but TrkA and peripherin negative (Supplementary Fig. 3).

To see if regulation of KCNS1 reflects a structure in the transcriptome related to changes in sensory function, we performed an unbiased network analysis of the microarray expression profiles (Fig. 2) (Oldham *et al*., 2006, 2008). We used KCNS1 as a seed, and identified its 30 nearest co-associated neighbours in the network. Among the neighbouring genes, 83% (24 of 29) were expressed by neurons, while 79% (23 of 29) were involved in membrane signalling. Furthermore, 45% (13 of 29) have a published link to pain (Supplementary Table 2). This gene analysis protocol, therefore, places KCNS1 in a group of neuronal signalling molecules whose injury-induced regulation may contribute to the pain phenotype.

To investigate if KCNS1 plays a role in determining pain thresholds and chronicity in humans, we genotyped a seven SNP panel spanning a 15-kb segment of chromosome 20q12, which completely encompasses the Kv9.1 gene (Fig. 3A). We first investigated a potential association of KCNS1 haplotypes with leg pain during the first postoperative year in 151 lumbar discectomy patients from the Maine Lumbar Spine Study (Atlas *et al*., 2005). Associations with two SNPs were statistically significant: rs734784, in which the allele coding for valine was associated with greater pain than the alternative allele coding for isoleucine ($P = 0.003$); and rs13043825, an adjacent synonymous SNP in which the uncommon allele was also associated with greater pain ($P = 0.03$) (Fig. 3B). In comparison with Ile homozygotes, the relative risk of failing to achieve a 1-year pain improvement following discectomy was 2.4 for two copies of the Val allele [95% confidence interval (CI): 1.2–4.5] and 1.3 for one copy (95% CI: 0.7–2.6).

