How Do Pain Genes Affect Pain Experience?

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From the Editors

Pain genetics is a relatively young field in pain research, and it faced much skepticism from both pain scientists studying basic mechanisms of pain who were unfamiliar with genetic approaches and human geneticists who considered pain to be too subjective, too immeasurable, and too unstable to be regarded as a genetic trait. Just ten years ago, we, the authors, had to defend our ground and gradually build the evidence to convince the scientific community that studying pain genetically is methodologically achievable and scientifically valuable. Furthermore, as for the other human traits, studying genetics of pain has substantial translational importance. Fortunately, genetic methodology and technology have revolutionarily advanced, decreasing cost, time, and efforts and allowing us to gather compelling data and uniquely claim that genes do contribute to pain. In fact, hundreds of genes have been already identified as pain genes, a couple of dozen have been intensively studied in animals and humans transnationally, and many more are expected to become part of a pain genome. The goal of this book is to present the most interesting, controversial, and fascinating aspects of pain genetics, as well as related fields. We selected the hot topics and asked the most recognized experts to discuss them. These investigators from Australia, Canada, Israel, Norway, and the United States are not only the finest scientists, but also world leaders in pain genetics and closely related fields. We hope that this book increases the visibility of pain genetics and further promotes the expansion of multidisciplinary pain research opening path to personalized pain medicine.

We open this book with the most important question, which inspired our personal dedication to this field of science as well as this publication: HOW DO PAIN GENES AFFECT PAIN EXPERIENCE? Professor Marshall Devor from the Hebrew University in Israel provides a rigorous overview on this topic, from the history of pain genetics to rationales and logistics of current and future pain genetic studies. Professor Devor, a pain research pioneer, has contributed considerably to our understanding of the neurophysiology, neuroanatomy, and genetics of pain. His ground-breaking discoveries in genetics of neuropathic pain research significantly advanced the knowledge of basic molecular, genetic and pathophysiological mechanisms of neuropathic pain development, and likely will eventually result in clinical implementation.
Introduction

Quite a few genetic polymorphisms have been identified that associate with painful medical conditions. The identification of many more can be anticipated. But genes code for proteins, not for perceptions. Through what mechanisms might genetic polymorphisms shape pain experience?

Variability in Pain Experience

There is remarkable variability in the amount pain that different people experience. Variability is the rule for acute pain in response to noxious stimuli; we each have our own “pain threshold.” It is also the rule for chronic pain associated with injury and disease, even when the provoking tissue damage is identical. A dramatic example is classical trigeminal neuralgia (TN) (type 1). TN is a severe neuropathic pain condition in which sufferers report feeling intense intermittent electric shock-like pain paroxysms in the face. The underlying nerve lesion in most cases is microvascular compression of the trigeminal root near its point of entry into the brainstem. Consistent with early postmortem reports, a recent magnetic resonance imaging (MRI) study found that 17% of mature adults have the lesion (Miller et al. 2009). However, only 0.01% of people suffer from TN pain (population prevalence ca. 10/100 000; Manzoni and Torelli 2005). That is, only 1 in every 2000 people with the lesion has TN pain. Other neuropathic pain conditions also show a “disconnect” between the supposedly causative lesion and the pain, although the ratios are rarely so extreme. More typical is pain after limb amputation where roughly 25% of amputees escape phantom limb pain and 75% escape severe pain even though in 100% of amputees all nerves in the amputated limb were severed (Sherman et al. 1996). What is different about those individuals whose lesion causes devastating pain?

Individual differences in pain response are traditionally attributed to psychosocial and cultural factors, personality, personal inclination and upbringing – in brief, to environmental factors. Think of all those jokes about the stoic Vikings of the North and the pampered screamers of Mediterranean countries. Variability is also irrevocably entangled with uncertainty about the extent to which the outward expression of pain actually reflects the pain experienced by the individual, inside. In some societies, children are taught from an early age not to express their pain, boys at least. But this does not necessarily mean that the perceptual experience of pain is any less. Even for a given individual, pain expression is strongly affected by context – did that slap in the face really hurt so much? Pain behavior is not the same as the “raw feel” of pain.

