OPINION

# The molecular epidemiology of pain: a new discipline for drug discovery

*Mitchell B. Max and Walter F. Stewart*

Abstract | Recent candidate gene studies have identified and replicated the first associations between several common polymorphisms and pain severity in humans. Moreover, human studies in twins suggest high heritability for responses to experimental pain stimuli. Human genome-wide association studies of pain phenotypes might identify novel analgesic targets, help to prioritize research among current targets, and increase the likelihood of success for analgesic candidates emerging from animal studies. However, clinical research in pain has largely focused on small neurophysiology-based studies, so expansion of epidemiological understanding will be essential to the success of genetic or proteomic dissection of complex pain disorders. This Perspective outlines how methods of molecular epidemiology, proved effective in the study of other diseases, can enhance the returns from human genomic studies and expedite the development of new drugs to prevent or treat pain.

Pain costs approximately US$1 trillion per year in medical treatment, loss of productivity and disability payments in developed countries[1,2], and so new treatments for treating pain are needed. Apart from new migraine treatments, appropriation of existing antidepressants and anti-epileptics, and the introduction of extended-release opioid preparations, common pain treatments have changed little since the introduction of non-steroidal anti-inflammatory agents (NSAIDs) three decades ago.

The anatomy and relevant basic science suggest that pain should be a tractable area for drug development. For example, peripheral and spinal neurons that carry pain signals are localized in a few nuclei that express unique chemical mediators that could be targets for selective drugs[3]. This accessibility of the pain system has facilitated a rapid growth in the understanding of molecular pain mechanisms. Animal models of pain[4] better resemble clinical conditions in their trait-evoking injuries and resulting behaviours than do animal models of anxiety or depression[5], or schizophrenia[6]. These pain models have shown effects of existing analgesics, including opioids, NSAIDs, gabapentin and tricyclic antidepressants[7]. The most notable therapeutic advance resulting from these models has been the new niche of analgesics that are administered into the spine directly, including opioids, clonidine and ziconitide[8].

However, the lack of clinical breakthroughs in oral or parenteral analgesics suggests that something is still missing (BOX 1). Traditional pain neuroscience studies in animals have been limited to only about 200 molecules[9], which represents less than 1% of the genome. Moreover, several novel drug classes — such as neurokinin 1 receptor ($NK_1$) antagonists, sodium channel blockers and glycine site NMDA (*N*-methyl-D-aspartate) antagonists — that appeared to relieve pain in animal studies have failed in the clinic[10–12].

Interspecies difference in how pain is processed and experienced is probably a major factor in translational failures. Pain arises from an interaction of many transmission, amplification and suppression systems, involving hundreds of molecules. Humans may have a slightly different mix of these mechanisms than other species. Moreover, pain assessment in animal models has focused on motor responses to painful stimuli. Direct reports from humans (BOX 2) provide richer data about the quality and persistence of pain in the absence of external stimulation. Data can also include temporal features, as well as associated mood disorders and side effects of analgesics.

Genomic studies of human pain might compensate for the limitations of animal studies. Specifically, genomic studies would test whether hypoexpression or hyperexpression of a molecule's activity affects human pain phenotypes, providing human validation before one embarked on the expensive animal and human safety studies that precede clinical trials of a putative new analgesic class. Recent genome-wide association (GWA) studies in humans have revealed novel genes and molecules in disorders such as macular degeneration, Crohn's disease, cancer, diabetes, lipid disorders and cardiac-related sudden death[13–15]. It is plausible that GWA studies of pain might discover new targets and help to prioritize work on known targets for drugs that are intended to prevent or treat pain.

However, GWA studies generally require thousands of patients at a cost of millions of dollars. Potential funders of such studies are likely to demand prior evidence that human pain variability is largely heritable and that the proposed study uses the most reliable and sensitive measures available to assess pain experience and to characterize pain phenotypes. This is in addition to evidence showing that other factors mediating the relationship between genes and the pain phenotype have been scrutinized.

In this article, we first review evidence on the heritability of pain from animal and human studies, and several recent findings of associations of candidate genes with human pain phenotypes. These findings suggest that a meaningful share of variation in human pain is heritable, providing a rationale for GWA studies. This is especially true for pain phenotypes for which the physical causes are clear, such as pain caused by laboratory stimuli, surgery or nerve injury. We also consider a longer-term need: large-scale genetic epidemiology studies to unravel

the causes of common pain conditions for which there are no obvious structural lesions. These conditions include fibromyalgia, chronic tension-type headache, irritable bowel syndrome, temporomandibular disorders and low back pain. We will then briefly describe how GWA studies might glean information about the molecular links between pain inputs and complications of pain such as depression, anxiety, insomnia and limitation of movement. Interested readers may consult Diatchenko *et al.*[16] for additional detail about the molecular mechanisms by which gene variants affect pain in humans and animals.

Although this article focuses on gene variants that affect pain, the principles discussed are also relevant to other molecular approaches that have not yet been applied to human pain conditions. These include the systematic cataloguing of proteins (proteomics); small molecules (metabolomics); mRNA transcripts (expression microarrays); or experience-induced modifications of DNA and adjacent histones (epigenetics).

## Heritability of pain susceptibility

To justify investments in large genetic studies of a trait, one must demonstrate that a significant proportion of the trait variability is inherited. The next section describes some of the growing body of evidence for pain heritability.

## Heritability of pain in animals

The Mogil and Devor laboratories established that pain sensitivity is substantially heritable in mammals[17–19]. They compared the thresholds and intensities of behavioural responses among 11 inbred mouse strains to 22 different types of pain stimuli, including heat, cold, noxious chemicals, inflammation and nerve injury. Heritabilities of these traits ranged from 30% to 76%, with a median value of 46%. The fact that pain-altering alleles in these inbred lines were originally derived from wild mice suggests that none of these alleles impaired survival. These findings raised the possibility that common pain susceptibility alleles are also conserved in humans.

## Heritability of pain in humans

Historically, studies in twins have been used to answer questions of human heritability. Case–control design can also complement these inferences. Both designs can be used to compare alternative definitions of the pain phenotype — to find the phenotype likely to be the most heritable — in order to follow-up with studies of candidate gene or GWAs.

Box 1 | **Why does pain research lag behind other medical fields?**

We propose that therapeutic progress will be accelerated by adapting proven molecular epidemiology methods to pain research. But why has pain research lagged behind other fields in adopting such approaches?

***Relatively low funding.*** An optimal health research funding allocation strategy would distribute resources according to the public health burden of the problem and the likelihood of gains from research. Pain does not follow this rational model. Pain is the reason for more than 20% of visits to physicians[1], and, according to IMS Health, almost 10% of drug sales were for pain indications. Pain is also by far the single largest contributor to lost productive work time[99], but is the subject of well under 1% of research funding by the National Institutes of Health (NIH)[100].

One explanation of this low funding level is that the field of pain research developed after most government funding agencies were created and their missions defined. At the NIH, there is no institute, centre or other entity with a dedicated resource of funds for optimizing pain research strategies and expanding the workforce. The topics studied depend almost entirely on applications from existing investigators. Without a major institutional champion, pain researchers have been disproportionately affected in difficult budgetary times (D. H. Bradshaw *et al.*, personal communication).

***Clustering of clinical pain research in a few clinical areas.*** Current grants for pain research are clustered into the few clinical areas favoured by the founders of pain research and their students, especially anaesthesiology, neurology, neurophysiology, psychology, dentistry and nursing. Supplementary information S1 (box) shows how the current distribution of NIH clinical pain research grants has tracked the specialty distribution of the original (1973) and current membership of the International Association for the Study of Pain. There have been few epidemiologists or geneticists, hence the lag in pain epidemiology of all types, and especially genetic epidemiology. There are also few clinical studies of cardiac, gastroenterological, gynaecological or urological pain, even though chest and abdominal pain are among the most common complaints patients make to physicians[101]. This is also despite the fact that basic pain scientists have discovered unique mechanisms of visceral pain that could be translated into compelling clinical studies[102].

Why has the pharmaceutical industry not filled this pain research innovation gap, especially as the industry spends more on pain research than governments? Even mediocre drugs could achieve billion-dollar markets as the first entry in the area. Unfortunately, industry executives have rarely been willing to commit many years to opening up a new area[103]. They prefer to use methods that are already validated by government-funded academic clinical research, but for many pain syndromes there are no trailblazers to follow.

Whether additional funding becomes available through the political process, industry investment or philanthropy, we would stress that the greatest public health gains (and for industry, the high profit margins of 'first in class' therapies) are likely to result from the recruitment of experts and approaches from areas of medicine that are under-represented in current pain research.

***Studies in twins.*** By measuring the difference in expression of phenotypes between monozygotic and dizygotic twins, one can estimate the proportion of variance in a trait explained by heredity versus environment. However, the caveat is that monozygotic and dizygotic twins may differ in the degree to which environments are shared. A much higher degree of concordance in the expression of the trait in monozygotic twins than in dizygotic twins would suggest high heritability. This can either result from large contributions of one or several genes, or small contributions of many. Several studies in twins suggest heritabilities of 50% or more for back pain[20], dysmenorrhoea[21] and irritable bowel syndrome[22]. Most of these studies do not distinguish heritability of mechanisms that lead to pain-initiating structural lesions from heritability of mechanisms of neural pain processing. In a study of 300 pairs of twins, about 25% of the genetically determined variance in chronic back pain could be explained by degenerative changes observed on the subjects' spine from magnetic resonance imaging scans[23]. The authors of the study speculated that the remaining 75% of the genetic variance included direct genetic effects on pain processing.

Recent studies indicate that there is substantial heritability of variation in human processing of pain that is evoked by uniform stimuli in the laboratory. In a study of 98 pairs of twins, 53% of the thermal pain thresholds were heritable, an estimate that the authors replicated using another 160 pairs of twins[24]. The intensity of pain provoked by six other quantified mechanical, thermal and chemical stimuli after a mild burn to the forearm was also significant, with heritabilities ranging from 22% to 55%. In another study involving 92 pairs of twins,

60% of the variance in cold-induced pain and 26% of the variance in heat-induced pain were heritable[25]. Genetic contributions to cold and heat pain had little overlap, which suggests that distinct genetic mechanisms for pain processing can be distinguished by laboratory testing (see also REF. 26).

***Case–control studies of familial aggregation.*** Case–control studies of familial aggregation are also used to implicate heritability. Although often used for rare conditions, the case–control design is suitable for the study of common pain conditions (for example, back pain and migraine). Random digit dialling phone survey methods can be used to identify cases and select appropriate controls, and to obtain pain histories from first-degree relatives to examine variation in family aggregation of pain phenotypes.