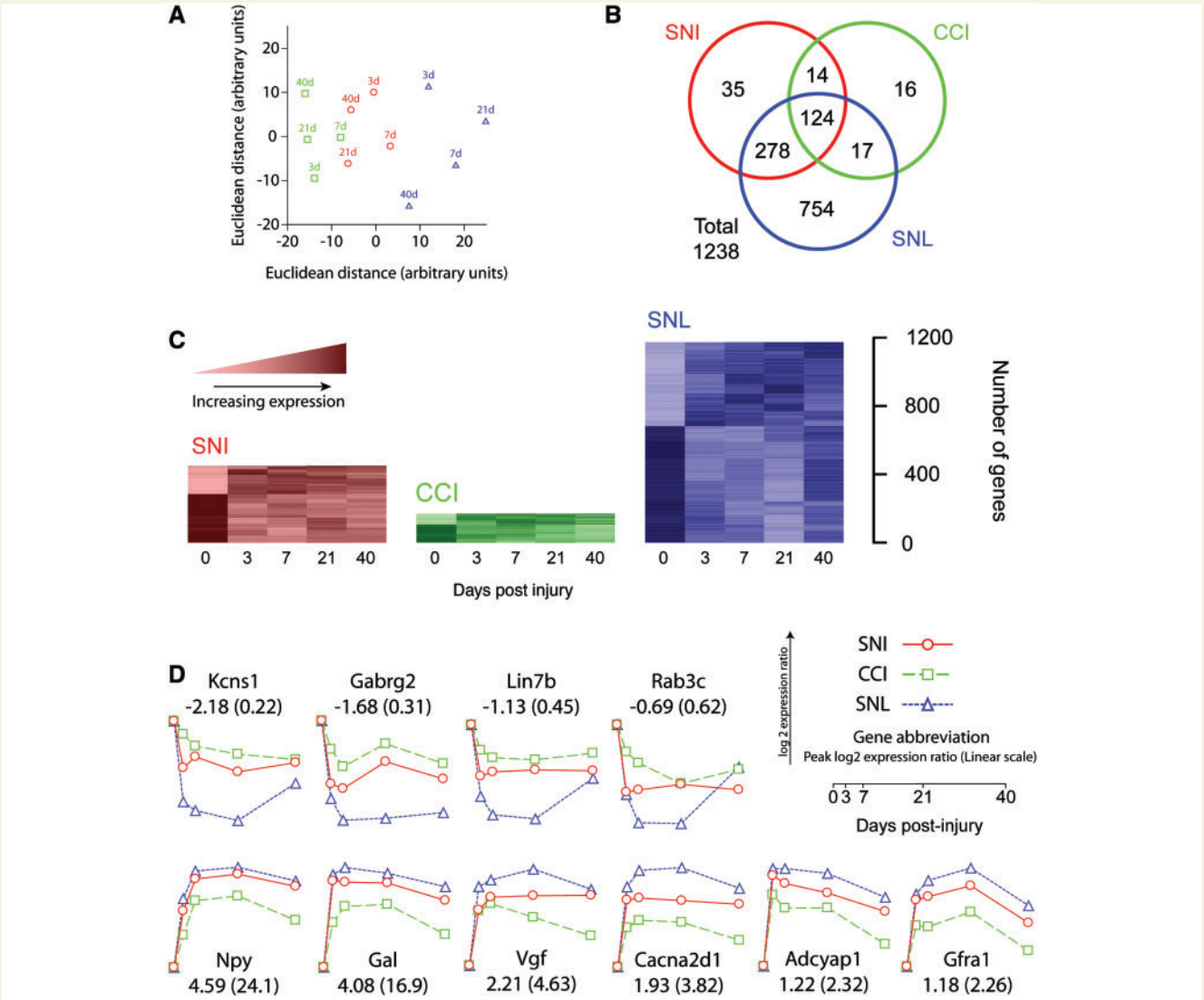

Figure 1 Global and functional DRG expression profiles in three neuropathic pain models. (**A**) Multidimensional scaling plot of the similarities among the microarrays. Data post-spared nerve injury (red circles), chronic constriction injury (green squares) and spinal nerve ligation (blue triangles) are shown with time points as indicated. (**B**) Venn diagram showing the number of regulated genes meeting fold difference and statistical thresholds in each pain model (spared nerve injury, chronic constriction injury or spinal nerve ligation). (**C**) Temporal expression patterns of genes regulated in these neuropathic pain models within the DRG. Each gene was normalized to mean 0, SD 1 and subjected to *k*-means clustering. Increased relative expression level is shown by increasing darkness. (**D**) Genes related to neurotransmission and neuronal excitability regulated in the DRG. Data shown for each gene are for spared nerve injury (red circles), chronic constriction injury (green squares), or spinal nerve ligation (blue triangles) post-injury. Each plot is on a $\log_2$ scale, with the origin at zero equivalent to 1-fold (i.e. non-regulation). The rat gene symbol, maximum difference from origin on the $\log_2$ scale, and in parentheses the maximum linear difference, are indicated. Genes are sorted from maximum downregulation to maximum upregulation. SNI = spared nerve injury; CCI = chronic constriction injury; SNL = spinal nerve ligation.

SNP rs734784 accounted for 4.6% of the variance in the pain endpoint.

The two significant SNPs were then genotyped in a cohort of 199 amputees with phantom limb pain. The Val allele at rs734784 was again associated with the intensity of phantom limb pain ($P = 0.00012$) as well as stump pain ($P = 0.0033$). SNP rs734784 accounted for 7.8% of the variance in phantom limb pain and 6.3% in stump pain, respectively. The adjacent SNP, rs13043825, was not significant for stump pain ($P = 0.056$) or for phantom limb pain ($P = 0.094$).

Detailed analysis of the haplotype structure of the genome in and around the KCNS1 gene, using three different algorithms, all produced essentially the same result. There is a strongly co-inherited 4.4 kb section of DNA in the middle of the KCNS1 coding region which contains both of the positive SNPs (Fig. 3 and Supplementary Fig. 4). We have therefore identified a KCNS1 haplotype variant associated with pain phenotype.