When trying to account for the variability of pain, there is no denying the importance of environmental factors. But environment isn’t everything. Pain genetics is premised on the idea that genetic factors contribute as much to individual differences in pain response as the environment, for some traits perhaps even more (Mogil 2004).

Heritability of Pain: Historical Roots

Most biological traits are affected by genes, environment, and interactions between the two. It should therefore come as no surprise that pain response is likewise affected by nature as well as nurture. Nonetheless, because of the striking influence of socialization in our day-to-day pain experience, the detection of an important genetic component took many people by surprise. The first solid indications of heritability came from rare familial disorders such as congenital insensitivity to pain with anhidrosis (CIPA), in which affected family members feel no pain, and Fabry’s disease
and familial hemiplegic migraine (FHM) where affected family members suffer from extreme pain (Kelly and Scherrer 2007). The heritable nature of these conditions was appreciated early in the history of medical genetics, and it must have been appreciated by the families themselves back into antiquity. The simple pattern of inheritance typical of such conditions points to the Mendelian inheritance of a single mutant gene of large effect. Using “linkage analysis,” today it is fairly straightforward to identify the specific causative mutant gene and the particular relevant base-pair change(s) within the gene that cause the disease. For Mendelian traits, the causative gene has already been pinpointed for all but the most obscure pain conditions.

Evidence for a genetic predisposition to pain on noxious stimulation in healthy individuals, and in painful medical conditions in which inheritance is non-Mendelian, came much later. Historically, the most common source of information on these (presumably polygenic) pain traits came from epidemiological research and the study of twins. For example, there are reports from the early 1900s of families with a higher-than-expected incidence of TN (Harris 1940). Likewise, it was noticed that pain as a normal trait (e.g., in response to experimentally applied heat and cold in healthy individuals) and pain in common medical conditions such as backache and sciatica are more concordant in identical (monozygotic) twins than in fraternal (dizygotic) twins (Nielsen et al. 2012). The idea here is that identical twins share all of their gene variants, while fraternal twins share only half. There remain caveats to this approach, however. For example, environment may be more similar for identical twins than for fraternal twins. Information can also be obtained from twins separated at birth and raised apart, in different environments. This occurred due to the disruptions of World War II but is now very rare.

Unlike Mendelian pain traits, it is still not straightforward to identify the genes responsible for these more complex traits. This is because they are caused by a combination of gene polymorphisms whose individual effects may be small. In addition, the pain traits themselves are not black and white. Pain is typically graded in intensity, and often inheritance is manifest as a statistical predisposition to developing a painful condition under particular circumstances. In recent years, the “association study” has emerged as an approach to identifying the genes involved in non-Mendelian inheritance. In association studies, cohorts of unrelated individuals with contrasting pain phenotype are compared for genetic differences. Ideally, all of the genes in the genome are searched for consistent differences between the cohort with and the cohort without pain (genome-wide association study (GWAS)). However, because of the high cost of carrying out a GWAS, comparisons are usually limited to one or more gene “candidates” that the investigator has prior reason to think might be relevant. A hybrid between these two methods is noted briefly in the succeeding text, where GWAS analysis was carried out inexpensively in mice using a neuropathic pain model, and the pain gene discovered was then tested as a candidate in a human association study (Nissenbaum et al. 2010).