Random digit dialling phone recruitment was used to identify individuals with migraine headache and matched controls[27,28]. Cases and controls were clinically evaluated to verify status and to characterize relevant pain features. First-degree relatives of cases and controls were contacted by phone to ascertain their history of migraine. Familial aggregation, expressed as the relative risk of migraine in case family members compared with control family members, was 1.88. Onset of migraine before the age of 16 (relative risk of 2.50) and severity of headache pain in the cases predicted family aggregation. A relative risk of 1.88 means that the prevalence of migraine is 88% higher in family members of cases compared with family members of controls. Given the relatively high prevalence of migraine in the general population (that is, 10–15%), a relative risk of 1.88 translates into an attributable fraction (that is, proportion of cases in the population explained by genetic factors) of 40–50%, depending on assumptions about penetrance. For a given relative risk, the attributable fraction will be lower if the disease occurrence is lower. These types of studies estimate the maximum heritability for a phenotype, as the estimate of family aggregation reflects both genetic factors and shared environments. The results of these headache studies are consistent with comparable studies in twins[29–31].

## Candidate gene studies

One can also demonstrate heritability of a trait by showing replicate evidence that common genetic polymorphisms explain a significant proportion of inter-individual variation in the trait. Max and collaborators started with candidate gene studies, skipping the traditional first step of twin or family studies, for several reasons. First, our choice of phenotypes was informed by the accumulated experience in analgesic clinical trials[32]. This 60-year experience suggests that one can most readily detect modest effects on pain in patients caused by major structural lesions such as surgery, nerve injury or joint degeneration. It is unusual that such lesions occur in both members of a pair of twins or in multiple family members. Second, even if the same painful injury or disease occurs in twins or first-degree relatives, they may be affected at different times, thus introducing estimation error because memory for pain severity deteriorates after the injury[33]. Third, family studies without genotyping only provide estimates of the heritability of the trait. Successful candidate gene studies provide some evidence for heritability and also identify the molecular mechanisms for part of this heritability. Below, we describe several such studies.

***Catechol-O-methyltransferase.*** The first candidate gene for which multiple studies reported association with pain traits was catechol *O*-methyltransferase (*COMT*), which codes for one of the enzymes that catabolizes noradrenaline, adrenaline and dopamine. The Met allele at the Val158Met polymorphism of *COMT* was previously associated with reduced thermostability of the enzyme and reduced monoamine degradation. Many clinical, cognitive processing and brain-imaging studies suggest that the Met158 allele produces higher levels of monoamines, greater anxiety and more effective cognitive processing. The Met158 allele was associated with lower levels of endogenous opioid release and higher affective pain ratings than the Val allele during infusion of hypertonic saline into the jaw muscles of normal subjects[34].

In another study of *COMT*, a series of experimental thermal, pressure and ischaemic pain stimuli were administered to 202 healthy young women[35]. A pain-enhancing trend for the Met158 allele alone was not

Box 2 | **A few key ideas about pain and pain genes**

**Does the subjective nature of pain make it difficult to study scientifically?**
Not necessarily. Even though there is considerable variability in how individuals report their experience of the same painful stimulus, thousands of studies have shown that various dimensions of pain are reported with high validity and reliability. For many common pain types, including experimentally administered pain, post-operative pain and nerve pain, standard pain rating scales (for example, none, slight, moderate, severe; 0–10; or 0–100) scales have reproducibly shown significant effects of even relatively weak analgesics. However, for pain conditions in which there is no clear physical cause and psychosocial complications are prominent, pain scales alone are less informative and our understanding has advanced more slowly.

**How different are the molecular mechanisms that underlie various types of pains?**
This is a fundamentally important but as yet unanswered question[104]. No one knows the proportion of pain-processing molecules shared by all types of pain, nor the amount of mechanistic overlap between any two pain diagnoses. The practical corollary is that drug regulators have little theoretical certainty to tell them whether a novel analgesic should be studied in one pain condition or 20. Some unique signalling mechanisms have been identified in cases of painful nerve injury[105] or visceral pain[102]. Even more distinct, and still poorly understood, are multisomatoform pain disorders[106] — such as fibromyalgia, irritable bowel syndrome, temporomandibular dysfunction and interstitial cystitis — in which there are no identifiable peripheral cause of pain. Moreover, for these disorders, the CNS appears to amplify many or all of the painful inputs[107,108]. These patients, whose symptoms account for 9% of visits to physicians, also appear to be biologically different from patients with structurally determined pain in that the lifetime rates of mood and anxiety disorders are two to three times that of the general population[109]. Genome-wide association studies in various types of clinical pains may be the best way to understand the relatedness of the types of pain.

**What do we mean by a pain gene?**
A literature search of the key words "pain" and "polymorphism" will yield two types of reports. In the first group, polymorphisms alter the susceptibility to develop a macroscopic structural lesion that causes pain. For example, polymorphisms in the collagen IX gene that disrupt collagen crosslinking[110] increase the likelihood that an intervertebral disc will herniate, causing a painful nerve compression. Pain researchers consider these to be "disease genes" not "pain genes". Pain researchers are more interested in the second group of reports: those that focus on polymorphisms coding for molecules that affect the neural processing of pain. The borderline cases are genes that affect the microscopic anatomy of injury caused by a disease; for example, a polymorphism that affects the amount of inflammatory cytokines released at an injury site. Because analgesic scientists can use this information to develop drugs, these "microstructural disease genes" can also be considered as pain genes.

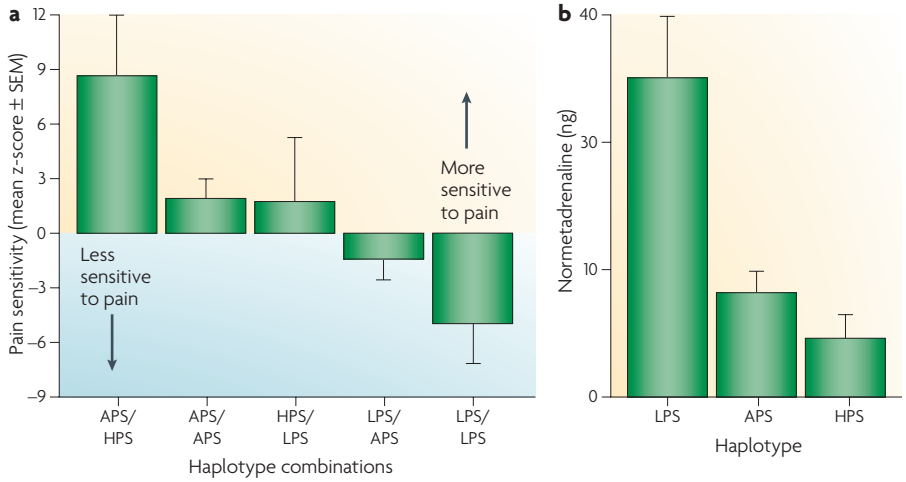


Figure 1 | **Association of *COMT* haplotypes with experimental pain sensitivity and with rate of metabolism of catecholamines. a** | The mean intensity of pain reported by 202 healthy volunteers to 16 types of experimental heat, pressure and ischaemic pain stimuli was associated with five combinations of three common haplotypes for catechol *O*-methyltransferase (*COMT*). These are termed high pain sensitivity (HPS, 10.7% frequency); average pain sensitivity (APS, 48.7%); and low pain sensitivity (LPS, 36.5%). Values on the y axis indicate the sum of z scores (the number of standard deviations above or below the mean) for the 16 tests. **b** | The investigators had proposed that pain report would be increased by genetic variants that impaired the breakdown of catecholamines. They transfected human embryonic kidney cells (HEK293) with full-length cDNA clones corresponding to the three *COMT* haplotypes, and incubated the cells with the catecholamine neurotransmitter noradrenaline. As predicted, cells with the LPS *COMT* haplotype metabolized noradrenaline to normetadrenaline more rapidly than the HPS haplotype ($p < 0.01$). Error bars in both panels represent SEM. This figure is modified with permission from REF. 35 © (2004) Oxford University Press.

statistically significant. Several other studies[36,37] have also failed to show an association between the Val158Met polymorphism and pain. However, Diatchenko *et al.*[35] went on to show that addition of a nearby synonymous single nucleotide polymorphism (SNP), rs4818, to the analysis explained a significant part of the variation in experimental-pain response. Three common combinations of alleles at these two SNPs were present on 96% of chromosomes. And according to experimental pain responsiveness were termed high pain sensitivity (HPS, 10.7% frequency); average pain sensitivity (APS, 48.7% frequency); and low pain sensitivity (LPS, 36.5% frequency) haplotypes (FIG. 1a). The HPS and APS haplotypes were also significantly associated with a higher likelihood of the subjects developing temporomandibular pain disorder during a 3-year follow-up period, with *COMT* haplotype and baseline psychological scales making independent contributions to the risk[38]. Because only 15 new cases occurred among the 202 subjects, these findings are tentative pending replication in a larger sample size. Additional studies to explain how *COMT* variation might affect pain are needed to translate this result into therapeutic interventions. One such example was provided by Nackley *et al.*[39], who used animal studies to suggest that the amplification of pain by low COMT activity appears be mediated through $\beta_2$-adrenergic and $\beta_3$-adrenergic receptor mechanisms, thus providing a rationale for clinical trials of β-adrenergic blockers as pain modifiers.

The HPS haplotype surprisingly included Val, not Met, at codon 158 (REF. 35). Human embryonic kidney cell lines (HEK293) transfected with the HPS haplotype broke down noradrenaline into normetadrenaline at only one-seventh of the rate of the LPS haplotype (FIG. 1b). Based on computer modelling of mRNA folding energies, it was proposed that the HPS haplotype produced mRNA that was highly stable in the hairpin configuration, in which mRNA cannot be translated into protein. A subsequent study confirmed this hypothesis by showing that directed mutagenesis at sites that released the mRNA from the stable hairpin structure, restored the rapid translation of mRNA into COMT[40].

These studies suggest that some pain-response traits may be among the most robust of psychological variables, which are measurable with enough precision to reveal novel molecular mechanisms of behaviour.

