Progress in Pain Genetics: A Meeting of Their Own

Miami symposium is first for field

by Pat McCaffrey

This is a report on the 10th IASP Research Symposium, The Genetics of Pain: Science, Medicine and Drug Development.

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Pain researchers convened on 7-9 February 2012 for a first-of-its-kind symposium dedicated to the genetics of pain. The meeting, sponsored by the International Association for the Study of Pain (IASP) and organized by the Genetics and Pain Special Interest Group, drew 120 registrants and 20 faculty to Miami Beach, Florida, US, for talks that ran the gamut from gene discovery in fruit flies to clinical development of ion channel blockers.

The meeting signaled a coming of age for the field of pain genetics. In his talk, Clifford Woolf, Children’s Hospital Boston, US, said that he is not a geneticist, but he joined the pain genetics train looking for research tools. “And it has been a wonderful ride—sometimes on a ghost train, sometimes stalled in a siding, and sometimes running out of control and almost derailed.” The meeting, he said, showed that the study of the genetics of pain “has become mainstream in neurobiology, and that’s exciting.”

Of mice (and rats) and men (and women)
The opening session, on preclinical studies of pain genetics, highlighted the central place of rodents in the discovery of pain genes, and in understanding the interplay of genes and environment. The talks showed the power of a translational approach that moves from animals to humans and back again to uncover candidate pain genes, elucidate mechanisms, and validate targets.

The first speaker of the conference, Marshall Devor, Hebrew University, Jerusalem, Israel, laid the groundwork for talks to come. Discovering genes that influence individual variation in pain sensitivity or risk for chronic pain serves two purposes, he said. First, it opens doors to understanding individual patients: making a diagnosis, illuminating a prognosis, predicting drug responses, or even comforting patients by telling them, “It’s not your fault.” Second, gene discovery and, in particular, unbiased genomewide methods will benefit many patients by uncovering novel pain mechanisms and potential new treatment targets. The real power, as Devor sees it, is the “promise for finding pathways in the physiology of pain that we never dreamed of before.”

The study of monogenetic pain diseases—rare occurrences like familial migraine, congenital insensitivity to pain, or congenital pain syndromes—has illuminated important players in pain pathways. But to identify genes that contribute to more common pain conditions, other approaches are needed. Linkage analysis is used when researchers have access to family members with and without pain. When it is impossible to do family studies (in post-operative pain, e.g., where every family member would have to have had the same operation), the alternative is association studies. Here, groups of unrelated people are compared on a case-control basis to identify genetic variants that are distributed unevenly in those with pain and those without. Association studies test either a limited number of pre-selected genes (a candidate gene approach) or all variants in an unbiased screen (a genomewide approach).

Devor said he believes genomewide association studies (GWAS) in humans are “the path to real discovery,” but such investigations are expensive, and so far very few have been funded for pain. He made the case that doing genomewide scans in animals is a way to “jumpstart” human studies, enabling the ultimate identification of human genes at a much lower cost. Identifying mouse strains that vary in the phenotype of choice (or making such lines by selective breeding) allows for the mapping of a phenotype to one or more quantitative trait loci (QTL). This can lead to the identification of the responsible pain gene or genes, which can then be confirmed in humans.

An example of this is the discovery that a variant of the CACNG2 gene is a risk factor for chronic pain after breast surgery (Nissenbaum et al., 2010). More than a decade ago, Devor collaborated with Jeffrey Mogil, McGill University, Montreal, Canada, and others to phenotype a panel of 12 common mouse strains in multiple pain tests, including autotomy after nerve injury, a model of neuropathic pain (Mogil et al., 1999). Further breeding and linkage analysis, and the use of inbred recombinant strains, led to the identification of a locus on chromosome 15 (Seltzer et al., 2001; Devor et al., 2005), which was ultimately narrowed to 155 candidate genes. Using expression data, functional annotation, and single nucleotide polymorphism (SNP) association, Devor and colleagues zeroed in on a single candidate, CACNG2. As the group reported in 2010, when they then looked at variants in the corresponding human gene, they found a haplotype of three SNPs that was associated with an increased risk of pain after mastectomy in a
sample of 549 Israeli women, some of whom had pain and some of whom did not.

“If this can be replicated, it’s useful,” Devor said. Possibly, if a woman is genetically more likely to develop pain, that information may be part of a decision on what type of surgery to have, for example.

**CACNG2** encodes the voltage-dependent calcium channel gamma subunit 2 (also known as stargazin), a protein which both regulates the trafficking of AMPA-type glutamate receptors and controls the excitability of neurons. The gene has been implicated in epilepsy, indicating a “deep connection between epilepsy and neuropathic pain having to do with excitability of neural networks,” Devor said.

**High-throughput phenotyping in a new mouse model**

**Ze’ev Seltzer**, University of Toronto, Canada, described an ambitious translational project involving high-throughput phenotyping in a mouse model of neuropathic spreading pain, and a large cohort of traumatic limb amputees he has assembled for genetic analysis of post-amputation neuropathic pain.

Seltzer described a new mouse model of unilateral damage to the infraorbital nerve (ION). In mice, as in other mammals and humans, the ION innervates the face from just below the eyebrow to the upper lip, and from the side of the nose to the cheek. In rodents, the ION also innervates the vibrissal pad that anchors the whiskers. Large parts of the rodent brain are dedicated to processing sensory input from the whiskers, and many labs have used ION injury in rats and mice as a model of neural plasticity, but not to study pain.

