

**Highlights of
The NIH Pain Consortium
4th Annual Symposium on Advances in Pain Research
NIH Natcher Conference Center
May 26, 2009**

This symposium focused on the genetics of pain. The three major areas covered were (1) genetic risk factors for chronic pain, (2) genetics of analgesic drug responsiveness and addiction, and (3) genetic tools and models for studying and treating pain.

Opening Remarks and Introduction of New Co-Leaders

Patricia A. Grady, Ph.D., R.N., FAAN, Director, National Institute of Nursing Research and NIH Pain Consortium Co-Leader

Dr. Grady reviewed the history and goals of the Pain Consortium, which serves as the coordinating group for the 20 NIH Institutes and Centers (ICs) that fund pain research. These Institutes and Centers are investing a total of \$279 million in pain research this year. Dr. Grady discussed two recent funding opportunities for pain research available through the American Recovery and Reinvestment Act: NIH Challenge Grants and Grand Opportunity Grants. She also presented a list of all NIH funding opportunities currently available for pain research, and noted that a topic in pain, “Harnessing Our Understanding of Neural Plasticity to Elucidate the Transition from Acute to Chronic Pain,” has been included in the Blueprint Grand Challenge (a new initiative from the Neuroscience Blueprint). Legislation is currently pending on Capitol Hill to increase recognition of pain as a public health issue, promote better pain treatment, and accelerate pain research.

The Pain Consortium has a new, revised website (<http://painconsortium.nih.gov/>) where information about research funding opportunities and Consortium activities can be found. A number of workshops in pain research were held this year, including workshops on Neuropathic Cancer Pain, Non-Pharmacological Treatments for Back Pain, and Genetics of Pain, and the Headache Research Planning Meeting.

Comments from New Co-Leaders

Josephine P. Briggs, M.D., Director, National Center for Complementary and Alternative Medicine (NCCAM)

Forty percent of Americans now use at least one form of complementary or alternative medicine (CAM), such as herbal dietary supplements, meditation, or massage. Pain is the leading reason by far for which individuals use CAM. Back pain is the leading form of pain for which CAM is used, followed by neck pain, joint pain, and arthritis. Use of CAM to treat pain has risen significantly in recent years. Interestingly, the use of CAM is more prevalent among people with higher education (i.e., college degrees or higher).

A number of CAM approaches show promise for treating pain. For example, meditation produces long-lasting changes in the brain that may help counteract those that occur in the transition from acute to chronic pain. However, there are cases in which excessive claims have been made for the effectiveness of CAM treatments, and the NCCAM encourages a skeptical approach and rigorous methodology in its investigators.

Nora D. Volkow, M.D., Director, National Institute on Drug Abuse (NIDA)

Fifteen to twenty percent of the NIDA budget is spent on research relevant to the basic biology and clinical aspects of pain. The leading drugs of abuse (nicotine, marijuana, and heroin) all bind to neurotransmitter receptors involved in pain perception. The mechanisms underlying addiction resemble those mediating the transition from acute to chronic pain in that both involve long-term changes in brain structure and function. In addiction, such “neuroplastic” changes mediate the transition from experimenting with drugs to being addicted to them.

Abuse rates have declined in this country for some substances, including nicotine and illicit drugs. However, the abuse of psychotherapeutics (opiate, stimulant, and sedative-hypnotic medications) has remained stable. Opiates are the most abused of these medications, with an estimated 4 million abusers in the US. Of most relevance to the pain field is the subgroup of pain patients being appropriately treated with opiate medications who become addicted to them. The possibility of addiction in turn creates stigma for these medications and reluctance to prescribe them for patients who could benefit from them. Thus, there is strong need to understand the variables that increase the risk of opiate abuse and dependence in patients for whom these medications are prescribed. This need is becoming even more pressing with the return from Iraq and Afghanistan of soldiers with conditions involving chronic pain.

PANEL SESSION

Genetic Risk Factors for Chronic Pain

Moderator: John Kusiak, Ph.D., National Institute of Dental and Craniofacial Research, National Institutes of Health

David Whitcomb, M.D., Ph.D., Chief, Division of Gastroenterology, Hepatology and Nutrition, University of Pittsburgh

Genetic Factors That Modulate Pancreatic Pain

Pancreatitis is inflammation of the pancreas, a large gland that lies next to the stomach and produces digestive enzymes. Pancreatitis can be either acute or chronic, and in either case can be extremely painful. In chronic pancreatitis, the pancreas is eventually destroyed. No restorative treatment is available for pancreatitis, and therapeutic options are limited to replacing lost function (e.g., by administering enzymes) and attempting to control pain.

Treatment of pancreatic pain has been challenged by lack of understanding of its underlying biological mechanisms. It is unclear, for example, why some patients with chronic pancreatitis experience no pain, while others with similar levels of pancreatic damage experience intense pain. It is clear, however, that chronic pain has a far more negative impact on mental and physical health than does acute pain, regardless of pain intensity. Thus, it is critical to identify the factors promoting the transition from acute to chronic pancreatitis.