After characterizing each patient's ethnic background by typing 186 ancestry informative markers (Pritchard *et al*., 2000; Enoch *et al*., 2006), there was no evidence that population stratification

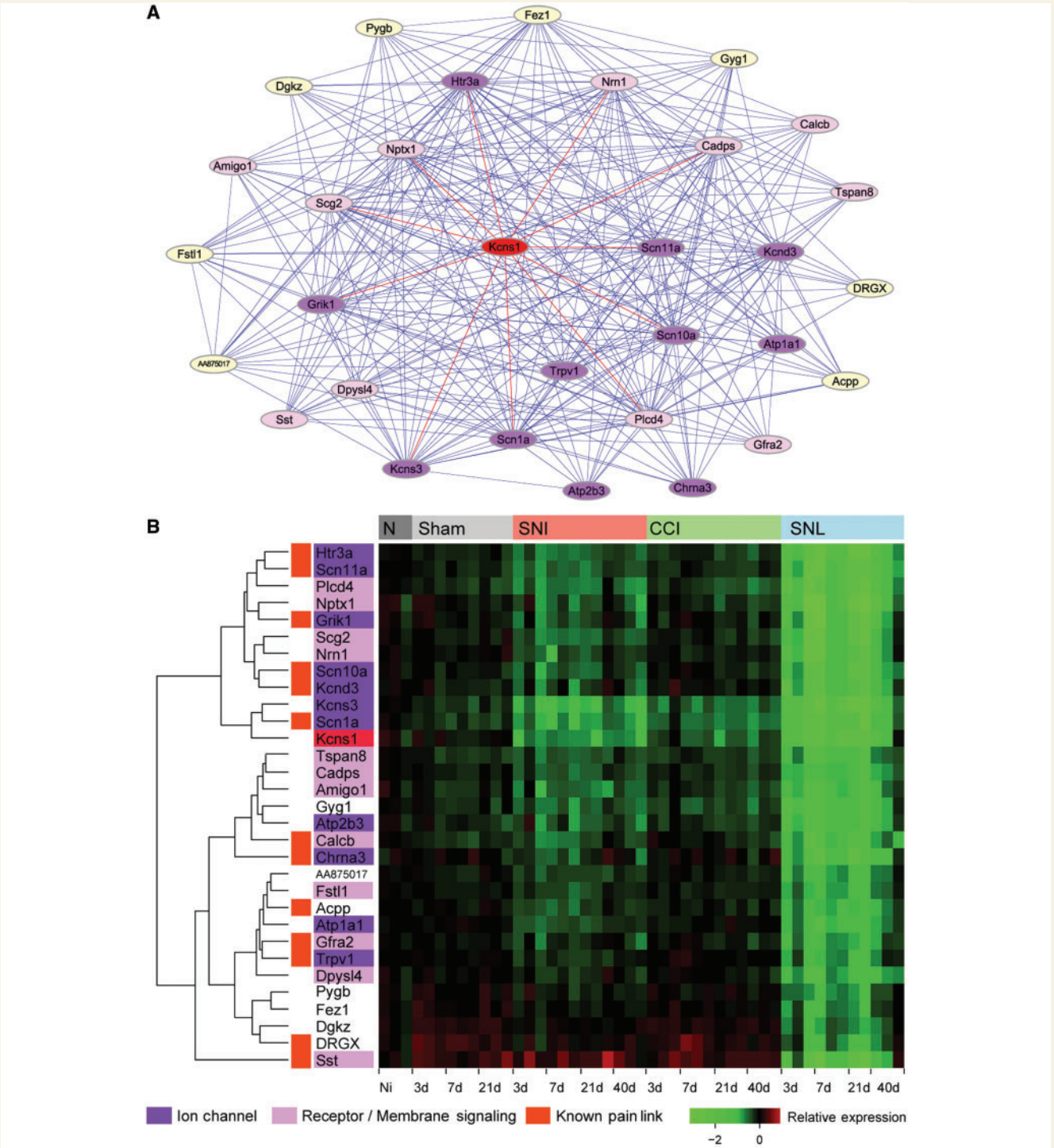

Figure 2 (**A**) Weighted gene co-expression network analysis/neighbourhood network analysis. KCNS1 was used as a seed and the 30 nearest neighbours were identified, using topological overlap as a measure of connection strength with directly connected genes identified by red links, ion channels identified as purple, receptors and membrane signalling in pink. (**B**) Heat map showing regulation of the genes in the KCNS1 30 nearest neighbours grouped by hierarchical clustering for differential expression. Red on this plot represents upregulated, with green representing downregulated. To the left are gene names highlighted for function (as above), orange indicates genes have a published link to pain.

biases contributed to the results in the Maine or Israel limb pain cohorts (Supplementary Fig. 5).