In new work, Seltzer and collaborators showed that they could use ION injury in mice to model the “extra-territorial” spread of neuropathic pain, a problem that occurs in many chronic pain patients where pain spreads beyond the field innervated by an injured nerve to surrounding regions. If they cut the ION, sensation was lost in the innervation area, including the whisker pad, but then over weeks some strains of mice developed increased pain sensitivity (alldynia and hyperalgesia) to mechanical and heat stimuli in the ears, contralateral whisker pad, forehead, nose, all four paws, and even the tail, “practically all over their body,” Seltzer said.

To investigate the genetic basis of pain spread, the researchers compared the levels of alldynia and hyperalgesia in male and female mice from three different genetic backgrounds (A/J, C57BL/6J, and DBA/2J strains). The highest contrast in the extent of spread of mechanical and heat alldynia and hyperalgesia was found between A/J and C57BL/6J mice of both sexes. Based on these differences, Seltzer’s group selected the AXB-BXA panel of 23 recombinant inbred lines, descendants of the two contrasting parental strains, for a detailed genetic mapping study. With publicly available genomic data on the strains, and Internet-based mapping software (WebQTL), Seltzer said, the task of associating each trait with a specific region of the genome becomes a simple statistical analysis.
However, it takes more than that to identify and validate the causative genes in each region. To narrow the list of candidate genes, Seltzer and colleagues at the University of Zhejiang, China, phenotyped an additional 15 strains of fully haplotype-mapped mice (the Peltz HapMap panel; see Zheng et al., 2012). The strength of this approach is that it fine-maps traits to chromosomal regions orders of magnitude smaller than the AXB-BXA panel. Combining the two assays is expected to decrease the candidate gene list to a manageable few.

What can they expect for results from these kinds of studies? Seltzer gave one example: the response of naïve animals to noxious heat, where his group sees a strong sex effect in some lines but not others. Mapping of the trait yielded five loci, including a major one on chromosome 7 that includes the gene for a metabotropic glutamate receptor. More work is needed, he said, to validate this gene.

The Toronto and Zhejiang teams have just finished phenotyping pain spread in approximately 1,600 mice from 40 strains or lines after ION injury or sham surgery. To help with the task, Seltzer and colleagues developed high-throughput avoidance tests for thermal or mechanical pain. They built a nine-chamber testing apparatus, where each chamber floor has a 30°C section and a 40°C section. Naïve animals show no temperature preference, but the higher temperature is painful to animals with neuropathic injuries. To test for mechanical allodynia, the chambers are fitted with smooth and spiked floor sections, and allodynic animals avoid the spiked surface. Video tracking is used to analyze animal behavior, and the set-up allows testing of nine animals in parallel in three to five minutes.

Moving to people
While mice can be marshaled by the hundreds or thousands for genetic studies, one of the biggest challenges in human pain genetics is achieving large, well-characterized cohorts to attain sufficient statistical power in association studies. To address that issue, Seltzer has been recruiting thousands of subjects for a genetic study of post-traumatic neuropathic pain in an unusual locale: Cambodia. He and his colleagues have enrolled 5,500 amputees who sustained injuries from land mines and unexploded munitions that still litter the country 20 years after the end of the last war. Approximately one in 230 Cambodians is an amputee (the highest rate in the world), and there are an estimated 60,000 amputees in the country. In Cambodia, one of the poorest countries in the world, the injured receive no first aid, no acute pain control, and cannot afford adequate treatment for chronic pain. However, the Cambodian government, with the help of foreign organizations, offers free prostheses and rehabilitation to land-mine victims. Seltzer’s collaborators have been working in the clinics where the replacement limbs are to recruit study volunteers and collect their DNA. The phenotyping consists of a three-hour interview that gathers demographic data, medical history, information on prior pain, amputation scenario, pain type, disability, and psychological state, as well as quantitative sensory testing (pin prick, cold pressor...
test, and thermal grill illusion, among other data). All the data, along with automated measurement of heart rate, blood pressure, and glucose levels, are beamed back to Toronto by satellite. In addition to the amputees, most of whom are male, Seltzer and colleagues have also collected DNA from 500 controls, plus 500 male family members of the affected group.

To achieve even larger sample sizes for genetic studies of neuropathic pain, Seltzer and collaborators have set up a consortium which they call the Tactical Union of Research Networks in Pain Genetics (TURN-PAGE). By combining seven cohorts, including amputees and post-surgical patients, the consortium now covers just over 10,000 subjects, by far the largest cohort assembled for pain genetics. The consortium includes amputees from Cambodia, Germany (collected by Herta Flor and coworkers at the Heidelberg University, Mannheim, Germany), and Israel (collected by Seltzer and his colleagues at Sheba and Beit Levinstein Medical Centers, Israel), five cohorts of post-surgical neuropathic pain, including two cohorts of women post-mastectomy (collected in the US by Inna Belfer and her colleagues at the University of Pittsburgh, and in Israel by Seltzer and colleagues at Hadassah and Sheba Medical Centers), a cohort of post-cardiac surgery patients (collected by Joel Katz, Hance Clarke, and Seltzer at Toronto General Hospital, Canada), and a cohort of neuropathic pain patients (the Canadian Multicenter Neuropathic Pain Database (NePDAT) Registry collected by Dwight Moulin, Western University, Ontario, Canada, and others). The consortium also collaborates with deCODE Genetics, Iceland, which has access to a large group of various chronic pain conditions. The total number of subjects is expected to increase to 14,000 by the end of 2013.