***GTP cyclohydrolase 1.*** The *GCH1* gene codes for GTP cyclohydrolase 1, a rate-limiting enzyme for synthesis of tetrahydrobiopterin, an essential cofactor for catecholamine, serotonin and nitric oxide production. *GCH1* was prespecified as one of the four highest priority candidates for human testing to emerge from a whole-genome microarray study of mRNA expression in the dorsal root ganglion of rats given three types of painful nerve injuries[41]. After nerve injury, levels of mRNA and protein for this molecule rose 6–10-fold (FIG. 2a). Administration of a GCH1 antagonist reversed behavioural signs of nerve pain within 60 minutes, and also inhibited inflammatory pain in other rat models.

Fifteen SNPs in *GCH1* were then genotyped in 168 patients who had undergone lumbar discectomy for lumbar nerve root compression. Uncommon alleles of five of the SNPs were significantly associated with lower scores of persistent pain over the year following surgery, as was a haplotype of 15% frequency that combined all five SNPs (FIG. 2b). The effects of this putative "pain-protective haplotype X" were then confirmed on experimental pain perception in the 202 normal subjects described above[35] and in 245 additional normal subjects (FIG. 2c). None of the significant SNPs caused obvious changes in protein coding. However, further experiments showed that the lymphocytes of the patients carrying the pain-protective haplotype who had undergone spine surgery produced significantly less mRNA, enzyme and enzyme product than those with the other haplotypes[41]. This finding was confirmed in two cohorts of normal German subjects[42] (FIG. 2d). Analyses of pain scores from two other experimental pain cohorts[42] (C. Campbell *et al.*, personal communication) support the effects of the pain-protective *GCH1* haplotype. On the basis of initial findings, a small pharmaceutical company (Solace Pharmaceuticals; see Further information) is developing GCH1 blockers as analgesics. However, there is also one published report showing no association of these SNPs with experimental pain or pain after oral surgery[43]. Additional studies are needed before the association of *GCH1* polymorphisms with pain perception reaches the high level of certainty attained by many polymorphisms associated with other diseases.

To summarize, common genetic variation accounts for much of the variability in pain responses in animals. Several replicated studies of candidate gene studies and in twins suggest that this heritability may

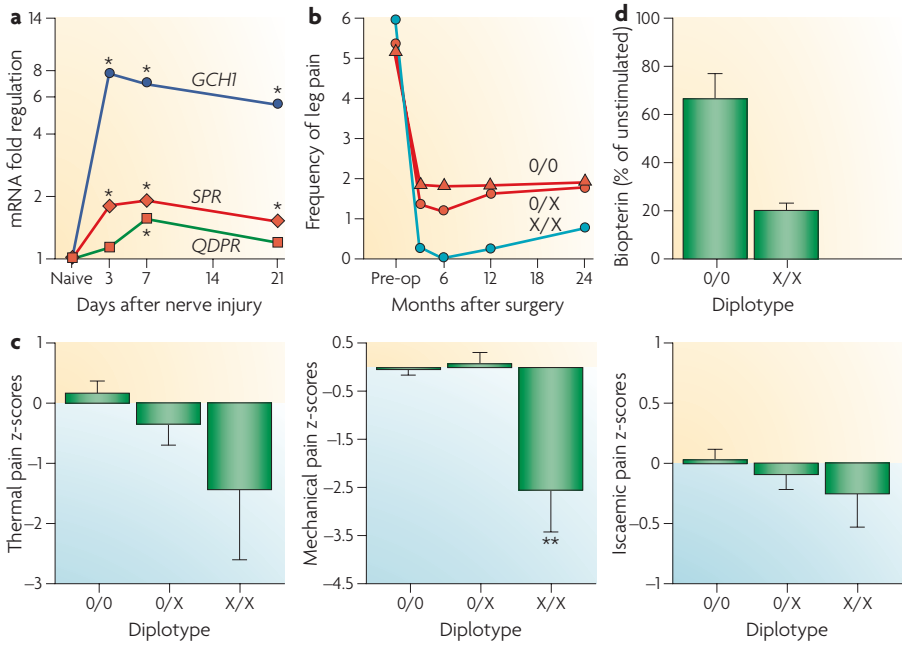


Figure 2 | **Association of *GCH1* haplotypes with human chronic spinal nerve root pain, experimental pain and synthesis of biopterin. a** | mRNA of GTP cyclohydrolase 1 (*GCH1*) was significantly upregulated for 3 weeks in $L_4$–$L_5$ dorsal root ganglia of rats that had undergone experimental painful nerve injury (spared nerve injury model). Levels of mRNA levels coding for two other enzymes in this pathway, sepiapterin reductase (*SPR*) and quinoid dihydropteridine reductase (*QDPR*), were only modestly increased (*$p < 0.05$). **b** | Pain-protective effect in the first post-operative year of a *GCH1* haplotype of 15% frequency in 168 patients who underwent discectomy for lumbar disc herniation with painful nerve irritation, compared with all other haplotypes. 0/0 ($n = 116$), 0/X ($n = 42$), and X/X ($n = 4$) denote patients with 0, 1 or 2 copies of the putative pain-protective haplotype X. Values on the y axis indicate pain frequency: always (6); almost always (5); usually (4); half the time (3); a few times (2); rarely (1); and not at all (0). Pre-op: before surgery. **c** | Effect of number of copies of pain-protective haplotype X on experimental pain sensitivity in healthy volunteers. X/X ($n = 10$), 0/X ($n = 153$), 0/0 ($n = 384$). Haplotype X tended to reduce ratings of thermal, mechanical and ischaemic pain; this was significant for mechanical pain (**$p < 0.01$ for X/X compared with the 0/0 group. **d** | Biopterin in supernatant of forskolin-stimulated whole blood from healthy volunteers. X/X ($n = 10$), 0/0 ($n = 11$). Consistent with the hypothesis that biopterin analogues are essential for synthesis of pain-producing nitric oxide in the dorsal root ganglion, the pain-protective haplotype X is associated with lower biopterin levels ($p = 0.002$). Moreover, in immortalized lymphocytes from another group of subjects, the pain-protective haplotype X is also associated with lower levels of mRNA expression, GCH1 protein on Western blot, and biopterin[41]. This figure is modified with permission from *Nature Medicine* REF. 41 © (2006) Macmillan Publishers Ltd. All rights reserved.

extend to humans. These results make it plausible that GWA studies might identify other novel pain mediators.

## Designing GWA studies for pain

GWA studies can either use a case–control design or a longitudinal design, in which the time-course and severity of pain is studied in relation to a provoking injury or disease. We now review important elements of these designs.

### Sample size needed

It has been calculated that one can independently test common genetic variants at one million loci in the genome (that is, every common variant) for association with a disease with only eight times as many subjects as it takes to test a single locus[44] (FIG. 3). For a single locus, the sample size required is largely driven by the prevalence of the at-risk genotype, the minimum odds ratio to be detected, the desired power to detect the odds ratio, and the acceptable false-positive rate. The sample size requirements are greater if multiple loci are to be evaluated. This is to allow a correction for the tendency to accrue more false-positive findings. To detect a SNP that doubles the risk of the disorder while correcting for the hundreds of thousands of SNPs tested, one needs at least a thousand cases and a thousand controls[45]. When cases are difficult and controls are easy to ascertain, it is sometimes sensible to select two or more controls per case to increase power. For example, given an allele with a population frequency of 10% and 1,000 cases, a control group of 2,000, 3,000 or 4,000 will provide the same power as a study with a 1:1 case–control ratio and approximately 1,390, 1,600 or 1,710 cases.

Publicly available GWA databases of thousands of healthy subjects offer a low-cost means of obtaining already genotyped population controls[46,47] with the caveat that the sample will be contaminated with cases. This contamination, which will bias estimates of associations towards the null, is more than compensated by the large size of the control group. However, there are other potential limitations. Publicly available GWA databases may differ from a particular case group under study in ancestral background[48], or may differ from the cases in other unspecified ways for which it is impossible to correct (that is, measures relevant to selection or confounding are not available). One must also estimate and correct for biases in allele frequency caused by genotyping cases and controls in different laboratories.

Many interesting disease-susceptibility polymorphisms are likely to have a relative risk that is substantially lower than 2.0 (REF. 46). To detect these smaller effects or gene–gene or gene–environment interactions, substantially more than 1,000 cases and 1,000 controls may be required. At the time of writing, we are not aware of any clinical cohorts of patients with DNA and pain data that approach even these minimal numbers recommended for GWA studies. Once such cohorts are available, the ability to detect smaller effects may be enhanced by integrating multiple sources of data. For example, replication across human cohorts or weighting *p* values according to information about the effects of each human SNP on gene function, or genome-wide syntheses of animal data on pain (BOX 3).

Typical phenotyping costs of several thousand dollars per patient and GWA genotyping costs of $500 per chip currently limit the size of fundable studies to several thousand individuals. However, as GWA genotyping costs decline over the next decade, it will be possible to conduct much larger studies if the appropriate clinical data is routinely collected by health-care systems and research networks. Therefore, researchers involved in pain genetics should contribute to next-generation pain measures that are under development[49], and advocate the inclusion of pain assessment tools in their local clinical databases or in population genetics studies that are ongoing or

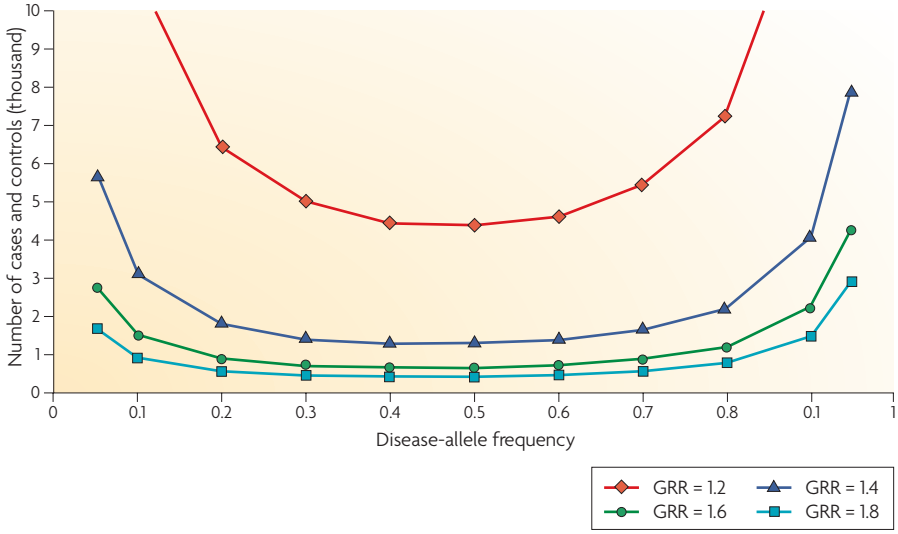


Figure 3 | **Required number of cases and controls for whole-genome association study.** The number of controls and cases required to detect varying disease allele frequencies and genotypic relative risks (GRRs) with 80% power — assuming a genome-wide association study of 500,000 independent single nucleotide polymorphisms (SNPs) — is shown. To correct for this number of comparisons and maintain a *p* value of 0.05, one must reach a nominal *p* value of $1 \times 10^{-7}$ or less. A multiplicative model was assumed — relative risk for a homozygote = (relative risk for a heterozygote)$^2$ — and numbers were adjusted for a mean $r^2$ of 0.97, implying a close correlation between the SNP on the array and the common disease allele. This figure is modified with permission from *Nature Protocols* REF. 45 © (2007) Macmillan Publishers Ltd. All rights reserved.

under consideration. If cases and controls are plentiful but the budget does not permit all individuals to be genotyped, one can maximize power by selecting extreme individuals for genotyping and analysis; for example, those with the most severe extent of the phenotype versus those who may have even fewer signs of the phenotype than the population average.