The earliest experimental study I am aware of that was specifically intended to test for pain heritability was remarkably recent. The senior investigator was Israel Lieblich at the Hebrew University of Jerusalem. Lieblich had done postdoctoral work at Caltech with James Olds. In the 1950s, Olds and Milner (1954) had discovered “pleasure centers” in the brain. Specifically, they showed that a rat would press a bar (“self-stimulate”), or learn any other operant, if rewarded by electrical stimulation delivered directly to limbic areas of the brain, notably the lateral hypothalamus. In the 1960s, the study of hypothalamic self-stimulation flourished, but then people began to run out of novel research ideas. Lieblich came up with a good one. Knowing that some rats are better self-stimulators than others, he mated high self-stimulating males and females and low self-stimulators and then tested their offspring for the trait. After several generations of such selective breeding, he obtained lines in which there was a reliable, inherited predisposition for high versus
low self-stimulation. Lieblich’s idea was to see what other brain traits – neurochemical, structural, or behavioral – co-selected with self-stimulation. In one such trial, he and I looked at neuropathic pain behavior. We discovered that the high-pleasure rats were also high-pain rats (Inbal et al. 1980). This result indicated a deep connection between the limbic networks mediating pain and pleasure (Leknes and Tracey 2008). In addition, it encouraged me to undertake a long series of experiments aimed at directly exploring heritability of the pain trait. This line of work was later taken up by other groups as well (Figure 1.1).

Why is Pain Genetics Interesting and Potentially Useful?

There are basically four reasons why a pain professional ought to be interested to know that pain response is affected by genes:

1. **Stigma**: People who report having severe pain in the absence of easily observed signs of injury and disease are often stigmatized as complainers and malingerers. Sometimes they are even suspected of being outright liars, trying to cheat “the system” in order to obtain undeserved
sympathy and benefits from caring family members, employers, insurance companies, and the government. Such stigmatization is usually unfair, it undermines self-image, and it is likely to add considerably to the patient’s distress and suffering. For the pain professional, stigma is almost always harmful to efforts at reducing pain. Knowledge, by the therapist and the patient, that one person may have much more pain than another not because of a character flaw but for genetic reasons is sure to provide comfort. You don’t choose your genes! “It’s not your fault!” Note that this benefit does not require actual knowledge of which pain genes are involved or even knowledge that in the specific patient at hand, excessive pain is indeed due to bad luck in the genetic draw. The simple knowledge that science has shown that genes affect pain response can reassure your patient. It can also provide him/her with a response to tormentors and maybe even help you, as a pain professional, to take the patient’s problem more seriously. This knowledge is an easy gift you can give your patient as he sets out on his journey to pain relief.

Diagnosis, prognosis, and guidance: Polymorphisms (variants) in pain genes affect pain response. Typically these polymorphisms amount to single-letter differences in the nucleotide sequence of A’s, T’s, G’s, and C’s that make up the genome. Such single base-pair differences are called single-nucleotide polymorphisms (SNPs) (pronounced “snips”). Quite a few SNPs have already been identified that might affect the amount of pain that an individual suffers. Although we are not there yet, it is fairly straightforward to identify, in a simple blood or saliva test, whether an individual patient carries one or more pain-related SNPs that might account for exacerbated pain. It can be anticipated that in the future, results of such lab tests will assist in the accurate medical diagnosis of the underlying pain condition and in making a prognosis. Such information may also guide treatment by genetic counseling or even form the basis of gene therapy.

One example of the potential medical usefulness of pain genetics comes from recent work from my research group (Nissenbaum et al. 2010). We began with inbred mice in which individuals of one strain consistently develop neuropathic pain behavior after a standard nerve lesion, while those of another strain don’t. Crossbreeding male and female mice of the two strains brought us to the conclusion that a gene of major effect was in play. Subsequent application of a set of modern analytical tools eventually allowed us to identify the gene in question. It is a gene called Caeng2 and is known to code for the gamma subunit of voltage-sensitive Ca2+ channels (the stargazin protein). Caeng2 had previously been implicated in epileptogenesis, but not in neuropathic pain. Virtually all mouse genes have a homolog in the human genome, and Caeng2 is no exception. The human version of the Caeng2 gene is designated CACNG2 (Figure 1.2).