The next challenge is to find the money to do the genetic studies. “Each item on the genotyping menu has a price tag,” Seltzer said. The consortium would like to do a genomewide association study for common variants, as well as look for rare trait-associated loci. All of these studies are costly, and Seltzer said he is now looking for support from pharmaceutical companies and from the governmental funding agencies in the US and Canada. The consortium has teamed up with Beijing Genetics Institute, the largest genotyping facility in the world, to do exome sequencing in several hundred amputees, comparing those with the highest levels of pain and pain-free subjects. Also in the works is a screening with a commercially available exomics array.

**It’s complicated**

How are we doing overall in pain genetics? Good in some ways, said Jeffrey Mogil, McGill University, Montreal, Canada. His Pain Gene Database currently lists data on 358 knockout mice with a pain phenotype, drawn from 839 publications. “What percentage of those genes will end up contributing to individual differences is anyone’s guess,” he said, but he believes it will be a pretty large one. In humans, association studies have exploded since 2005, implicating approximately 120 genes. But, he pointed out, many associations have proven hard to replicate.
The problem, Mogil suggested, is that “we have been too simpleminded.” Chronic pain is the result of many interactions, and it is known that genes interact with other genes and with environmental factors. Unaccounted-for interactions may explain why association studies don’t replicate more often than they do now. However, Mogil said, “If we position ourselves to look at these interactions, we’re going to find them more often than not.” That means collecting as many data as possible to increase the chances of identifying hidden complexities.

As evidence, he presented his recent work on vasopressin and stress-induced analgesia (see PRF related news story on Mogil et al., 2011). Mogil and colleagues first identified an association of the vasopressin receptor gene Avpr1a with inflammatory pain sensitivity in mice. But, following up on the result in humans, they unearthed a three-way interaction among the receptor gene variants, stress, and sex. All three factors determine whether mice, or humans, display an analgesic response to administered vasopressin. The study revealed that vasopressin mediates stress-induced analgesia, but only in males. Along the way, it served up a cautionary tale for gene hunters: The original finding, an association between the receptor gene and inflammatory pain in mice, was only detected because the animals were stressed during the testing procedure. Animals that were acclimated to the testing scenario beforehand did not show the association.

“I don’t think we can get away from the complexity, because it’s part of the system,” Mogil said.

**Mice made for the job**

A pair of talks in later sessions expanded on the utility of mouse genetics. In the years since Mogil’s original analysis of strain differences in pain phenotypes, the development of panels of recombinant inbred strains and advances in sequencing have given researchers ever more powerful genomewide tools to reveal the genetic underpinnings of pain.

**William Lariviere**, University of Pittsburgh, US, described the use of recombinant inbred strains as genetic tools. These strains serve as useful reference populations, because their genotype, phenotype, and expression profiles are stable over time and data can be pooled and shared among different labs. He has utilized the BXD recombinant inbred panel (a set of strains derived from crosses between C57 Black 6/6J and DBA/2J strains) to identify QTLs for mechanical sensitivity and nociception or acetic acid-induced abdominal extensions (a model of inflammatory pain). In each case, combining linkage mapping with publicly available expression data has helped to narrow down the candidate gene list (e.g., see Nair et al., 2011). Lariviere is now also studying the genetic determinants of the response to melittin in honeybee venom, an alternative therapy used to treat arthritis (reviewed in Chen and Lariviere, 2010). He reported that some mouse strains are hypersensitive to the painful effects of melittin, a trait that maps to an interval on chromosome 18 containing only five known genes. Other strains are hyposensitive, and that trait maps to a two-gene
interval on chromosome 19. For the hyposensitivity trait, expression covariance data implicate the gene *Atrnl1*, which encodes a protein that interacts with the melanocortin 4 receptor, but whose function is unclear and has never been implicated in pain before.

“We are just at the beginning of exploiting these models,” Lariviere said. At the same time, he said, the BXD model is "going out of style." Because the panel results from the cross of just two strains, there is limited genetic diversity. However, a new model is nearing completion. The collaborative cross is the product of breeding between eight founder strains, of which three are derived from wild-type mice. The resulting strains together will represent greater than 90 percent of mouse genetic diversity and offer higher-precision genetic mapping. Results using the new strains in tests of thermal sensitivity (hot plate latency) reveal a locus on chromosome 5 containing six genes, and for mechanical nociception (tail clip test), a locus on chromosome 2 containing only five known genes (Philip et al., 2011). For new genetic studies, Lariviere said, this is the model researchers should be proposing in their grant applications, unless they can justify needing all the additional supporting information already available for the BXD strains.

Roy Levitt, University of Miami Miller School of Medicine, US, has tapped into another mouse resource to identify genes that contribute to the risk of persistent pain after surgery. The mouse HapMap project has genotyped 94 strains of mice and identified eight million SNPs. To look for genetic variants associated with pain after surgery, Levitt and colleagues measured thermal withdrawal in 16 inbred strains of mice (using the Hargreaves test) at baseline, 1, 7, 14, and 21 days after chronic constriction injury of the sciatic nerve. They plotted the withdrawal latency over time, and used the area under the curve to calculate a persistent pain index that they showed was heritable. Levitt presented unpublished data on haplotype association mapping using three-SNP windows that revealed loci on chromosome 5 (covering five genes) and 12 (containing one gene) associated with persistent post-surgical pain.

The gene on chromosome 12 encodes Nova-1 (neuro-oncological ventral antigen-1), a tissue-specific RNA-binding protein that regulates alternative splicing in the central nervous system. Nova-1’s targets include many proteins in the inhibitory synapse (Ule et al., 2005). Nova-1 protein regulates the splicing of kinases and phosphatases, and the phosphodomains in many proteins (Zhang et al., 2010). Therefore, the protein has the capacity to affect overall phosphorylation patterns in the central nervous system.