## Choice of pain condition

Given the sample size demands for GWA studies, common pain disorders may offer an advantage in terms of a large sample size available for study. However, several other considerations will ultimately inform this choice.

***Study a relatively uniform cause and type of pain.*** There are compelling reasons to focus on pain symptoms that are defined by a common lesion or clinical syndrome.

First, the more homogeneous the inciting lesion and type of pain, the better the chance of finding that a genetic variant affects patients similarly. The reduction in error variance will increase the power to detect a small gene effect.

Second, patients with different pain conditions will vary in other ways that increase the variance and reduce the power to detect gene effects. For example, a completely different set of factors may mediate the onset of chronic daily headache (female preponderance and no variation by age) versus chronic osteoarthritis (that is, increasing risk with age)[50,51].

Third, completely different interviews are needed to adequately characterize the pain phenotype in different conditions, for example, in chronic daily headache versus irritable bowel syndrome.

***Weigh the trade-offs between the most precise pain model and generalizability to a large number of pain patients.*** If the processing of all types of pain were the same, the obvious choice for genetic studies would be experimentally inflicted pain in normal young adults. This is because the intensity and site of noxious thermal, electrical, mechanical or chemical stimuli can be the most precisely controlled. In addition, the neural processing mechanisms are probably quite similar, and responses of the subjects are least likely to be affected by chronic disease or cognitive impairment. Several groups have already phenotyped several thousand normal subjects for responses to laboratory pain[25,35]. Within several years, GWA studies of these and other experimental pain cohorts may provide clues to novel pain-processing molecules. Studies in mice[13] and humans (for example, the *COMT* and *GCH1* examples above) suggest that in some cases, genetic variants that influence acute experimental pain will also affect some chronic pain conditions.

However, studies of laboratory-induced pain have serious limitations. Pain caused by disease or surgery activates mechanisms that differ from those triggered by experimental pain protocols[52]. Moreover, the cognitive and affective processes and consequences of clinical pain differ substantially from those of experimental pain. Experimental pain studies may miss key phenomena for which we want to develop drugs.

Surgery and other procedures provide a uniform trauma in a controlled environment, and evoke some of the co-morbidities that are important in chronic pain such as anxiety, depressed mood, impaired movement and insomnia.

Analgesic studies in well-standardized conditions such as third molar extraction[53] or orthopaedic procedures such as total joint replacement[54] or bunionectomy[55] approach laboratory pain in their precision in detecting analgesic drug effects.

Studies of acute pain may successfully model some features of chronic pain. For example, success of drugs such as NSAIDs, cyclooxygenase 2 (COX2) antagonists, opioids and tramadol in acute-pain studies have predicted efficacy in some chronic pain conditions. For these reasons, we consider short-term pain caused by surgery to be an attractive phenotype for initial studies of clinical pain in GWA studies. As in analgesic pharmacology, post-operative pain can serve as a model for other clinical pain conditions, as well as to directly suggest treatments that could treat perioperative pain or prevent its chronic persistence[56].

Whichever pain condition is chosen, we urge researchers to take a fresh look at assessing any factor that might explain variance in the primary pain outcome. Comprehensive measurement of a broad set of variables is common in epidemiological studies, but rare and potentially valuable in biomedically oriented pain studies such as analgesic clinical trials. For example, variance in perioperative pain is likely to be influenced by the amount of surgical trauma, variations in administration and response to general and local anaesthetics. Perioperative pain can also be influenced by concomitant use of a rescue opioid, propensity for pain-related catastrophizing and sensitivity to experimental pain.

A chronic pain example is the *GCH1* spinal pain study described above[41]. Incorporation of important clinical variables

into the analysis — workers' compensation status, general health as related on the short-form-36 measure of quality of life and the delay of surgery after enrollment — explained 18% of the variance in the chronic pain score before any genetic analyses. This gave an improvement in power equivalent to that which would have resulted from increasing the sample size by more than 20%.

Despite the attractiveness of the controlled perioperative environment, many chronic episodic and chronic pain disorders, including nerve injury pain and pains without known peripheral lesions, cannot be modelled acutely on the surgical ward. There is greater complexity in studying these chronic pain phenotypes, including a much larger component of variance that is contributed to by environmental factors and by effect-modifying constitutional factors. Therefore, the sample size required to evaluate genetic factors will be substantially greater than that required for experimental pain studies. However, GWA studies of chronic pain conditions could have enormous impact if mediating molecules found in acute pain studies are replicated or new mediators are discovered.

## Design of chronic pain GWA studies

The authors' professional traditions lead us to prefer different approaches to GWA studies of chronic pain. That is, clinical pharmacologists seek to minimize variance as part of the design, choosing cohorts with similar, measurable structural lesions that initiate pain (for example, as in the study of lumbar nerve pain cited above). The primary outcome and case definition is determined by the patient's report of pain severity at a given time after injury.

By contrast, epidemiologists prefer to retain variance in a study sample to reflect real-world complexity, and would use GWA studies to examine refractory pain-related disorders without a clear-cut inciting lesion. Pain epidemiologists have adopted a widely used model of disease progression to study how exogenous and genetic factors influence transitions between different stages of pain (for example, episodic versus chronic). In principle, case and control definitions in this context are relative, based on the history of pain experience (for example, symptoms, episodicity, persistence and intensity), and reflect phenotypes that differ by stage of progression. Genetic, environmental (for example, physical stress and diet) and other (for example, mood) factors are studied to understand why individuals do or do not transition from an episodic to a chronic persistent state of pain. In the remainder of this section, we consider how this framework is used to examine the role of genetic factors in pain disease progression.

Adopting a model used for other diseases[57], epidemiological studies of pain assume that individuals can progress through different stages of pain, in which the end stage is a chronic persistent state that may or may not be reversible. In this model, the full variability of expression of a given pain condition is defined (FIG. 4). How each stage is defined may differ by pain condition. For example, Von Korff *et al.*[58] empirically defined four stages in which current status (that is, level of pain and impairment) predicts future status. Fluidity in moving from one stage to another and back again is reflected by the relative size of the arrows (FIG. 4). Movement from left to right is less reversible because as one moves further to the right, changes in molecules and cellular and tissue structures increase the resistance to reversibility. The definitions of each stage in this model are arbitrary at first, but increasingly informed by evidence. More generally, the notion of progression inherent to the model is not arbitrary. Population-based longitudinal studies[58–60] demonstrate that most individuals with a given pain disorder are in dynamic flux, in which frequency and duration of acute episodes vary[60]. Even the persistent pain state (for example, back pain every day) is dynamic for most individuals. Only a minority will remain irreversibly in the chronic persistent end-stage. Pain clinicians and researchers agree that pain of long duration tends to become more difficult to reverse, but the mechanisms for this remain unclear[59,61]. We suggest that the elucidation of these mechanisms should be a major aim of the molecular epidemiology of pain.

### Box 3 | Integrating GWA data with other convergent translational approaches

FIGURE 3 shows that a single genome-wide association (GWA) study has limited power on its own to detect small genetic effects of common single nucleotide polymorphisms (SNPs). But the drug developer is interested in small effects and rare variants because they validate the target as a pain mediator. If the effect is small because the SNP alters function of the product by only 2%, it does not matter, as the synthetic chemist may be able to devise an agonist or antagonist with a 100% effect on the target molecule's function. How can pain researchers use other sources of data to confirm small effects despite the severe statistical penalty of correcting the *p* value for tests of 500,000 or more SNPs? Suggestions are listed below.

***Replications in other human cohorts.*** Reviewers of GWA studies often demand the availability of other human cohorts for replication, ideally with the same phenotype.

***Weighting of GWA SNP p values.*** Weighting of SNP *p* values can be supplemented with data from outside the study[111,112]. These weights may include:

- Human genome annotation data showing that the SNP affects the biochemical function of a cell, or is likely to have such an effect. This could be because it alters amino-acid coding, or is located in a site crucial for initiation of mRNA transcription or splicing, or because its common allele is conserved across species.
- Animal data showing that a variant in that gene explains variability in pain behaviour among strains[17,18]. Although humans and mice are unlikely to share identical variation at a particular SNP, such data give a double boost to a human candidate gene. This is because it suggests that the gene is critical for mammalian pain processing, and that variation in the gene did not impair the survival of the wild mice that were recent ancestors of the inbred laboratory strains.
- Other animal data linking a molecule to pain. For example, altered levels of mRNA expression at the time the animal develops pain[113], alteration of pain behaviour by gene knockout[114] or hyperexpression, or more traditional anatomical or pharmacological studies.
- Re-analysis of GWA data to integrate small effects that have occurred in the same biochemical pathway into a *p* value for the whole pathway[115].

Genomic purists might prefer that the primary statistical analyses stick closely to the raw data, pointing out that a major advantage of the GWA method is to uncover new types of human genetic regulation. An example described in the main text, although it did not come from a GWA study, are the studies of the association of catechol *O*-methyltransferase (*COMT*) with pain. If SNP rs4818 had been down-weighted in a GWA study because it had no obvious function, the investigators may not have let this result lead them to a new mechanism for regulation of mRNA translation into protein.

Regardless of the method by which one integrates diverse human and animal databases, it is clear that the interpretation of either animal or human 'omics' studies are enhanced by interactions among experts who can translate these results across species and methods.