Having determined the importance of polymorphisms in this gene for neuropathic pain in mice, we asked whether it might also have a role in neuropathic pain in humans. This was checked by comparing SNPs in the CACNG2 gene using DNA obtained from blood samples of 549 women who had undergone complete or partial mastectomy due to breast cancer. Among these women, 215 reported persistent neuropathic pain on the chest wall and 334 did not. We found that if a particular woman had the nucleotides A-C-C at three particular adjacent SNPs in the base-pair sequence of the CACNG2 gene, this would predict with a fair likelihood that she will develop chronic postoperative pain (odds ratio = 1.7 on a baseline likelihood of roughly 40%). The increased chance is sufficiently large that it could be a factor in the decision on which surgical procedure to choose or at least to inform the surgeon that special care is needed to minimize nerve injury during the procedure. Our experimental result needs to be reproduced independently by other investigators using additional cohorts of women.
Figure 1.2  Pain behavior in the neuroma model of neuropathic pain (Devor 2007) is a heritable trait. Inbred C58/J and C3H/HeN mice are consistently low or high, respectively, in the autotomy trait. F1 offspring are low, indicating dominance of the low-pain allele(s). Results of backcrossing (BC) F1 individuals onto the parental low and high strains yielded trait segregation suggestive of the presence of a single gene of major effect, probably with additional modifiers (data in figure from Raber et al. 2006). The gene was later identified as Cacng2 (Nissenbaum et al. 2010).
before it would make sense to introduce CACNG2 genotyping into clinical practice. But this type of application likely lies in the not too distant future.

(3) Pharmacogenetics and individualized medicine: A third arena in which pain genetics could make a difference is in predicting which patients are likely to respond to which analgesic drugs. This capability is called “pharmacogenetics.” Like pain itself, response to therapeutic options varies among individuals, and a good deal of the variability is thought to be due to genes. Pharmacogenetics promises a new era of individualized pain medicine where the choice of drugs will be tailored to the specific patient, providing increased efficacy with decreased unwanted side effects.

(4) Discovery of novel pain mechanisms: Research on pain genetics has already begun to contribute to the understanding of pain mechanisms and will probably continue to do so. Past experience indicates that this will ultimately lead to improved pain medicine. Indeed, pain genetics has a special potential to uncover novel and unexpected insights about pain, insights that might not be achieved by step-by-step pursuit of our current pain physiology. This is because, if done right, the genetic approach permits a broad and unbiased scan of genes and gene-related pain mechanisms independent of prior knowledge about the physiology of pain (Figure 1.3).
What Are Pain Genes?

Genes are parts of DNA molecules that contain the information used by cells to construct protein molecules. They “code” for proteins. Proteins are “products” of gene transcription and translation, the two cellular processes that exploit the information encoded in the sequence of nucleotide base pairs in the DNA molecule to manufacture particular proteins. It is these proteins that carry out the work of the cell, such as enzymatic action, motility, and electrical impulse generation. It is essential to realize that genes code for protein molecules, not for sensory and emotional experiences such as pain. Likewise, when we read in the popular (and in the scientific) press about “genes for generosity,” “genes for risk-taking,” “genes for social awkwardness,” and “genes for empathy” (Ebstein et al. 2010), nobody is actually proposing that these high-order cognitive and emotional phenomena are due to the action of individual gene products, that is, protein molecules. Pain and these other traits are complex downstream effects of protein function. Only a conscious brain can experience pain or feel empathy for someone else who is in pain. But this does not mean that all of the 25,000 odd genes that are required to make a human body and brain are pain genes. Variation in the action of certain protein species makes a difference to how pain is perceived by a conscious brain, while variation of other proteins doesn’t. A “pain gene” is a gene for which there are one or more polymorphisms (i.e., variations in the sequence of DNA base pairs) that affect the expression or the functioning of its protein product in a way that affects pain response. The causal link between sequence polymorphism and pain response is discussed in the succeeding text.

How Many Pain Genes Are There?