Current evidence suggests that chronic pancreatitis results from an abnormal inflammatory response to pancreatic injury. This abnormal response reflects immune system alterations, which in turn result from environmental stressors and/or genetic risk factors. Among environmental stressors, alcohol does not appear to play as big a role as was previously believed. Alcohol appears to be a significant risk factor for chronic pancreatitis only in very heavy drinkers (5 or more drinks/day). Smoking, on the other hand, is a major risk factor, and individuals with chronic pancreatitis tend to smoke more than average because it reduces pain.

A multi-center collaborative study, the North American Pancreatitis Study 2, has been initiated to identify genetic risk factors for chronic pancreatitis. This study has so far recruited and collected detailed clinical information for over 1000 patients. This patient population was used to look at the possible role in pancreatic pain of a variant in the gene for the enzyme GTP cyclohydrolase (GCH1). This genetic variation had previously been shown to contribute to back pain. However, it was found not to have a major impact on the pain associated with chronic pancreatitis. Hence, there may be different mechanisms underlying neuropathic pain and visceral pain (i.e., the kind associated with pancreatitis). Further efforts are underway to identify genes associated with the latter.

Roger Filligim, Ph.D., Professor, College of Dentistry, University of Florida;
Psychologist, Gainesville VA Medical Center
Genetic Contributions to Ethnic Differences in Chronic Pain

An individual's experience of pain can be influenced by his or her ethnicity. (Note: the term "ethnicity" can be defined as a combination of genetic and environmental influences, including ancestral geographical origins, socioeconomic status, education, and access to healthcare.) For example, African Americans and Hispanic whites have greater sensitivity to experimentally administered heat, cold or ischemic pain than do non-Hispanic whites. There are also ethnic differences in the extent to which individuals report themselves to be disabled by pain.

Individual differences in pain responses are determined in part by genetic factors. Dr. Filligim and colleagues found previously that a variant in the gene for the μ -opioid receptor (OPRM1) is associated with individual differences in pain responses. OPRM1 is the major target of opiate drugs, and also for the opiate-like neuropeptides produced naturally in the body. A rare variation in a single nucleotide (i.e., one "letter" of the DNA code) of the OPRM1 gene reduces sensitivity to experimental pressure pain in non-Hispanic whites. Recently, Dr. Filligim and colleagues discovered that this OPRM1

gene variant is much less common among African Americans than among whites. Moreover, this variant does not reduce pain sensitivity in either African-Americans or Hispanic whites as it does in non-Hispanic whites. Thus, the effect of a particular genetic variation on pain sensitivity can depend on the ethnicity of the individual in whom it occurs. The ethnicity-dependent effect of the OPRM1 gene variant on pain sensitivity also highlights the importance of including ethnicity as a variable when studying the genetics of pain.

Samuel McLean, M.D., M.P.H., Director, TRYUMPH Research Program, Center for Neurosensory Disorders, University of North Carolina

Influence of Adrenergic System-Related Genotypes on Posttraumatic Pain and Drug Response

Persistent pain after motor vehicle accidents is a significant public health problem. About 10-20% of individuals involved in minor vehicle accidents experience persistent pain afterwards, with neck pain being the most common type reported. Individuals who report moderate to severe pain upon arrival at the emergency room following an accident are more likely to develop chronic pain later. However, early treatment with physical therapy following an accident does not appear to reduce the incidence of subsequent chronic pain. Hence, there is a pressing need to understand the biological mechanisms underlying the transition from acute to chronic pain.

Previous studies have shown that the risk of developing chronic pain can be influenced by variations in the gene encoding catechol-O-methyltransferase (COMT). COMT is an enzyme that degrades catecholamine neurotransmitters, such as epinephrine and norepinephrine. A relatively common variation in this gene confers reduced sensitivity to experimental pain and also reduced risk of developing certain chronic pain conditions, such as shoulder and temporomandibular joint (TMJ) pain. Dr. McLean and colleagues found that individuals bearing this variant of the COMT gene (called the Low Pain Sensitivity, or LPS, allele) are less likely to report neck pain at the time they arrive at the emergency room. They are also less likely to experience cognitive symptoms after the accident (such as feeling dazed or out of touch with their bodies) and are more optimistic about the time it will take them to recover.

The LPS allele produces a more active form of COMT and reduced levels of catecholamine neurotransmitters. This fact raises the possibility that treatment with drugs that block catecholamine receptors could reduce chronic pain. To test this idea, patients with chronic TMJ pain were treated with a low dose of the beta-blocker propranolol. (A beta-blocker is a drug that blocks catecholamine receptors of the beta-adrenergic type.) Interestingly, treatment results depended on how many copies of the LPS allele patients carried: substantial improvement was seen in patients who did not carry the allele, whereas little or no improvement was seen in those with two copies of it. Thus, there may be subgroups of chronic pain patients for whom beta-blockers will be effective therapeutics.