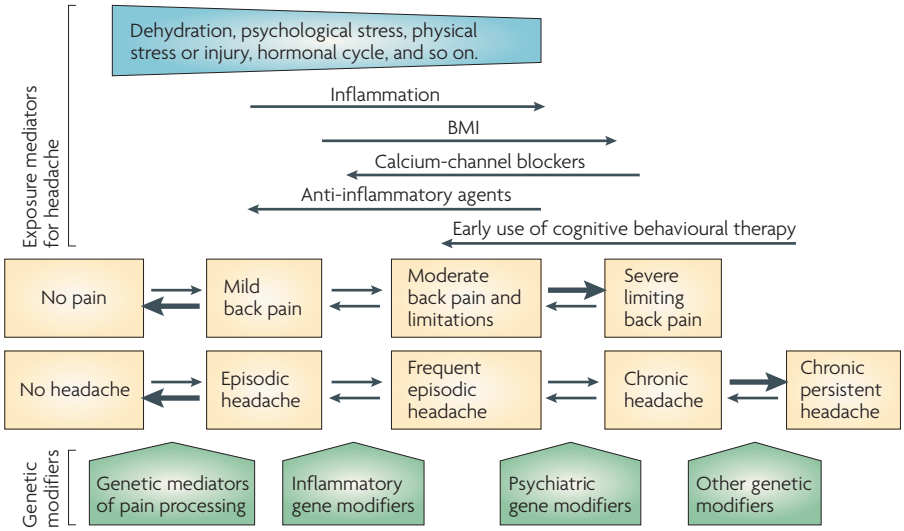


Figure 4 | **Models of back pain and headache pain progression.** The role of exposure mediators and genetic modifiers of risk in transitioning from one state to another in models of back pain[58] and headache pain is shown. The several types of genetic modifiers below may be involved at multiple transitional stages. Epidemiological studies use standard terms to characterize how a particular measure influences transition among stages. Mediators are variables that directly influence transition back and forth between stages (that is, reflected by arrows linking the stages). Environmental exposures (for example, diet, psychological stress and percent of body fat) are typically viewed as mediators. Often, however, the measurement of a variable is distantly related to the underlying molecular mediator. In this case, the measurement is denoted as a surrogate for a latent or underlying process. For example, body-mass index (BMI) is known to be associated with higher frequency of migraine headache[120]. But BMI itself may be a surrogate for percent of body fat, and both of these variables are positively associated with circulating levels of inflammatory mediators[121]. The term 'effect modifier' is used to characterize a variable that modifies the influence of a mediator. Genotypes are usually defined as modifying the role of an environmental exposure in disease expression and progression, whether or not the exposure is explicitly defined. Specifically, genotypes modify the influence that exogenous factors (for example, stressors, inflammation and head injury) have on the likelihood that a pain condition will progress or regress and on variability in expression at a given point in time. Thus, the term effect modification is intended to be somewhat intuitive, indicating that the relationship between a mediator and stage of a pain condition differs for those with the at-risk genotype versus those who do not have the at-risk genotype. Depending on the stage, a single variable may act as a mediator or modifier.

The cohort or case–control design can be used to study the factors that influence change from one stage to another. For simplicity, we describe use of the case–control design. For instance, the case–control design can be used to compare individuals from two or more different stages on genetic, environmental and other factors. Selection of cases and controls depends on the stages and questions of interest. The case group is typically defined by the stage at the far right of interest, whereas the control group(s) is defined by one or more stages to the left of the case group (FIG. 4). We first consider an example for which the initiating insult is well defined, followed by an application for which the initiating insult is not well defined.

After surgery, some patients experience pain at the site of the lesion beyond the time period that would be expected (that is, chronic pain). Fewer patients experience post-surgical pain for unusually long periods of time (that is, chronic persistent). To understand mediators between these different states, the case group is defined as having chronic persistent pain as a consequence of surgery. Controls must have had the initiating insult. In addition, more than one control group (for example, transient pain only versus chronic pain, but not long-term chronic persistent pain) may be sensible, depending on the question (for example, transition to chronic pain versus persistence in a chronic pain state). Data collected from cases and controls can include history of psychiatric disorders, assessment of traits (for example, personality), exposure to stressors, genome-wide profiles and measures that are relevant to other domains. Cases and controls are then compared on individual domain-specific measures as well as interactions among measures from different domains (for example, gene and environment) to understand the combination of factors that maximally distinguish each of the defined case and control subgroups. We think that studies that are more comprehensive in scope will accelerate understanding of the interactions between mediators and genetic modifiers of variability in pain phenotype expression.

This same model is used for common pain conditions (for example, chronic back pain, chronic daily headache) in which the overt initiating event is not known[62,63]. For instance, the following three groups would be studied to understand factors that are involved in transitioning to persistent chronic daily headache: individuals with persistent chronic daily headache, individuals with a history of chronic daily headache, and individuals with episodic migraine or tension headache but without a history of chronic daily headache. The case–control design is well suited for this type of study as current headache status and historical episodes of chronic daily headache (that is, headaches at least every other day for 6 months) are likely to be reasonably well recalled. If the focus of interest is onset and persistence of migraine then an optimal design would involve a comparison of the following groups: individuals with no history of migraine, individuals with a history of migraine that has remitted, and current migraine sufferers. As a majority of new onset migraine occurs before the age of 35 (W.F.S., unpublished observations) it may be sensible to impose an age criteria for the three groups. Moreover, as genetic loading is associated with age of onset[27] or duration of time with a condition, a duration of time criteria may be imposed on the active migraine case group.

### Ascertaining cases and controls

The method used to ascertain cases and controls will depend on the incidence and the prevalence of the condition and whether incident or prevalent cases are required. It will also depend on the proportion and representativeness of those with the phenotype of interest who seek care, concerns about selection bias, and a range of other factors.

Selection bias can pose a problem if the phenotypes of interest are ascertained through specialty or even primary-care clinics versus direct ascertainment of the general population. In specialty-care centres, patients typically represent a highly self-selected sample and may not be representative of the phenotype of interest. Numerous

other factors (for example, psychiatric and behaviours related to use of care) that may be genetically mediated influence who seeks care. For example, patients with migraine who are treated by a specialist (that is, versus a primary-care physician) are more likely to be treatment-resistant, to have psychiatric co-morbidities, and to have other traits related to use of care than a population-based sample of patients with the disorder of interest[64–66]. More importantly, use of specialty care for selecting cases poses significant challenges in identifying appropriate controls who are equally select. Numerous traits, other than those directly related to the pain phenotype could yield significant identification of SNPs that have little or no molecular relation to the pain disorder of interest.

Choosing incident (that is, new onset) or prevalent cases will depend on whether the primary focus of interest is in mediators of onset of the condition or persistence and variability of pain experience. Recruitment of prevalent cases offers the advantage in that they are considerably more common than incident cases. However, recall of crucial pain events can be a challenge if it is not reliably reported (see below).

Large representative samples of potential cases can be ascertained through the use of insurance or health-plan claims' databases, or electronic health record databases from integrated delivery systems[64,67,68]. However, documentation of case or control status in these databases will not be complete, requiring supplemental data collection to confirm case status and to characterize phenotypes that will serve as controls. Supplemental data collection might include phone interviews to obtain history of pain, longitudinal observation of pain or clinical examinations. Once initial contact is established with cases and controls, follow-up mailings of questionnaires and saliva DNA kits offer a highly efficient means of obtaining other data (for example, co-morbidities and other traits) and DNA.

If the pain disorder is relatively common, other means of ascertainment may be sensible. Mailed questionnaire surveys sent to randomly selected households offer a relatively low-cost means of ascertaining phenotype variants of common disorders. For example, the prevalence of chronic daily headache is 4%. Diagnostic questionnaires can be used to ascertain headache by large-scale mailed questionnaire surveys[69]. Finally, a blend of clinic-based and population-based cases may be sensible when cases are rare and when patients are highly likely to seek care (for example, those with persistent chronic daily headache).

Box 4 | **Data collection for genome-wide association studies of pain**

Obtaining data directly from patients suffering from pain is usually essential to phenotyping. Where possible, standardized instruments and questionnaires with proven reliability should be used. If instruments must be created *de novo*, measurement reliability should be proven before initiating data collection. To do so otherwise is needless gambling. A study of reliability (that is, collect the same data twice on the same group) and internal consistency in several hundred individuals is relatively inexpensive, usually highly feasible, can be completed in a relatively short period of time, and is invaluable in guiding whether data collection should proceed or data collection procedures and instruments should be improved. As a benchmark, test–retest correlations (that is, Spearman's or Pearson's) and Cronbach's alpha of phenotype measures should be at or above 0.8. Often, accuracy is given priority over reliability. In general, however, a measure will not be useful if it is accurate, on average, but not reliable. For example, if the average duration of a pain episode is reliably reported but, on average, is overestimated, the strength of associations will not be affected by the bias.

The time and cost of collecting data directly from patients varies substantially, depending on the type of data needed and the method used. Collecting data in a single interview to obtain a retrospective history of pain experience (for example, age of onset, pain features and changes over time) is considerably less costly and demanding to obtain than repeated prospective interviews. A detailed retrospective history may be the only reasonable option if phenotyping requires an understanding of how the pain condition evolved over time (for example, age of onset and history of chronic pain episodes). On the other hand, a prospective design may be optimal if the phenotype is largely defined by how the pain condition evolves over months or several years from initial onset. For example, post-surgery pain phenotypes may be defined by the variability of pain experience (for example, frequency of acute exacerbation and persistence) for which prospective data are required.

There are no overarching rules for which method of data collection from patients is optimal. Phone interviews or face-to-face interviews are substantially more costly than mailed questionnaire surveys. Participation rates are higher and quality control is usually better when using the former methods, especially phone interviews. These different methods are often used in combination, as there are often complementary strengths. Retrospective recall of history (for example, age of onset or worst pain in past 4 weeks) may be used in combination with prospective measures. Brief phone interviews are often used to ensure a high participation rate and to establish a rapport with the participant, but are followed with mailed questionnaires to reduce the cost of obtaining detailed data. Increasingly, psychologists and epidemiologists are using electronic diaries (for example, using a Palm Pilot-like device) to capture high-resolution data on pain experience. Such diaries can alert patients at appropriate intervals, collect relevant data, time stamp when data were collected and wirelessly transmit data to facilitate monitoring of partcipation[116–119].