DNA sequence variants (SNPs) that are common among humans are called “polymorphisms.” Rare variants, occurring in <1% of the population, are called “mutations.” Genes that differ slightly because of these variants are “alleles” of the gene. In human populations, most genes have a handful of common alleles and many rare variants. Nonetheless, only a tiny fraction of all genes have allelic variations with known effects on either cellular function, organ function, or the behavior of the individual, including pain response. Although more will surely be found, it is a safe bet that overall, most allelic variants in humans will prove to have no significant effects on pain processing. Only a small fraction of human genes are likely to be pain genes. However, some allelic variants in some genes do have measurable effects on pain, and the effect is sometimes substantial. As noted earlier, individual SNPs cause certain Mendelian diseases associated with severe pain or congenital insensitivity to pain. A particularly interesting example involves the SCN9A gene that codes for the alpha-subunit protein of the voltage-gated Na+ channel Nav1.7. Some alleles of this gene cause severe pain, others cause complete pain insensitivity, and still others only slightly affect the likelihood of an individual’s developing one or another painful condition (Fischer and Waxman 2010; Reimann et al. 2010). Evolution has probably weeded out from the genome most alleles that directly cause painful diseases, but many alleles remain that can predispose to conditions with a pain signature.

Overall, putting aside the rare Mendelian mutations, pain phenotype is likely determined by polymorphisms in a few hundred genes, with only a fraction of these contributing in any particular individual or painful condition. Some pain genes enhance pain (or the likelihood of developing pain), and some protect from it. Some likely have a relatively large effect, and some have a small effect. At this point, it is impossible to estimate how many of each sort there may be.
Complicating things still further, it is unlikely that the final pain phenotype results from simple summation of the various allelic effects present. There is probably a complex calculus where the various alleles inherited by an individual interact with one another nonlinearly and with the environment (Mogil 2004; Mogil et al. 2011). Thus, for example, a genetic polymorphism may have no effect on pain response except under unusual environmental circumstances (e.g., at high altitudes), or if your kids made you particularly exasperated. The growing realization that the genetics of complex traits, pain among them, is a subtle and complex affair has been the source of considerable frustration recently and even gloominess about the value of the enterprise as a whole (Goldstein 2009; Mogil 2009). After all, pain genes are of medical and scientific interest to the extent that individual allelic variants have a reasonably strong and predictive effect on pain response. It is not enough to find pain genes. One wants to find significant ones. This will require additional work. It is possible to incorporate in the search for pain genes strategies for enhancing the likelihood of finding functionally significant polymorphisms. One such strategy is the mouse–human hybrid approach noted earlier. It is much easier to screen mice for major heritable pain phenotypes than to screen people.

How Do Pain Genes Affect Pain Experience?

Genetic polymorphisms do not affect pain response by an action on the psyche, but rather by altering cellular functions. They can do this in several ways. Some polymorphisms occur in the nucleotide sequence that actually encodes for the gene’s protein product (i.e., in exons). Depending on the details, this may alter the amino acid sequence of the protein, changing its shape or charge configuration and hence its functioning. A polymorphism of a single nucleotide (SNP) in the exonic sequence can be enough to significantly alter a protein’s function, depressing it, enhancing it, or eliminating it entirely. Exonic polymorphisms can also affect the “bar code” address of a protein, affecting “trafficking.” This is the process that determines where in the neuron the protein will be delivered. The affected protein may work normally, but if it is sent to the wrong part of the cell, it can’t do its job properly. Sequence polymorphisms that affect pain can also occur in noncoding parts of a gene (introns) and noncoding intergenic regions. Such polymorphisms can affect the protein product itself by altering gene splicing. But more commonly the effect of non-exonic polymorphisms is on the regulation of gene expression, for example, changing how much of the gene product is synthesized (copy number, abundance).