Further validation was obtained in two other independent neuropathic pain cohorts. In the first cohort, of patients with sciatica pain (the Finnish cohort), the Val allele at rs734784 was associated with more severe sciatica pain prior to discectomy ($P = 0.04$, adjusted for a significant gender effect); however, pain post-surgery or change in pain following surgery was not

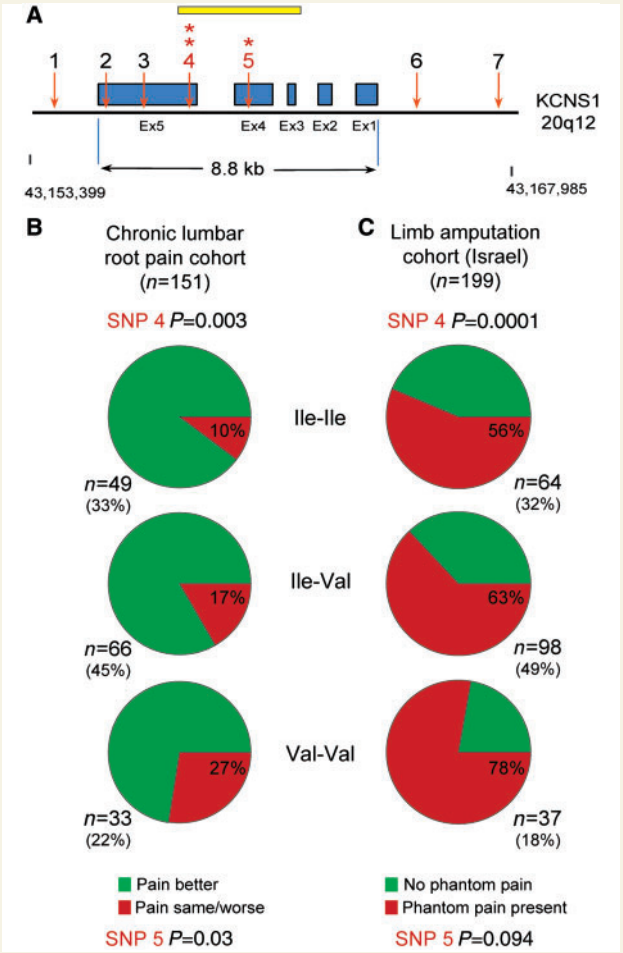

Figure 3 (**A**) Locations of seven genotyped SNPs on coding DNA strand of KCNS1. Coding exons are shown as solid blocks. The SNPs with significant association of pain phenotype are marked. Also marked in yellow is the position of the haplotype block identified in this study. **Most associated SNP, *lesser associated SNP. (**B**) Association of Val allele of KCNS1 with persistent sciatica after discectomy (Maine chronic lumbar root pain cohort). At one year after surgery, the proportion of patients describing their leg pain as improved falls from 90%, for those with no copies of the Val allele, to 73% of those homozygous for Val. (**C**) Association of Val allele of KCNS1 with phantom limb pain following leg amputation (Israeli limb amputation pain cohort). Association of Val allele of KCNS1 with proportion of amputee patients reporting no phantom pain falls from 45% for those with no copies of Val to 22% of those homozygous for Val.