## Measurement and pain phenotypes

Obtaining self-reported data on pain experience, which is essential to characterize pain phenotypes, is usually the most costly aspect of a GWA study of pain, and potentially its Achilles' heel (BOX 4). Errors in measurement at this stage reduce overall statistical power, can be financially costly and can easily mask the detection of real associations. For any disorder, measurement of pain features is essential to characterize each phenotype of interest. In addition, measurement of past exposures is important to identify non-genetic causal mediators, and measurement of patient traits (for example, personality and mood disorders) is important to identify other factors that influence risk. General measures of interest that are relevant to most studies include response to treatment, age of onset and nature of onset (for example, gradual or sudden). They can also include duration of time with pain, frequency and duration of episodes, persistence of pain and common pain descriptors (for example, hot, cold or burning).

Treatment studies represent a special case for GWA studies. In these studies, the phenotype is defined by their response to the intervention. Prospective data collection is essential and random errors in measurement can be costly. Considerable thought should be invested in selecting the right tool for measuring pain experience. Only a handful of reports have compared the sensitivity of various pain measures in showing the effects of analgesic drugs in clinical trials, and no studies have compared the sensitivity of pain measures in detecting the effects of genetic variants on pain. Bellamy *et al.*[70,71] compared the variability of eight pain scales in assessing reduction of pain when cohorts of about 100 patients with osteoarthritis or rheumatoid arthritis had anti-inflammatory treatment for 1 month. The smallest standard deviations in pain-reduction scores resulted

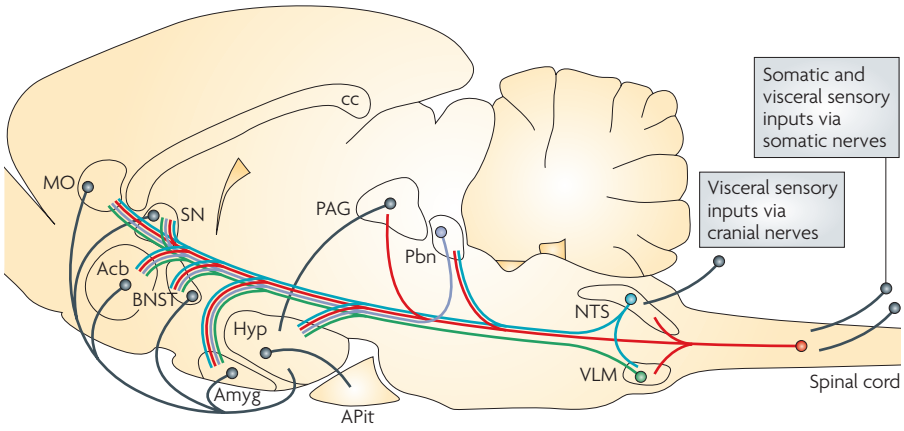


Figure 5 | **Neural pathways of pain.** A sagittal view of a rat brain showing the pathways of pain conveying somtosensory and visceral information to the hypothalamus and other limbic structures. These pathways also mediate mood, sleep, appetite, endocrine and cardiovascular functions. Sites of origin (circles) and termination of neural pathways are indicated. The dense innervation of these mood and vegetative centres by pain inputs suggest that pain-mediated depression, anxiety, insomnia, appetite and libido changes might have distinct neurochemical mechanisms from disorders caused by other types of stress. A better understanding of the neurotransmitters linking these pain inputs to mood and vegetative centres might facilitate the development of drugs to prevent or reverse these disabling complications of pain. Acb, nucleus accumbens; Amyg, amygdale; APit, anterior pituitary gland; BNST, bed nucleus stria terminalis; cc, corpus callosum; Hyp, hypothalamus; MO, medial orbital cortex; NTS, nucleus tractus solitarius; PAG, periaqueductal grey; Pbn, parabrachial nuclei; SN, septal nuclei; VLM, ventrolateral medulla. This figure is modified with permission from REF. 122 © (1996) Elsevier Science.

from the 0–10 numerical scale and a 100 mm visual analogue scale. Other studies, however, suggest that elderly patients are sometimes confused by visual analogue scales[72], thus potentially decreasing the study efficiency in this age group. From the data of Bellamy *et al.* one can calculate that because of a higher variance relative to these two most-efficient scales, one would need 25% more patients to achieve the same level of statistical significance for pain reduction using a 5-point categorical scale, and double the number of patients using the full McGill pain questionnaire. This question of relative scale efficiency is so crucial to almost every type of pain study that further comparative work is warranted.

Researchers may consider augmenting pain report with quantitative sensory testing in GWA studies of chronic pain, although this may increase the cost and logistical complexity of the study. Studies in twins have shown that these laboratory measures of pain processing have high heritabilities, at least in expert hands in single-centre studies[24,25]. Expenses might be reduced by focusing the sensory examination on a measure established by prior work in that disorder. For example, many groups agree[73,74] that brief-heat pain-threshold testing of patients with post-herpetic neuralgia may subdivide them into subgroups with relatively intact or severely diminished innervation of the affected skin. These subgroups may have different responses to drugs[75].

Errors in data entry or transfers, sample identification and processing, and laboratory measurements routinely contaminate large genetic epidemiology studies, and may cause a loss of power or a false-positive result. Investigators who are new to genetic epidemiology should consult with colleagues experienced with standard practices for quality management in epidemiological and genetic research[76–78].

## Genetics of pain disorders

The literature in pain psychology emphasizes that chronic pain itself is not necessarily life-destroying or financially ruinous, and many people bear the pain and carry on their activities. However, the common co-morbidities of depression, anxiety, insomnia, restriction of movement, cognitive impairment or substance abuse disable many patients suffering from pain.

Like the fine points of pain measurement, assessment and mechanistic study of pain co-morbidities have been neglected in drug development efforts. This gap has recently been addressed in a consensus document published by regulators and experts involved in chronic pain clinical trials[79].

The multibillion-dollar question for each of these co-morbidities is whether their mechanisms and drug treatments are unique in the patient, or similar in patients with and without pain. If there are specific pain-evoked mechanisms, then there may be novel classes of antidepressants, anxiolytics, hypnotics or cognitive enhancers for the patients suffering from pain awaiting discovery.

The neuroanatomy of the pain pathways and some clinical observations make it plausible to hypothesize some unique pain-related mechanisms of co-morbidity. FIGURE 5 shows pathways that are anatomically situated to make specifically pain-related neurochemical contributions to disorders of mood, sleep, reward or other vegetative functions. In the 1980s, several laboratories[80–83] described these powerful spinal, trigeminal and solitary tract pathways by which pain inputs are multiplied by several orders of magnitude at synapses in the parabrachial and solitary tract nuclei. Projections from these nuclei then synapse in the hypothalamus, amygdala, nucleus accumbens, septal nuclei and medial orbital cortex, structures that mediate the pain co-morbidities described above. These animal data describing powerful limbic connections of pain inputs have been confirmed by human functional imaging studies of pain[84,85].

Some clinical studies support distinct mechanisms for pain-related co-morbidities. For example, in a large randomized trial of selective serotonin reuptake-blocking antidepressants, depression was less likely to improve in patients with pain than in those without pain, and severity of pain was inversely correlated with treatment response[86]. However, the discovery of specific drugs to prevent or treat pain co-morbidities will take a great deal of work. Studies of the neurochemical links between pain afferents and limbic brain structures are just beginning[87–89]. Furthermore, there are no established animal models of pain-mediated anxiety, depression, insomnia or cognitive disorders.

Clinical genetic studies might be used in parallel with animal model development to identify how pain causes its co-morbid symptoms or how these symptoms exacerbate pain. Such an approach to the pain–mood interaction has recently been illustrated in the lumbar discectomy cohort described above in the *GCH1* study[90]. A simple analysis was demonstrated by which one could search for genetic polymorphisms that only produce depression or anxiety

in the presence of pain. This group has also used data on everyday motor activities collected with the same lumbar discectomy cohort to screen motor control genes for clues to developing new muscle relaxants for patients with musculoskeletal pain[91]. Using this approach, a $GABA_{B1}$ (γ-aminobutyric acid β1) receptor haplotype was identified that seems to protect against excessive reduction in movement in the presence of subacute low back pain with sciatica. Such analyses, together with molecular studies in animal models, could also be used to look for molecules that mediate links between pain and sleep[92–94] or cognitive dysfunction[95–98].

## Conclusions

A systematic approach to the molecular epidemiology of pain might accelerate the development of new treatments for pain and its co-morbidities, including depression, anxiety, insomnia and cognitive dysfunction. Whole-genome studies can provide human data to validate targets studied in animal models, potentially improving the chances that drug candidates will succeed in the clinic. Although this discussion has focused on drug discovery, the elucidation of genes that alter the risk of severe pain or pain-related co-morbidities might lead to diagnostic tests that better estimate the benefits and risks of medical treatments or environmental exposures to individuals.

Because clinical pain research has historically focused on small, intensive sensory physiology studies, new investigators will need to be recruited to apply epidemiological principles that have been proven in other disease areas. Optimizing measures of pain and its co-morbidities and extraction of contributing covariates can vastly improve the cost-effectiveness of pain treatment trials and genomic studies. Because many of the same neural systems process pain, nausea, dyspnoea, depression, anxiety and drug-induced reward, molecular studies of well-characterized types of human pain may advance the fundamental understanding of other medical symptoms and behavioural disorders.