Direct Effects of Allelic Variation on Pain Mechanisms

By altering cellular functions, pain genes can affect pain response in a direct manner or indirectly. Typical direct mechanisms include (i) increased intrinsic excitability of pain signaling neurons, due, for example, to altered ion channels and membrane receptors, or (ii) hyperexcitability in pain processing networks due, for example, to reduced inhibitory neurotransmission. Neuronal and network excitability are determined by a very large number of different molecules. In principle, genetic polymorphisms affecting any of them could directly affect pain. However, this is only a theoretical possibility as the genome of natural human populations probably does not contain significant allelic variants for most mission-critical genes. It is likely that only a small fraction of the genes that build the pain system actually contribute to individual pain variability. In this way, the search for natural pain genes differs greatly from the creation of artificial gene variants in
transgenic animals for scientific research (e.g., knockout mice). The Pain Genes Database (http://www.jbldesign.com/jmogil/Enter.html) currently lists 390 genes that yield a pain phenotype in transgenic mice. Many of these genetic variants probably do not occur in the homologous gene in human populations.

Sometimes, however, a functional allelic variant occurs in a gene that sits at a nodal point in pain physiology. A prime example is the Nav1.7 Na+ channel gene (SCN9A). As noted earlier, some mutations in this gene cause the extreme pain conditions familial erythromelalgia and paroxysmal extreme pain disorder. Other mutations in the same gene produce congenital insensitivity to pain (Catterall et al. 2008; Fischer and Waxman 2010). Investigators have expressed both the pain-inducing and the pain-preventing mutations of the human SCN9A gene in rodent sensory neurons and in human nonneural cells that do not otherwise express Na+ channels. Recordings from these cells showed that the pain-inducing mutations render the neurons electrically hyperexcitable, prone to generate nerve impulses in excess. They are “gain-of-function” mutations. In contrast, the mutations that produced pain insensitivity in humans caused experimentally modified cells to be hypo-excitable (“loss-of-function” mutations). What is more, the cellular hyperexcitability and hypo-excitability have been directly linked to relevant alterations in specific parts of the Na+ channel molecule itself. This is a rare example where clinical pain phenotypes can be directly associated with point mutations in a particular gene and its protein product. It is also a highly informative example, adding considerably to our understanding of Na+ channel gating and pain processing.

Polymorphisms also occur in the genes that code for types of Na+ channels other than Nav1.7. These, however, apparently do not cause severe pain or pain insensitivity; at least no such families have been identified yet. This suggests that the Nav1.7 Na+ channel plays a special role in pain processing. In light of this, investigators have asked whether additional polymorphisms in the Nav1.7 gene, ones that do not directly cause a painful disease, might nonetheless have detectable effects on pain. Indeed, association was found between the A allele of a particular fairly common SCN9A SNP (rs6746030) and pain scores in a variety of medical conditions including osteoarthritis and pancreatitis (Reimann et al. 2010). The A allele of this SNP even predicts pain threshold in healthy individuals. Surveying common polymorphisms of small effect in genes that have already been found to carry rare mutations of large effect may be a productive strategy in general.

As strong as the case is for SCN9A variants having a direct action on pain, the information in hand nonetheless leaves major unknowns. For example, the nature of the mutations does not explain why in erythromelalgia pain occurs mainly in the hands and feet (the mutation affects Nav1.7 proteins everywhere), why different gain-of-function mutations in the same gene cause very different clinical symptoms (erythromelalgia vs. paroxysmal extreme pain disorder), or why the loss of Nav1.7 function leads to pain insensitivity, but does not cause a deficit in nerve conduction or sensation in most other modalities (touch, vibration, vision). We also have no solid clues at present as to why the Nav1.7 gene polymorphism identified by Reimann et al. (2010) appears to have a small predictive effect on many different painful conditions.