Levitt and colleagues looked at Nova-1 protein and activity in dorsal root ganglia associated with injury. They found that the protein is expressed in nociceptors, and that its activity differs among mouse strains. A comprehensive RNA profiling revealed statistically significant differences in the splicing patterns of Nova-1 targets among strains, which might account for differences in susceptibility to post-operative pain.
Is Nova-1 associated with pain in people? Levitt found preliminary evidence that says yes. In three cohorts (osteoarthritis, pain one year after hernia surgery, and post-herpetic neuralgia), there was a statistically significant association between one or more Nova1 SNPs and pain. No association was seen in a sciatica group.

Levitt says the next steps are to identify SNPs that explain the varied function of the gene and understand how they affect persistent pain susceptibility in animal models and in human populations. He added that, down the road, this could lead to diagnostics that help determine who is at risk for persistent post-operative pain, and better interventions.

**Human studies move ahead**

Mice and rats led off in Miami, but before long, talk turned to human genetic studies. In contrast to the large body of mouse work, human studies are still in their infancy, most agreed. Numerous challenges—recruiting patient cohorts, defining phenotypes, accounting for clinical and genetic heterogeneity, finding funding for large genetic studies for pain—all affect the pace of discovery. Getting to see the big picture may be some time away, but bits and pieces are coming into focus.

**Inna Belfer**, University of Pittsburgh, US, reviewed work on the best established and most published pain gene, catechol-O-methyltransferase (*COMT*). First studied in 2003, *COMT* is now the subject of more than 100 publications relating to pain (reviewed in Belfer and Segall, 2011). Belfer reviewed what is known about the gene, and presented some new data suggesting that *COMT* variants play different roles in nociceptive versus neuropathic pain.

The *COMT* protein metabolizes catecholamine neurotransmitters including dopamine, epinephrine, and norepinephrine. As a terminator of neural signaling, *COMT* has been implicated in mood, cognition, stress response, and pain. *COMT* genetic variation involves over 28 common single nucleotide polymorphisms (SNPs) that are inherited in complex patterns. For one highly studied missense variant, Val158Met (or SNP identifier rs4680), the Met allele is associated with decreased thermal stability and enzymatic activity of *COMT*, and thus higher levels of dopamine and other neurotransmitters. The Met allele has been studied extensively for its effect on dopamine-related cognitive phenotypes, including mental processing speed, mood, and schizophrenia.

Belfer’s previous work established that four SNPs in *COMT* define three haplotypes that account for low, medium, and high sensitivity to experimental pain, differences in endogenous pain control via the μ-opioid receptor, and susceptibility to common musculoskeletal pain conditions. High pain risk is linked to extremely low enzyme activity, and average and low pain risks are associated with higher activity. Interestingly, both high and low pain haplotypes contain the Val158 variant, but differ at other SNPs that influence mRNA structure and protein expression levels. This shows that evaluating haplotypes,
rather than individual SNPs, is necessary to understand functional variation in genes.

Dopamine, epinephrine, and norepinephrine have both pro- and anti-nociceptive actions, and so the exact pathways by which variations in COMT enzyme activity affect pain are complex and mostly unknown. Belfer pointed to two negative association studies (Armero et al., 2005; Mylius et al., 2010) that got her thinking about a possible dichotomy in catecholamine signaling in nociceptive and neuropathic pain. High-activity COMT variants protect against nociceptive pain, but their potential to lower epinephrine and norepinephrine in the spinal cord could lead to less anti-nociceptive signaling through β-adrenergic receptors and more pain. Could high-activity COMT alleles be a risk factor for spinal cord or neuropathic pain?

To investigate this idea, Belfer and colleagues did a post-hoc analysis of COMT variants in 11 common strains of mice that had been phenotyped previously (Mogil et al., 1999). Strains of mice vary in their COMT activity, and this has been shown to be associated with pain sensitivity (Segall et al., 2010). Belfer presented unpublished data suggesting that, across the strains, COMT haplotypes were differentially associated with nociceptive and neuropathic pain modalities. A low COMT haplotype was a risk factor for nociceptive pain, and high COMT was protective, as observed previously in human studies. However, in the mice, high COMT alleles conveyed a risk for neuropathic pain. The mechanism likely involves signaling through the adrenergic receptor, but dopamine signaling or effects on the immune system may also play a part.

**Intermediate phenotypes: breaking it down**

**William Maixner**, University of North Carolina, Chapel Hill, US, presented results from the genetics portion of OPPERA, the largest prospective human study of chronic pain and its risk factors to date (see PRF related news story). OPPERA (Orofacial Pain: Prospective Evaluation and Risk Assessment) is following 3,276 initially pain-free adults for seven years, watching for onset of temporomandibular (jaw) pain. Data were recently published from a case-control study within OPPERA that analyzed 3,295 SNPs in 358 candidate genes in 348 cases and 1,612 controls (Smith et al., 2011). In the study, no SNP exceeded the statistical threshold for significance, although previously reported associations of the COMT and HTR2A genes were supported. Suggestive associations were seen for five genes (NR3C1, CAMK4, CHRM2, IFRD1, and GRK5) not implicated before in risk of pain or nociceptive pathways.