*Mitchell B. Max is at the Center for Pain Research, Departments of Anesthesiology and Medicine, University of Pittsburgh, A-1305 Scaife Hall, 3550 Terrace St, Pittsburgh, Pennsylvania 15261, USA.*

*Walter F. Stewart is at the Center for Health Research, Geisinger Health System, 100 N Academy Ave, Danville, Pennsylvania 17822, USA.*

*Correspondence to M.B.M.*
*e-mail: maxmb@upmc.edu*
doi:10.1038/nrd2595
Published online 27 June 2008

1. Schappert, S. M., National Ambulatory Medical Care Survey: 1992 summary. *Adv. Data* **253**, 1–20 (1994).
2. Stewart, W. F. *et al*. Lost productive time and cost due to common pain conditions in the US workforce. *JAMA* **290**, 2443–2454 (2003).
3. Willis, W. D., Jr. The somatosensory system, with emphasis on structures important for pain. *Brain Res. Rev.* **55**, 297–313 (2007).
4. Negus, S. S. *et al*. Preclinical assessment of candidate analgesic drugs: recent advances and future challenges. *J. Pharmacol. Exp. Ther.* **319**, 507–514 (2006).
5. Kalueff, A. V., Wheaton, M. & D. L. Murphy, What's wrong with my mouse model? Advances and strategies in animal modeling of anxiety and depression. *Behav. Brain Res.* **179**, 1–18 (2007).
6. Powell, C. M. & Miyakawa, T. Schizophrenia-relevant behavioral testing in rodent models: a uniquely human disorder? *Biol. Psychiatry* **59**, 1198–1207 (2006).
7. Kontinen, V. K., Meert, T. F. in *Proc. 10th World Congress Pain* 1–937 (eds Dostrovsky, J. O., Carr, D. B. & Koltzenburg, M.) (IASP Press, Seattle, 2003).
8. Schug, S. A. *et al*. Neuraxial drug administration: a review of treatment options for anaesthesia and analgesia. *CNS Drugs* **20**, 917–933 (2006).
9. Belfer, I. *et al*. Candidate gene studies of human pain mechanisms: methods for optimizing choice of polymorphisms and sample size. *Anesthesiology* **100**, 1562–1572 (2004).
10. Hill, R. NK1 (substance P) receptor antagonists — why are they not analgesic in humans? *Trends Pharmacol. Sci.* **21**, 244–246 (2000).
11. Wallace, M. S. *et al*. A multicenter, double-blind, randomized, placebo-controlled crossover evaluation of a short course of 4030W92 in patients with chronic neuropathic pain. *J. Pain* **3**, 227–233 (2002).
12. Wallace, M. S. *et al*. A randomized, double-blind, placebo-controlled trial of a glycine antagonist in neuropathic pain. *Neurology* **59**, 1694–1700 (2002).
13. Manolio, T. A., Bailey-Wilson, J. E. & Collins, F. S. Genes, environment and the value of prospective cohort studies. *Nature Rev. Genet.* **7**, 812–820 (2006).
14. Gudmundsson, J. *et al*. Common sequence variants on 2p15 and Xp11.22 confer susceptibility to prostate cancer. *Nature Genet.* **40**, 281–283 (2008).
15. Lusis, A. J. & Pajukanta, P. A treasure trove for lipoprotein biology. *Nature Genet.* **40**, 129–130 (2008).
16. Diatchenko, L. *et al*. Genetic architecture of human pain perception. *Trends Genet.* **23**, 605–613 (2007).
17. Mogil, J. S. *et al*. Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. *Pain* **80**, 67–82 (1999).
18. Lariviere, W. R. *et al*. Heritability of nociception. III. Genetic relationships among commonly used assays of nociception and hypersensitivity. *Pain* **97**, 75–86 (2002).
19. Devor, M. & P. Raber, Heritability of symptoms in an experimental model of neuropathic pain. *Pain* **42**, 51–67 (1990).
20. Bengtsson, B. & Thorson, J. Back pain: a study of twins. *Acta Genet. Med. Gemellol.* (Roma) **40**, 83–90 (1991).
21. Treloar, S. A., Martin, N. G. & Heath, A. C. Longitudinal genetic analysis of menstrual flow, pain, and limitation in a sample of Australian twins. *Behav. Genet.* **28**, 107–116 (1998).
22. Morris-Yates, A. *et al*. Evidence of a genetic contribution to functional bowel disorder. *Am. J. Gastroenterol.* **93**, 1311–1317 (1998).
23. Battie, M. C. *et al*. Heritability of low back pain and the role of disc degeneration. *Pain* **131**, 272–280 (2007).
24. Norbury, T. A. *et al*. Heritability of responses to painful stimuli in women: a classical twin study. *Brain* **130**, 3041–3049 (2007).
25. Nielsen, C. S. *et al*. Individual differences in pain sensitivity: genetic and environmental contributions. *Pain* **136**, 21–29 (2007).
26. Bhalang, K. *et al*. Associations among four modalities of experimental pain in women. *J. Pain* **6**, 604–611 (2005).
27. Stewart, W. F. *et al*. Familial risk of migraine: variation by proband age at onset and headache severity. *Neurology* **66**, 344–348 (2006).
28. Stewart, W. F. *et al*. Familial risk of migraine: a population-based study. *Ann. Neurol.* **41**, 166–172 (1997).
29. Mulder, E. J. *et al*. Genetic and environmental influences on migraine: a twin study across six countries. *Twin Res.* **6**, 422–431 (2003).
30. Russell, M. B., Iselius, L. & Olesen, J. Migraine without aura and migraine with aura are inherited disorders. *Cephalalgia* **16**, 305–309 (1996).
31. Russell, M. B. *et al*. Migraine without aura and migraine with aura are distinct disorders. A population-based twin survey. *Headache* **42**, 332–336 (2002).
32. Max, M., Portenoy, R. K. & Laska, E. M. *The Design of Analgesic Clinical Trials (Advances in Pain Research and Therapy)* Vol. 18 (Raven Press, New York, 1991).
33. Dawson, E. G. *et al*. Low back pain recollection versus concurrent accounts: outcomes analysis. *Spine* **27**, 984–993; discussion 994 (2002).
34. Zubieta, J. K. *et al*. COMT Val158Met genotype affects μ-opioid neurotransmitter responses to a pain stressor. *Science* **299**, 1240–1243 (2003).
35. Diatchenko, L. *et al*. Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Hum. Mol. Genet.* **14**, 135–143 (2005).
36. Armero, P. *et al*. COMT (Val158Met) polymorphism is not associated to neuropathic pain in a Spanish population. *Eur. J. Pain* **9**, 229–232 (2005).
37. Hagen, K. *et al*. No association between chronic musculoskeletal complaints and Val158Met polymorphism in the catechol-*O*-methyltransferase gene. The HUNT study. *BMC Musculoskelet. Disord.* **7**, 40 (2006).
38. Slade, G. D. *et al*. Influence of psychological factors on risk of temporomandibular disorders. *J. Dent. Res.* **86**, 1120–1125 (2007).
39. Nackley, A. G. *et al*. Catechol-*O*-methyltransferase inhibition increases pain sensitivity through activation of both $\beta_2$- and $\beta_2$-adrenergic receptors. *Pain* **128**, 199–208 (2007).
40. Nackley, A. G. *et al*. Human catechol-*O*-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science* **314**, 1930–1933 (2006).
41. Tegeder, I. *et al*. GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence. *Nature Med.* **12**, 1269–1277 (2006).
42. Tegeder, I. *et al*. Reduced hyperalgesia in homozygous carriers of a GTP cyclohydrolase 1 haplotype. *Eur. J. Pain* 26 Mar 2008 (doi:10.1016/j.ejpain.2008.02.004).
43. Kim, H. & Dionne, R. A. Lack of influence of GTP cyclohydrolase gene (*GCH1*) variations on pain sensitivity in humans. *Mol. Pain* **3**, 6 (2007).
44. Risch, N. & Merikangas, K. The future of genetic studies of complex human diseases. *Science* **273**, 1516–1517 (1996).
45. Zondervan, K. T. & Cardon, L. R. Designing candidate gene and genome-wide case-control association studies. *Nature Protoc.* **2**, 2492–2501 (2007).
46. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**, 661–678 (2007).
47. Luca, D. *et al*. On the use of general control samples for genome-wide association studies: genetic matching highlights causal variants. *Am. J. Hum. Genet.* **82**, 453–463 (2008).
48. Cardon, L. R. & Palmer, L. J. Population stratification and spurious allelic association. *Lancet* **361**, 598–604 (2003).
49. Cella, D. *et al*. The patient-reported outcomes measurement information system (PROMIS): progress of an NIH roadmap cooperative group during its first two years. *Med. Care* **45** (5 Suppl. 1), S3–S11 (2007).
50. Reginster, J. Y. The prevalence and burden of arthritis. *Rheumatology* (Oxford) **41** (Supp. 1), 3–6 (2002).
51. Scher, A. I. *et al*. Prevalence of frequent headache in a population sample. *Headache* **38**, 497–506 (1998).
52. Woolf, C. J. & Salter, M. W. Neuronal plasticity: increasing the gain in pain. *Science* **288**, 1765–1769 (2000).
53. Barden, J. *et al*. Relative efficacy of oral analgesics after third molar extraction. *Br. Dent J.* **197**, 407–411; discussion 397 (2004).