**Indirect Effects of Allelic Variation on Pain Mechanisms**

Allelic variants sometimes have effects that appear on the face of it to link directly to pain mechanisms, but in fact don’t. Consider an oncogene which causes the formation of a tumor that compresses a nerve and induces a painful compression neuropathy. The cancer gene appears to have caused the pain, but it actually had no direct connection to pain mechanisms. The factor compressing the nerve could just as well have been a tight-fitting shoe. Some cases
are less obvious. The mutation in the gene for the major protein component of peripheral nerve myelin that causes painful Charcot–Marie–Tooth (CMT-2) neuropathy is an example (Kelley and Scherrer 2007). In CMT-2, a Thr124Met nucleotide substitution alters the chain of amino acids that make up a key protein in the nerve’s myelin sheath, the myelin-associated protein P0. The mutation in the \( P0 \) gene places a Met(hionine) amino acid in place of the normal Thr(eonine) at position 124 of the protein’s amino acid sequence. The resulting protein functions abnormally causing myelin damage and pain. Thus, the mutation appears to have a direct link to pain. The problem is that the mutation does not cause pain in all CMT-2 families, and the degree of pain tends to vary considerably from individual to individual. Moreover, several additional (Mendelian) mutations are also known to cause demyelinating peripheral neuropathies, with different names and different clinical features, but most of these neuropathies are painless. Only a few demyelinating neuropathies, most notably CMT-2, often cause pain (Kelley and Scherrer 2007). The reasons for this are not clear. The mutated P0 protein has a \textit{direct} effect on the health of the sensory axon’s myelin. But the process that links the gene product (the P0 protein) and pathology (demyelination) to the phenotype (pain) includes a number of intermediate steps that still need to be worked out. The reasons why demyelination may cause pain are complex and probably have to do with protein trafficking (Devor 2013). To add to the confusion, painful CMT-2 in some families derives from mutations of different genes that code for different nerve proteins. Nonetheless, the end result is a clinically similar type of neuropathy and functional deficit. The simple fact that the nerve has been damaged does not in itself explain the pain.

The examples of the Nav1.7 channelopathies and CMT-2 illustrate how knowledge of allelic variants associated with a painful medical condition may or may not provide insight into pain processing itself. In general, given the current state of pain science where many of the underlying processes and molecules are not yet known, it is difficult to go from the discovery of a pain gene to the mechanism by which it causes pain. \textit{A priori} appearance of a link may be misleading. One of the major potential contributions of pain genetics is to highlight genes that are empirically (clinically) important for pain experience. The challenge for pain scientists is then to determine \textit{how} these genes cause the effects that they cause.

The CMT-2 case is an example of a mutation that appears to have a direct link to pain but where the link is actually indirect. Most allelic variations that tip the balance toward pain can be expected to operate in ways that are indirect. Consider this imaginary, but not unrealistic, scenario. Imagine an allele that encodes for a muscle protein that tends to make a young man more muscular. Being more muscular, he is likely to be more athletic and perhaps more attractive to young women. A probable outcome is that he will be more popular, more self-confident, and perhaps more likely to engage in risky sports. As such, he will be more likely to suffer injury and pain. After all, the more risks you take, the more your chances of being hurt. The genetic variant that codes for the muscle protein is therefore, by definition, a variant of a pain susceptibility gene. Its presence leads to an enhanced likelihood of pain, however indirectly. Indeed, a gene allele associated with increased risk-taking has been reported, not in the pain genetics literature but in the literature on the genetics of personality traits (Knafo et al. 2008; Ebstein et al. 2010). It is the vasopressin receptor gene \( AVPR1A \). But by predisposing to taking risks, the allele also predisposes to injury and pain. There is every likelihood that risk-taking genes would come up in an appropriately large association study of pain. However, due to the very indirect link between pain and polymorphisms in both risk-taking genes and the gene for our hypothetical muscle protein, it is unlikely that either will lead to better understanding of pain mechanisms or to the development of better analgesic drugs.
Disease Susceptibility Genes Versus Pain Susceptibility Genes