The data, Maixner said, “may not be as rosy as
we would like them to be, and the interpretation is problematic.” In pain, as in other fields, genomewide association results have not been robust or reliable. Many findings point to SNPs or genes without known function, and with low odds ratios that explain only a very small portion of the trait variation. One problem, Maixner said, is that SNPs are often treated as a univariate phenomenon, and researchers need to start looking at gene-gene interactions and using pathway analysis and other computational methods.

Another complication of association studies in pain and other conditions is that phenotypes such as case status (whether subjects have a disease or not) are complex. When people talk about “pain genes,” Maixner said, they use the term “pain” to mean a favorite phenotype or disease of interest. But each gene determines only a small part of the overall complex phenotype. That is where endophenotypes, or intermediate phenotypes, come in.

For example, underlying a persistent pain condition such as temporomandibular joint disease and contributing to its complex phenotype are signs and symptoms that can be measured individually. These include mood, anxiety, depression, tissue injury, state of pain regulatory systems, proinflammatory state, and others. These more defined endophenotypes or intermediate phenotypes are each affected by genes that may have a dramatic effect on the endophenotype, despite having a small effect on the overall complex phenotype. So, by defining endophenotypes that are more proximal to genes, it should be possible to better identify genetic associations with complex pain conditions.

**Not all patients are created equal**

One way to “clean up” phenotypes, and discover endophenotypes, is by dividing heterogeneous populations into homogeneous subgroups. To do that, Maixner is using statistical clustering techniques to group patients based on detailed characterizations including quantitative sensory testing, and psychological and physiological measures. Using a cohort of 199 chronic temporomandibular disorder (TMD) patients and 200 controls, the analysis identified three clusters: One was mostly controls, the second had an approximately sevenfold increased chance of having TMD, and the third had a 60-fold increased chance of TMD. The clusters had different phenotypic profiles: The second cluster had more pressure pain sensitivity than the first, and the third had more psychological distress, autonomic differences, and pain sensitivity relative to the other two. Patients in cluster three had more severe pain clinically, more comorbid pain conditions, and more headaches, indicating that the clusters defined a gradation of severity.

When the analysis was applied to the OPPERA cohort, Maixner and colleagues attained similar results. If a patient was in cluster three at baseline, he or she was 2.5 times more likely to develop TMD, and 85 percent of the TMD patients fell into cluster three. The researchers are now looking at genetic profiles within the clusters.

Even when individual polymorphisms do not achieve statistically significant
scores in association studies, they can suggest hypotheses for further testing. When Maixner and colleagues compared 129 people with chronic TMD and 231 very healthy “supercontrols,” they found no hits that attained significance on their own. However, two of the top three genes were for proteins involved in the epidermal growth factor (EGF) signaling pathway, namely, the EGF receptor (EGFR) and one of its ligands, epiregulin (EREG). Maixner showed unpublished data from a collaboration with Jeffrey Mogil indicating that EGF and the EGFR are involved in producing acute and inflammatory pain, but not neuropathic pain, after nerve injury in mice.

Continuing the theme of pathways, Maixner’s colleague at the University of North Carolina, Luda Diatchenko, presented new data on pathway and phenotype analysis of the OPPERA case-control cohort genetic data. To better detect genetic associations, Diatchenko divided patients into two phenotypic groups: One group had only localized facial pain (believed to result from direct activation of muscle nociceptors), and the other had widespread pain (presumably due to central sensitization). In a discovery cohort, none of the top SNPs approached statistical significance after correction for multiple testing. Interestingly, the top SNPs in the two groups were different.

Diatchenko then proceeded to pathway analysis, where genes are grouped into pathways based on their functions and relationships, and genetic data are queried for associations to pathways, rather than individual SNPs or genes. The approach posits that biological pathways, rather than specific polymorphisms, are important for clinical effects, and in a given population there may be associations with different genes, but they will cluster into a limited number of biologically important pathways. Starting with commercially available software (Ariadne Genomics Pathway Studio) that was made for analyzing RNA expression, Diatchenko and colleagues modified it to analyze SNPs. They first combined p values for all SNPs in one gene to give a gene level p value, and from there calculated a pathway p value from all genes in any of 248 pathways.

In this analysis, patients with just facial pain showed a significant association with the serotonin receptor pathway, including two serotonin receptor genes and two MAP kinase genes. In a discovery cohort, four SNPs in the serotonin pathway combined to cause a 2.3-fold increased risk of TMD with local pain, and the same four SNPs replicated in the OPPERA cohort, giving a significant 1.6-fold increased risk. In patients with widespread pain, a T cell receptor signaling pathway was significantly associated in the discovery cohort, although that did not hold up in the replication cohort.

**Beyond genetics**

Jörn Lötsch, Goethe Universität, Frankfurt am Main, Germany, has studied the effects of genetic variation on the response to opioids. But in his talk, Lötsch presented a different kind of data—on the response of genes to opioids. His recent work suggests that opioid treatment leads to epigenetic changes in pain patients, which could potentially contribute to pain.
Epigenetic changes are stable, environmentally induced modifications of the genome, such as methylation, that affect gene expression without changing DNA base sequence (reviewed in Doehring et al., 2011). There is a small but growing literature on epigenetics and pain (see PRF related news story). It had been shown that methadone-maintained opioid addicts have increased methylation near OPRM1, the gene for the μ-opioid receptor (Nielsen et al., 2009), but it was unclear whether the change was a cause or an effect of drug use. That inspired Lötsch and Alexandra Doehring to look at pain patients, and Lötsch reported unpublished results showing the same increase in methylation in chronic opioid-treated subjects.