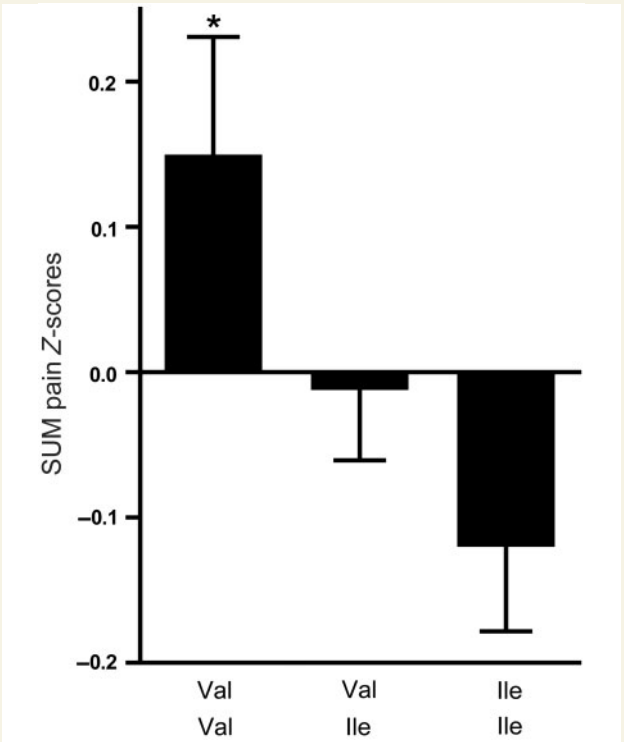

Figure 4 Association of Val allele of KCNS1 with acute pain in healthy volunteers (UNC experimental pain cohort). Combined z-score of all experimental assays (18 measures) shows additive correlation of differences in pain thresholds with those homozygous for the Val allele the most sensitive and those homozygous for the Ile allele the least. *$P<0.05$.

significantly associated with this locus ($P=0.98$ and 0.12, respectively). In the second cohort, comprising Danish limb amputees, the rs734784 SNP was associated with more severe phantom limb pain ($P=0.01$, adjusted for age and gender). We also genotyped SNP rs734784 (Val) in a post-mastectomy pain cohort, but found no evidence of an association with pain intensity ($P=0.74$).

To investigate if KCNS1 plays a role in determining pain thresholds in healthy individuals, we genotyped Kv9.1 SNPs rs734784 in a group of female volunteers subjected to experimental pain stimuli (Shabalina *et al*., 2009). The scores of all 16 experimental pain measurements were normalized and an aggregate pain score was calculated for each subject. The effect of rs734784 genotype was significant ($P=0.0360$), using ANOVA with a genotypic model [F (2,182) = 3.3851], with homozygotes for Val allele showing greater sensitivity to painful stimuli (Fig. 4).

We then tested the hypothesis that 'the KCNS1 valine allele is associated with increased pain sensitivity' over all six independent cohorts. To do this we combined the five positive associations with the negative post-mastectomy result to determine a study-wide P-value, using the truncated product method. The combined P-value for the six cohorts (1359 subjects), using the 5% truncation threshold, was $1.14E-08$.

Discussion

Rodent and human studies suggest that neuropathic pain susceptibility is genetically linked (Diatchenko *et al*., 2007; Lacroix-Fralish and Mogil, 2009; Costigan *et al*., 2009*b*). We used a convergent experimental approach that implicates KCNS1 as a gene marking the risk of developing neuropathic pain. First, we identified co-regulated genes within the DRG across three partial peripheral nerve injury models. KCNS1 was among those involved in neurotransmission or neuronal excitability, and was the most downregulated gene in this functional category. KCNS1 expression and function in the somatosensory system has not been described

previously. This prompted our focus on this gene as a possible pain-related gene.