In any discussion of pain genes, an essential distinction needs to be made between two situations: (i) alleles that cause (or predispose to) a disease that may be painful and (ii) alleles that are, at least partly, responsible for the fact that two different individuals subjected to identical noxious stimuli, or who have suffered an identical injury or disease, may report very different levels of pain. Some gene variants (alleles) cause painful disease. Examples are the PO gene underlying CMT-2 neuropathy and genes that increase the likelihood of developing type 2 diabetes (Wolfs et al. 2009). These are “disease susceptibility genes.” In diabetes, metabolic abnormalities sometimes lead to painful diabetic polyneuropathy, although usually diabetic neuropathy is non-painful. Sometimes the diabetes causes no neuropathy at all. The connection between genes, disease, neuropathy, and pain in type 2 diabetes is complex and still largely obscure. It will probably remain so until we have a better understanding of neuropathic pain mechanisms in general (Devor 2013). Identification of disease susceptibility genes may be of considerable medical interest for disease diagnosis and prevention, but such genes are not certain to contribute much to an understanding of the underlying pain process. It is difficult to know in advance. Therefore, if the aim is to understand pain rather than disease, a more likely approach is to identify genes in which sequence variants cause one person to develop pain, while another doesn’t, in the presence of the same disease or injury. These are “pain susceptibility genes.”

Finding genes that alter pain response to experimentally applied stimuli is relatively straightforward using the GWAS approach. One simply compares cohorts with high versus low pain response. Finding genes that predispose to pain due to disease or injury is more challenging. Here it is not sufficient to compare allelic differences in groups of individuals with more pain versus less pain. It is also necessary to insure that the groups being compared have the same underlying pathology. The likelihood of developing type 2 diabetes, for example, is affected by genes (as well as by lifestyle). But as noted, some people who suffer from diabetes develop severe or mild diabetic polyneuropathy, while others don’t. The factors that determine the severity of the neuropathy may have nothing to do with pain mechanisms per se. Thus, the individuals who develop diabetic pain may simply be the ones who developed a more severe neuropathy. A GWAS comparing diabetics with and without pain would therefore most likely yield genes that predispose to neuropathy, that is, disease susceptibility genes rather than pain susceptibility genes. In principle, this problem might be solved by matching the comparison groups for the extent of neuropathy present using criteria that are independent of diabetic pain. However, at this stage, we do not really know which criteria to chose.

A similar caveat holds for many other painful conditions, among them low back pain, postherpetic neuralgia, and temporomandibular joint disorder. There are a few conditions, however, where matching groups for equal pathology is feasible. Examples are neuropathic pains that develop (or do not develop) following standard surgical procedures that injure the nerves, such as thoracotomy or the harvesting of blood vessels in the calf for cardiac grafting (Devor 2004), TN where the lesion can be visualized with MRI (Miller et al. 2009), and osteoarthritic pain where the groups compared can be balanced for the degree of cartilage erosion assessed radiologically. Limb amputation presents a particularly interesting potential opportunity. As noted earlier, some amputees develop severe phantom limb pain, while others, with the same amputation level (i.e., the same disease), have none. Pain variability in the face of identical pathology provides confidence that pain susceptibility, and not just disease susceptibility, is heritable. Some pain susceptibility genes have already been discovered using this strategy including CACNG2, as noted earlier.
**Perspective**

The study of pain genes has only just begun. The basis for expecting substantial payoffs in terms of new understanding and medical applications is firm. This remains true even though results so far have not met early, perhaps unrealistically optimistic expectations in terms of ease of replication and magnitude of effects. One reason is that the methods and the choice of comparison groups used to date have often not been optimal. This includes both the underpowered scope of many of the trials undertaken so far and the focus on candidate genes selected on the basis of preconceptions rather than unbiased GWAS. In particular, one needs to recall that genes which predispose to developing a disease that tends to be painful may be only distantly related to the neural mechanisms of pain processing itself. This makes for weak genetic inference.

It is reasonable to predict that pain susceptibility genes, when found in the course of a systematic search, will show stronger association to pain phenotype than will disease susceptibility genes and will bear a closer relationship to pain mechanisms. At present, we should celebrate the success stories that can be told and hope that in time these will increase in number. In the meanwhile, the simple knowledge that heritability plays an important role ought to reduce the stigma that often attaches unfairly to individuals whose pain appears to be excessive. In this way, pain genetics can contribute, here and now, to better pain management.

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