The results suggest that opioids have epigenetic effects, and open up the possibility that the observed changes in DNA methylation could contribute to pain. “As DNA methylation has been shown in animal experiments to produce pain, it would be reasonable to ask whether drug-induced methylation affects pain in patients treated with these drugs,” Lötsch said. Those experiments are ongoing.

**Problematic Phenotyping**

In a session on pain assessment, several presenters took up a critical issue in studies looking to relate genes to pain, and that is defining and validating phenotypes. “Poor phenotypes are one of the main reasons genetic studies fail,” said Christopher Sivert Nielsen, Norwegian Institute of Public Health, Oslo, Norway.

What makes a good phenotype? Nielsen said the measure must be reliable, valid, stable over time, heritable, genetically homogeneous, and consistently used across studies. The last two are particular challenges for the pain field, where it is not clear if different types of chronic pain (idiopathic, neuropathic, or nociceptive) have common genetic bases, although recent data from a twin study indicate that pain reporting at different body sites can be explained by a single genetic factor (Williams et al., 2010). In addition, the definition of phenotypes across studies is very inconsistent, making it hard to compare results.

Can experimental pain measures stand in as endophenotypes of chronic pain? While all experimental pain measures are heritable, they are not all equally so. Twin studies show that most clinical pain conditions have a heritability of 40-50 percent, while experimental measures of pain had varying heritability from 22-55 percent in one study (Norbury et al., 2007). In addition, not all experimental pain measures are created equal. Several studies have shown that sensitivity to different painful stimuli in an individual is not consistent across modalities (heat, pressure, cold), suggesting different underlying genetic determinants for each response (Nielsen et al., 2008; Neziri et al., 2011; Lariviere et al., 2002).
That leaves the question of which measures correlate best with clinical pain. Nielsen argued for the cold pressor test, in which subjects immerse their hands in ice water to determine cold pain threshold and tolerance. He presented preliminary data from a study of 10,566 subjects in a population-based sample drawn from citizens of Tromsø, Norway. In the study, subjects with chronic pain (defined as pain lasting three months or longer) were more sensitive in the cold pressor test, and those with longer duration of pain (more than five years) were more sensitive than those with shorter duration. The frequency of pain made little difference, but intensity did—people who experienced moderate or strong pain were more sensitive in the cold pressor test. People who had pain at two or more body sites showed more sensitivity. The results suggest that increased sensitivity to cold pain does mark a population of people with strong, long-lasting pain and widespread pain. In contrast, heat pain threshold (tested in 4,094 people) and pressure pain (n = 4,807) did not show significant correlations with clinical pain. Repeat measures of heat pain and the cold pressor test in a small number of subjects suggested that responses to heat were less stable over time. Nielsen concluded that the cold pressor test may be a better marker for a general pain condition than other measures. Overall, he said, phenotype selection should be based on empirical evidence of measurement characteristics, relationship with clinical pain, and genetic factor structure from twin or family studies.

According to Roger Fillingim, University of Florida, Gainesville, US, the genome is dwarfed by the phenome. “We can train a chimpanzee to do genotyping, but you have to be really smart to do phenotyping,” he said. Like Nielsen, he sees quantitative sensory testing (QST) measures as a source of endophenotypes that may provide increased power to identify genetic interactions. But, in the biopsychosocial model of pain, biological factors, genetic and otherwise, interact with psychological and social factors to produce the ultimate outcome of pain and disability. Other factors also influence genetic effects, including sex, age, and ethnicity. That means that researchers need to model these interactions as well, and remember that psychological, as well as biological, factors fall under genetic control.

How to deal with the complexity? Ignore it, omit it by design, or anticipate and model it, Fillingham said.

Defining phenotypes depends on standardized descriptions of pain. Researchers in the field of spinal cord injury (SCI) have made progress on that front with a recently published pain taxonomy (Bryce et al., 2011), said Eva Widerstrom-Noga, University of Miami, US. Pain after spinal cord injury, consisting of neuropathic and other types of pain, is very common, and is one of the most significant factors that lead to reduced quality of life. The consensus taxonomy covers all the pain that patients experience, whether resulting directly from their injury or not, to allow clinicians and clinical researchers to classify uniformly and to study patient-reported pain after SCI. Widerstrom-Noga showed how both pain reports and QST can be used to define patient subtypes in SCI. She also presented data using magnetic resonance spectrometry to look for changes in
brain biochemistry associated with pain. She reported preliminary results in a small group of patients suggesting that SCI patients with severe pain that highly impacted their lives in a negative way showed changes in brain metabolites in the anterior cingulate cortex, consistent with a role for that region in the experience of pain and distress.

One model of chronic pain that is considered the most promising for genetic studies is persistent post-surgical pain, because studies can be done prospectively, before subjects have chronic pain, and subjects are plentiful. In his talk, surgeon Henrik Kehlet, Copenhagen University, Denmark, reviewed work on the factors that contribute to persistent pain after hernia operation. In a prospective study, he identified both patient-related and surgery-related factors. Both are important: Surgical techniques that cause more or less nerve damage can result in higher or lower rates of post-operative pain. But an identical technique can present a different risk to individual patients, depending on patient factors including pre-operative responsiveness to pain (Aasvang et al., 2010). The take-home message, Kehlet said, is that all the details—pre- and post-surgical, patient-specific and procedure-specific—matter to pain outcome, and thus to genetic studies using outcome as a phenotype. “This setup can be used [for genetic studies],” he said, “but you have to take into account many factors.”