Potassium channels have many functions in neurons, including setting the membrane resting potential and controlling action potential shape and frequency. Voltage-gated potassium channels are formed by alpha and beta subunit tetramers. Alpha subunits are numerous, with at least twelve families (Kv1–12) containing many members (Gutman *et al*., 2005). There are three Kv9 subunits (KCNS1, KCNS2, KCNS3), but each is incapable of forming functional homo-multimeric channels in heterologous expression systems (Stocker *et al*., 1999). Instead, Kv9 subunits modulate potassium channel subunits from other families as heteromers. Expression of Kv9.1 and Kv9.3 suppress the currents mediated by Kv2 and Kv3 alpha-subunit families (Salinas *et al*., 1997; Shepard and Rae, 1999; Stocker *et al*., 1999).

The effect of a decrease in KCNS1 in injured neurons would depend on which K(+) channel KCNS1 heteromerizes with, their inactivation kinetics and changes in expression of other K(+) channel transcripts after injury. Several studies have noted a reduction in potassium channel transcript expression in the DRG after peripheral nerve lesions (Ishikawa *et al*., 1999; Kim *et al*., 2002; Yang *et al*., 2004). Potassium channel openers such as retigabine, a KCNQ (Kv7.2–7.5) potassium channel opener, and BMS-204352, a K(Ca) and KCNQ potassium channel opener, are analgesic in neuropathic pain in animal models (Blackburn-Munro and Jensen, 2003; Dost *et al*., 2004).

To determine which genes are most closely co-regulated with KCNS1 we performed a neighbourhood network analysis, where a candidate gene is used to seed a network comprising its closest neighbours in the global expression profile by an iterative process, to uncover structure in the transcriptome (Oldham *et al*., 2006, 2008). The genes most closely related to KCNS1 are overwhelmingly involved in membrane signalling, including many ion channels with published functional links to pain (45%). Together, the preclinical data point to a possible role for KCNS1 in neuropathic pain.

Significant SNP associations with an altered pain phenotype may occur by chance or be due to population stratification or linkage to a neighbouring causative gene. However, we argue that this is not the case for KCNS1 because associations were tested across multiple independent cohorts and replication was found in five of six studies. Support for an association is reflected by the study-wide *P*-value for association with pain phenotype of 1.14*E*−08, a value low enough to be considered significant, even in genome-wide analyses. The sodium channel subunit Nav1.7 has also recently been shown to contain a risk marker allele (rs6746030) originally identified by Estacion *et al*. (2009). Null mutations within this gene cause complete loss of pain (Fischer and Waxman, 2010); however, this more subtle function changing SNP identified across some of the same cohorts as used in this study ($n = 1277$) achieved a combined *P*-value of only 1.00*E*−04 (Reimann *et al*., 2010), highlighting the relative predictive strength of the current findings. Lack of replication in the post-mastectomy cohort may be due to the difference in aetiology and pain phenotype, different outcome measures or differences in patient treatment protocols (Belfer and Dai, 2010). False positives due to population stratification are unlikely, as we performed an analysis of population structure and found no evidence of stratification (Pritchard *et al*., 2000; Enoch *et al*., 2006). Three further associations obtained in ethnically diverse cohorts minimize the possibility that these data represent false positives due to this confounder. Finally, the possibility that the significant associations with KCNS1 SNPs flag a neighbouring gene is unlikely because multiple haplotypic analyses find the two SNPs identified in this study contained within a 4.4 kb block completely encompassed within the KCNS1 gene. Further work is required to define if, and how, the 'valine risk' allele alters KCNS1 function or if another change in the haplo-block is responsible, and how this silent potassium channel modulates endogenous potassium currents in sensory neurons following nerve injury. In any event, we have found a common allele occurring in homozygous form in ~20% of the populations assayed (predominantly Caucasian) that strongly associates with pain following nerve injury and should prove useful in shaping treatment strategies (Kehlet *et al*., 2006). Individuals at higher risk for developing neuropathic pain need special effort to avoid nerve damage at surgery, as well as aggressive early treatment in the presence of an unavoidable nerve lesion, to prevent a transition from acute to chronic pain.

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Supplementary material

Supplementary material is available at *Brain* online.

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