54. Malan, T. P., Jr *et al*. Parecoxib sodium, a parenteral cyclooxygenase 2 selective inhibitor, improves morphine analgesia and is opioid-sparing following total hip arthroplasty. *Anesthesiology* **98**, 950–956 (2003).
55. Pollak, R. *et al*. Analgesic efficacy of valdecoxib for acute postoperative pain after bunionectomy. *J. Am. Podiatr. Med. Assoc.* **96**, 393–407 (2006).
56. Kehlet, H., Jensen, T. S. & Woolf, C. J. Persistent postsurgical pain: risk factors and prevention. *Lancet* **367**, 1618–1625 (2006).
57. Committee on Biological Markers of the National Research Council. Biological markers in environmental health research. *Environ. Health Perspect.* **74**, 3–9 (1987).
58. Von Korff, M. *et al*. Grading the severity of chronic pain. *Pain* **50**, 133–149 (1992).
59. Dunn, K. M. & Croft, P. R. The importance of symptom duration in determining prognosis. *Pain* **121**, 126–132 (2006).
60. Von Korff, M. & Miglioretti, D. L. A prognostic approach to defining chronic pain. *Pain* **117**, 304–313 (2005).
61. Sullivan, M. J. *et al*. Stage of chronicity and treatment response in patients with musculoskeletal injuries and concurrent symptoms of depression. *Pain* **135**, 151–159 (2008).
62. Scher, A. I., Lipton, R. B. & Stewart, W. Risk factors for chronic daily headache. *Curr. Pain Headache Rep.* **6**, 486–491 (2002).
63. Scher, A. I., Stewart, W. F. & Lipton, R. B. Caffeine as a risk factor for chronic daily headache: a population-based study. *Neurology* **63**, 2022–2027 (2004).
64. Bigal, M. E. *et al*. Patterns of medical diagnosis and treatment of migraine and probable migraine in a health plan. *Cephalalgia* **26**, 43–49 (2006).
65. Kolodner, K. *et al*. Pharmacy and medical claims data identified migraine sufferers with high specificity but modest sensitivity. *J. Clin. Epidemiol.* **57**, 962–972 (2004).
66. Lipton, R. B. *et al*. Patterns of health care utilization for migraine in England and in the United States. *Neurology* **60**, 441–448 (2003).
67. Engel, C. C., von Korff, M. & Katon, W. J. Back pain in primary care: predictors of high health-care costs. *Pain* **65**, 197–204 (1996).
68. Saunders, K. W. *et al*. Prediction of physician visits and prescription medicine use for back pain. *Pain* **83**, 369–377 (1999).
69. Lipton, R. B. *et al*. Prevalence and burden of migraine in the United States: data from the American Migraine Study II. *Headache* **41**, 646–657 (2001).
70. Bellamy, N., Campbell, J. & Syrotuik, J. Comparative study of self-rating pain scales in rheumatoid arthritis patients. *Curr. Med. Res. Opin.* **15**, 121–127 (1999).
71. Bellamy, N., Campbell, J. & Syrotuik, J. Comparative study of self-rating pain scales in osteoarthritis patients. *Curr. Med. Res. Opin.* **15**, 113–119 (1999).
72. Herr, K. A. *et al*. Pain intensity assessment in older adults: use of experimental pain to compare psychometric properties and usability of selected pain scales with younger adults. *Clin. J. Pain* **20**, 207–219 (2004).
73. Rolke, R. *et al*. Quantitative sensory testing in the German research network on neuropathic pain (DFNS): standardized protocol and reference values. *Pain* **123**, 231–243 (2006).
74. Rowbotham, M. C. & Petersen, K. L. Zoster-associated pain and neural dysfunction. *Pain* **93**, 1–5 (2001).
75. Tella, P. K. *et al*. in *Proc. 11th World Congress Pain*. 1–882 (eds Flor, H., Kalso, E. & Dostrovsky, J. O.) (IASP Press, Seattle, 2006).
76. Edwards, B. J. *et al*. Power and sample size calculations in the presence of phenotype errors for case/control genetic association studies. *BMC Genet.* **6**, 18 (2005).
77. Elliott, P., Peakman, T. C. & UK Biobank. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. *Int. J. Epidemiol.* **37**, 234–244 (2008).
78. Rothman, N. *et al*. Misclassification of genetic susceptibility biomarkers: implications for case-control studies and cross-population comparisons. *Cancer Epidemiol. Biomarkers Prev.* **2**, 299–303 (1993).
79. Turk, D. C. *et al*. Core outcome domains for chronic pain clinical trials: IMMPACT recommendations. *Pain* **106**, 337–345 (2003).
80. Bernard, J. F., Peschanski, M. & Besson, J. M. A possible spino (trigemino)–ponto–amygdaloid pathway for pain. *Neurosci. Lett.* **100**, 83–88 (1989).
81. Burstein, R., Cliffer, K. D. & Giesler, G. J. Jr. Direct somatosensory projections from the spinal cord to the hypothalamus and telencephalon. *J. Neurosci.* **7**, 4159–4164 (1987).
82. Cechetto, D. F., Standaert, D. G. & Saper, C. B. Spinal and trigeminal dorsal horn projections to the parabrachial nucleus in the rat. *J. Comp. Neurol.* **240**, 153–160 (1985).
83. Hylden, J. L., Anton, F. & Nahin, R. L. Spinal lamina I projection neurons in the rat: collateral innervation of parabrachial area and thalamus. *Neuroscience* **28**, 27–37 (1989).
84. Apkarian, A. V. *et al*. Human brain mechanisms of pain perception and regulation in health and disease. *Eur. J. Pain* **9**, 463–484 (2005).
85. Talbot, J. D. *et al*. Multiple representations of pain in human cerebral cortex. *Science* **251**, 1355–1358 (1991).
86. Bair, M. J. *et al*. Impact of pain on depression treatment response in primary care. *Psychosom. Med.* **66**, 17–22 (2004).
87. Braz, J. M. *et al*. Parallel "pain" pathways arise from subpopulations of primary afferent nociceptor. *Neuron* **47**, 787–793 (2005).
88. Ikeda, R. *et al*. NMDA receptor-independent synaptic plasticity in the central amygdala in the rat model of neuropathic pain. *Pain* **127**, 161–172 (2007).
89. Neugebauer, V. *et al*. The amygdala and persistent pain. *Neuroscientist* **10**, 221–234 (2004).
90. Max, M. B. *et al*. A clinical genetic method to identify mechanisms by which pain causes depression and anxiety. *Mol. Pain* **2**, 14 (2006).
91. Mishra, B. K. *et al*. Do motor control genes contribute to interindividual variability in decreased movement in patients with pain? *Mol. Pain* **3**, 20 (2007).
92. Taylor, D. J. *et al*. Comorbidity of chronic insomnia with medical problems. *Sleep* **30**, 213–218 (2007).
93. Smith, M. T. & Haythornthwaite, J. A. How do sleep disturbance and chronic pain inter-relate? Insights from the longitudinal and cognitive–behavioral clinical trials literature. *Sleep Med. Rev.* **8**, 119–132 (2004).
94. Palesh, O. G. *et al*. A longitudinal study of depression, pain, and stress as predictors of sleep disturbance among women with metastatic breast cancer. *Biol. Psychol.* **75**, 37–44 (2007).
95. Karp, J. F. *et al*. The relationship between pain and mental flexibility in older adult pain clinic patients. *Pain Med.* **7**, 444–452 (2006).
96. Apkarian, A. V. *et al*. Chronic pain patients are impaired on an emotional decision-making task. *Pain* **108**, 129–136 (2004).
97. Apkarian, A. V. *et al*. Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. *J. Neurosci.* **24**, 10410–10415 (2004).
98. Weiner, D. K. *et al*. The relationship between pain, neuropsychological performance, and physical function in community-dwelling older adults with chronic low back pain. *Pain Med.* **7**, 60–70 (2006).
99. Stewart, W. F. *et al*. Lost productive work time costs from health conditions in the United States: results from the American Productivity Audit. *J. Occup. Environ. Med.* **45**, 1234–1246 (2003).
100. Bradshaw, D. H., Nakamura, Y. & Chapman, C. R. National Institutes of Health grant awards for pain, nausea, and dyspnea research: an assessment of funding patterns in 2003. *J. Pain* **6**, 277–293 (2005).
101. American Academy of Family Physicians. Facts About Family Practice. Kansas City, Mo; American Academy of Family Physicians (1987).
102. Fioramonti, J. & Gebhart, G. F. *In vivo* and transgenic animal models used to study visceral hypersensitivity. *Neurogastroenterol. Motil.* **19** (Suppl. 1), 20–28 (2007).
103. Bartfai, T. & Lees, G. V. *Drug Discovery: From Bedside to Wall Street*. 1–328 (Academic Press, Burlington, MA, 2006).
104. McMahon, S. & Koltzenburg, M. *Wall and Melzack's Textbook of Pain*. 5th edn (Elsevier Churchill Livingstone, London, 2006).
105. Campbell, J. N. & Meyer, R. A. Mechanisms of neuropathic pain. *Neuron* **52**, 77–92 (2006).
106. Kroenke, K. Physical symptom disorder: a simpler diagnostic category for somatization-spectrum conditions. *J. Psychosom. Res.* **60**, 335–339 (2006).
107. Gracely, R. H. *et al*. Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. *Arthritis Rheum.* **46**, 1333–1343 (2002).
108. Price, D. D. *et al*. Peripheral and central contributions to hyperalgesia in irritable bowel syndrome. *J. Pain* **7**, 529–535 (2006).
109. Katon, W., Sullivan, M. & Walker, E. Medical symptoms without identified pathology: relationship to psychiatric disorders, childhood and adult trauma, and personality traits. *Ann. Intern. Med.* **134**, 917–925 (2001).
110. Solovieva, S. *et al*. Intervertebral disc degeneration in relation to the COL9A3 and the IL-1ss gene polymorphisms. *Eur. Spine J.* **15**, 613–619 (2006).
111. Roeder, K., Devlin, B. & Wasserman, L. Improving power in genome-wide association studies: weights tip the scale. *Genet. Epidemiol.* **31**, 741–747 (2007).
112. Scott, L. J. *et al*. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* **316**, 1341–1345 (2007).
113. Costigan, M. *et al*. Replicate high-density rat genome oligonucleotide microarrays reveal hundreds of regulated genes in the dorsal root ganglion after peripheral nerve injury. *BMC Neurosci.* **3**, 16 (2002).
114. Lacroix-Fralish, M. L., Ledoux, J. B. & Mogil, J. S. The Pain Genes Database: an interactive web browser of pain-related transgenic knockout studies. *Pain* **131**, 3.e1–3.e4 (2007).
115. Wang, K., Li, M. & Bucan, M. Pathway-based approaches for analysis of genomewide association studies. *Am. J. Hum. Genet.* **81**, 1278–1283 (2007).
116. Begg, A., Drummond, G. & Tiplady, B. Assessment of postsurgical recovery after discharge using a pen computer diary. *Anaesthesia* **58**, 1101–1105 (2003).
117. Peters, M. L. *et al*. Electronic diary assessment of pain, disability and psychological adaptation in patients differing in duration of pain. *Pain* **84**, 181–192 (2000).
118. Roelofs, J. *et al*. Electronic diary assessment of pain-related fear, attention to pain, and pain intensity in chronic low back pain patients. *Pain* **112**, 335–342 (2004).
119. Stone, A. A. *et al*. Intensive momentary reporting of pain with an electronic diary: reactivity, compliance, and patient satisfaction. *Pain* **104**, 343–351 (2003).
120. Bigal, M. E. *et al*. Body mass index and episodic headaches: a population-based study. *Arch. Intern. Med.* **167**, 1964–1970 (2007).
121. Peterlin, B. L. *et al*. Migraine and adiponectin: is there a connection? *Cephalalgia* **27**, 435–446 (2007).
122. Burstein, R. Somatosensory and visceral input to the hypothalamus and limbic system. *Prog. Brain Res.* **107**, 257–267 (1996).



### Acknowledgements

We thank D. Goldman and his colleagues in the Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism (NIAAA), for generous mentorship, training and collaboration. A. Kingman, S. Diehl, M. Devor, J. Mogil, W. Lariviere and Z. Seltzer for advice and encouragement. Also, I. Belfer, T. Wu, S. Atlas, C. Woolf, M. Costigan, W. Maixner, L. Diatchenko, R. Fillingim and the many other collaborators in the studies described above. M.B.M.'s contribution to the scientific reports cited in this work was funded by a National Institute of Dental of Craniofacial Research intramural grant ZO1 DE00366, NIAAA Intramural Grant Z01 AA000301, and the Comprehensive Neuroscience Program Grant USUHS G192BR-C4.


### Competing interests statement

The authors declare competing financial interests: see web version for details.

### DATABASES

**Entrez Gene:**
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
*COMT* | *GCH1*

### FURTHER INFORMATION

**Solace Pharmaceuticals:**
http://www.solacepharma.com/content/projects

### SUPPLEMENTARY INFORMATION

**See online article:** S1 (box)

**ALL LINKS ARE ACTIVE IN THE ONLINE PDF**