**Translational Value**

There are two main reasons to track down genes involved in pain. One is to help identify people at risk for pain, and the other is to illuminate new mechanisms and, ultimately, novel targets for therapy. The translational value of genetic research as a pathway to drug discovery was on display in several presentations throughout the meeting.

Clifford Woolf, Children’s Hospital Boston, US, showed the twists and turns that ensue in moving from gene to potential treatment. In his case, a novel gene led researchers to consider a new use for an old drug.

The novel gene is *GCH1*, which encodes GTP cyclohydrolase, the rate-limiting enzyme in the synthesis of tetrahydrobiopterin (BH4). BH4 is a cofactor required for enzymes that produce catecholamines (dopamine, epinephrine, norepinephrine), serotonin, and nitric oxide. Woolf and colleagues discovered *GCH1* in a genomewide expression profiling of genes that were upregulated in rat models of neuropathic pain, and went on to show that the enzyme, and BH4, control neuropathic and inflammatory pain in rodents. That finding translated to humans, where they showed that a haplotype for human *GCH1* that caused decreased enzyme activity was associated with a lower risk of chronic pain, and with reduced sensitivity to pain (Tegeder et al., 2006; Tegeder et al., 2008). This result has since been replicated (Campbell et al., 2009; Lötsch et al., 2010; Kim et al., 2010).
BH4 synthesis is complicated, involving de-novo, salvage, and recycling pathways. Woolf mentioned unpublished data indicating that genes involved in all three pathways show changes in expression in neuropathic pain models in rodents. Based on the accumulation of human genetic and animal results, Woolf suggested that BH4 levels could serve as a biomarker of pain: People who make more BH4 have a greater risk of chronic pain.

Are there targets for new analgesics in the BH4 synthetic pathway? Recently, an independent group using a yeast screen to detect novel targets for approved drugs identified sulfasalazine, a mixed antibiotic and salicylate, as an inhibitor of the last enzyme in the BH4 synthetic pathway, sepiapterin reductase (SPR) (Chidley et al., 2011). The drug, which is over 70 years old, is widely used as an anti-inflammatory to treat rheumatoid arthritis (RA) and inflammatory bowel disease. Rheumatologists view sulfasalazine as an anti-inflammatory and disease modifier, not as an analgesic, but clinical trials have shown it does reduce pain in patients with RA. In addition, the compound blocks allodynia in diabetic rats (Berti-Mattera, 2008).

“We think this is pretty encouraging,” Woolf said, noting that he and his colleagues are working to make novel SPR inhibitors. Using a crystal structure of the enzyme to guide drug design, they have synthesized an inhibitor with μM potency in cells. And, they are in the planning stages of studies that will look at the activity of sulfasalazine itself in people with neuropathic pain.

Woolf speculated that SPR inhibitors might have dual anti-inflammatory and analgesic actions. GCH1 and BH4 upregulation was recently documented in T cells of the immune system (Chen et al., 2011), a cell type that Woolf and colleagues showed contributes to neuropathic pain (Costigan et al., 2009; see also coverage of Michael Costigan’s talk, below).

“The data encourage us to think that BH4 has activity on neuronal excitability and on immune cells, and thus that reducing BH4 will provide new tools to treat pain and inflammation,” Woolf concluded.

In another talk, Josef Penninger, Austrian Institute of Molecular Biology, Vienna, spoke about alternative models for gene discovery. Penninger collaborated with Woolf and others to identify pain genes in the fruit fly Drosophila melanogaster using avoidance of noxious heat as a high-throughput behavioral screen. In all, they found 580 candidate genes. Among them was straitjacket, a gene encoding a calcium channel subunit that the researchers went on to show is required for central processing of pain signals in flies and in mice (Neely et al., 2011; and see PRF related news story). Variants in the human straightjacket homolog CACNA2D3 were associated with the risk of chronic pain in people (Neely et al., 2011; and see PRF related news story). The screen also identified the fly transient receptor potential channel A1 (TrpA1) as a mediator of painful heat sensation in flies (Neely et al., 2011) whose nociceptive
function is conserved in humans. Penninger mentioned unpublished results on another gene, the phosphoinositide-3 kinase γ subunit (PI3Kγ), which also appears to function in mice (the knockout is hypersensitive to thermal stimuli and capsaicin), and humans (a single nucleotide polymorphism [SNP] in the human gene for PI3Kγ gene is associated with risk of chronic pain).

The results raise the question of how to evaluate the hundreds of other candidate pain genes from the fly screen. Knocking out each gene in a higher organism like the mouse takes a long time. Is there a quicker way? One reason that mammalian genetics take so long is that cells are diploid, containing two copies of each gene. Detecting recessive, loss-of-function effects requires removing both gene copies. Penninger asked if it was possible to develop a mammalian haploid cell that could be used for rapid genetic screens.

The answer, perhaps surprisingly, is yes. As they recently reported, Penninger and colleagues have succeeded in isolating haploid pluripotent embryonic stem cells (ESCs), and have developed a method to do saturating mutagenesis in the cells for genomewide screening for recessive traits (Elling et al., 2011). To derive the cells, the researchers induced parthenogenic cell division in unfertilized mouse oocytes, and then used standard methods to produce blastocysts, from which they isolated haploid ESCs. The two lines they established could be differentiated into all three germline tissues, and, Penninger said, to astrocytes and neurons. The lines could also be used to make chimeric mice. Similar results have been reported by another group (Leeb and Wutz, 2011).

Penninger and colleagues also developed a viral vector that they could use to induce reversible insertional mutations genomewide. With a single infection, they achieve what Penninger believes is near-saturation mutagenesis of every gene, producing up to 10 million independent mutations, one per cell. They used the mutants to identify for the first time a protein essential for cell death induced by ricin, a plant toxin and potential bioterrorism weapon.

Yeast genetics in a pluripotent embryonic stem cell background will allow the construction of a genomewide mutant ESC bank, Penninger said. Rather than use knockout mice to evaluate candidate genes, tests can be done in vitro, he said. “I would love to take this into pain models, into whatever we can model in cell culture to help us untangle all these data we have,” he said. Other exciting possibilities include the ability to "knock in" genes carrying mutations or combinations of common SNPs.

Michael Costigan, Children’s Hospital Boston, US, has pioneered the approach of using differential gene expression in rodent models to identify pathways and genes involved in neuropathic pain (Costigan et al., 2002). One pathway is immune activation, and Costigan and colleagues showed that T cell infiltration into the central nervous system (CNS) plays an important role in producing hypersensitivity after nerve injury in rats (Costigan et al., 2009). Since then, they have found that reconstituting immune-deficient animals with T cells, but not B
cells, can produce hypersensitivity.

In support of the importance of T cells in neuropathic pain, Costigan mentioned unpublished GWAS data on 180 patients enrolled in a prospective study of pain after surgery for lumbar disc sciatic pain. The second strongest association seen in the study, he said, was with an unnamed gene that controls T cell activity. Other genes identified in the study included known pain genes GCH1, KCNS1, and Nav1.7.

To produce pain in rats, T cells need to cross the blood-brain barrier, and Costigan proposed that preventing T cells from entering the CNS might be a new therapy for neuropathic pain. Although there is as yet no direct evidence for T cell infiltration associated with neuropathic pain in people, Costigan said it is intriguing that one of the symptoms of the autoimmune disease multiple sclerosis (MS) is trigeminal neuralgia (a neuropathic chronic pain syndrome). New data about how the nervous system regulates immune cell access through the blood-brain barrier in animal models of multiple sclerosis (see Arima et al., 2012, and associated comment by Costigan) raise the intriguing possibility that treatments for multiple sclerosis aimed at keeping T cells out of the nervous system might also have utility in chronic neuropathic pain, he said.

Into the clinic
A sure sign of enthusiasm for the meeting was how many participants stayed on through the last session, turning out for the final talks on new clinical approaches to pain. David Fink, University of Michigan, Ann Arbor, US, spoke about targeted gene delivery for treating localized pain. He has been developing herpes simplex virus (HSV)-based vectors that can retrogradely carry therapeutic genes from sensory nerve endings in the skin into the dorsal horn of the spinal cord. In published Phase 1 trial data, an HSV-proenkephalin vector appeared safe and even showed some evidence of efficacy in cancer patients (Fink et al., 2011). Enrollment in a Phase 2 randomized, placebo-controlled trial is nearly complete, he said, and results should be available later in 2012.

Genes that are close to clinical testing include GAD67, which encodes glutamic acid decarboxylase, a GABA-synthesizing enzyme. In rats, HSV vector-based transfer of GAD67 to dorsal root ganglion neurons results in the continuous release of GABA and reductions in hyperalgesia and allodynia in a model of painful diabetic neuropathy (Chattopadhyay et al., 2011). Fink also reported he recently received a grant to produce a neurotropin-3 vector as a potential therapy for chemotherapy-induced peripheral neuropathy.

Simon Tate, Convergence Pharmaceuticals, Cambridge, UK, presented data on a selective voltage-gated sodium channel (Nav1.7) blocker that recently began clinical trials. He showed unpublished data supporting the drug-like properties of the compound (orally dosed, achieves adequate blood levels, and well tolerated with few CNS side effects), and a unique activity profile of state-dependent channel inhibition that is relatively selective for Nav1.7 compared to other
voltage-gated sodium channels. The clinical plan for CNV1014802 includes two proof-of-concept studies with novel designs. “If Nav1.7 blockers have a chance in the clinic, this molecule will tell us,” Tate said.

After Phase 1 testing in healthy volunteers, CNV1014802 moved to Phase 2 in July 2011, in a crossover design in lumbosacral radiculopathy. Results are expected in the second half of 2012. A second Phase 2 trial is planned in trigeminal neuralgia. In that condition, the pain is so intense and carbamazepine offers an effective (but poorly tolerated) therapy, so patients will not be randomized immediately to placebo or active treatment. The trial will have an initial three week open-label phase, and then responders will be randomized to four weeks of placebo or drug in a double blind design. The primary outcome of the study will be the number of failures on CNV1014802 compared to the number of failures on placebo during the double blind treatment period. Results of that trial are due in early 2013.

It didn’t take human genetic data to interest researchers in Nav1.7, but the discovery in 2004-2006 of mutations in the gene for the channel in the painful conditions erythermalgia and paroxysmal extreme pain disorder (Yang et al., 2004; Cummins et al., 2004; Fertleman et al., 2006), and in congenital insensitivity to pain (first reported in Cox et al., 2006), helped to validate the target. “We were already working on the channels, but if we hadn’t been, we would have started,” Tate said.

With a look forward to these exciting clinical prospects, the meeting ended, leaving the attendees with much to ponder. If you missed the meeting, but want to learn more about pain genetics, never fear. The Pain Genetics Special Interest Group is sponsoring a satellite symposium this summer at the IASP World Pain Congress in Milan, Italy.

*Brain and helix image credit: A. Latremoliere and I. Chu, Children’s Hospital Boston*