Tony Buffington, D.V.M., Ph.D., Professor of Veterinary Clinical Sciences, Adjunct Professor of Urology, Ohio State University
Genetics, Epigenetics, and Chronic Pelvic Pain

Interstitial cystitis (IC) is characterized by bladder pain and urinary frequency. The causes of this condition are still unclear. IC occurs naturally in cats as well as in humans, with nearly identical symptoms and co-morbidities. Thus, both the causes and treatment of IC can be studied in cats. Dr. Buffington and coworkers found that the adrenal glands of cats with IC are reduced in size compared to those of healthy cats. (The adrenals are small glands near the kidney that produce stress hormones.) This decrease in adrenal size can occur if the cat's mother experienced an environmental threat when her offspring were developing *in utero*: maternal stress hormones cross the placenta and interfere with the development of the embryonic adrenal glands.

Cats with IC also have increased stress responses, and these too may reflect long-term consequences of maternal stress. Maternal stress produces permanent, heritable changes in the DNA of their offspring. These so-called "epigenetic" changes can increase the offspring's sensitivity to stress. Increased activity of the autonomic nervous system (the system responsible for "fight or flight" and other reactions to stress) in turn causes increased wear and tear on organs like the bladder, and may contribute to IC and co-morbid conditions. Recent clinical studies have shown that stressful experiences during early life, such as abuse or parental separation, can also contribute to chronic pain syndromes in humans. Work in cats with IC shows that the implementation of specific changes in their living environments aimed at stress reduction (such as providing more activities and visual stimulation for the cats, keep their litter boxes clean, and not punishing them) can produce dramatic reductions in IC symptoms.

PANEL SESSION

Genes: Analgesic Response, and Abuse Potential

Michael Camilleri, M.D., Professor of Medicine and Physiology, Division of Gastroenterology and Hepatology, Mayo Clinic
Genotypes Associated with Mechanisms Influencing Pain in Functional GI Disorders

Irritable bowel syndrome (IBS) is a condition of chronic abdominal pain associated with changes in bowel function. Bowel function is regulated by the neurotransmitters norepinephrine, serotonin, and acetylcholine, and also by certain inflammatory factors. A number of researchers have explored the potential role in IBS of genes in these neurotransmitter signaling and inflammatory pathways, but no strong evidence had been found previously that any of these genes are associated with IBS.

Genetic studies of IBS are complicated by the complex clinical presentation of this syndrome. Dr. Camilleri and colleagues therefore developed methods to measure specific isolated features of the syndrome, known as "intermediate phenotypes." These include the elasticity of the bowel wall, rate of transit of material through the bowel, and pain sensation associated with bowel distension. Use of such intermediate phenotypes

can uncover genetic associations not evident when studying a disease as a multi-symptomatic entity, and also enables the analysis of smaller patient sample sizes. Dr. Camilleri's group has now shown that a gene for an enzyme that metabolizes endocannabinoids (marijuana-like substances that occur naturally in the body) is associated with rapid colon transit. They also found that a gene for an enzyme that inactivates serotonin is involved in rectal pain sensation. Finally, they showed that variants in genes for three proteins lying in neurotransmitter signaling pathways affect individual responses to clonidine, a drug used in treating IBS.

Raymond Dionne, D.D.S., Ph.D., Scientific Director, Division of Intramural Research, National Institute of Nursing Research
Genetic Contributions to Clinical Pain and Analgesia: Avoiding Pitfalls on the Road to Clinical Applications

Individuals often differ markedly in their responses to drugs, including pain medications. Individual differences in responsiveness to a particular drug can result from differences in the activities of proteins that bind or metabolize that drug, molecules in the disease pathway targeted by the drug, or even behavioral factors. The field of "personalized medicine" seeks to identify genes, proteins, and other molecular markers that predict which drugs will work best for a given patient. For example, Dr. Dionne and colleagues have identified a variant in the COX-2 gene that predicts whether patients with oral pain will respond better to COX-2 inhibitors (such as celebrex) or ibuprofen. (COX-2 is an enzyme involved in inflammation and pain.)

Opportunities for personalized analgesia are currently limited by the relatively few classes of analgesics available. New classes of analgesics might be developed by targeting genes involved in pain responsiveness. Dionne and colleagues identified a gene called "zinc finger protein 249" that is associated with responsiveness to ketorolac. This gene was not previously recognized as being involved in inflammatory pain, and therefore represents a potential novel target for drug development. Another way to discover novel drug targets is to identify genes that are turned on during the pain response. This can be done using microarrays, tools that enable one to monitor the activity of thousands of genes simultaneously. Microarray analyses have identified several genes that are up-regulated after oral surgery and down-regulated in response to analgesics. These genes not only represent potential novel drug targets, but may also serve as early markers for the inflammatory pain response.

Ann Marie McCarthy, Ph.D., R.N., FAAN, Professor, University of Iowa College of Nursing
Genetic Predictors of Child Distress Responses to a Painful Procedure

Children often experience pain and anxiety during medical procedures, and the psychological impact of such experiences can continue into adulthood. A number of interventions have been assessed for minimizing child distress during painful procedures. Distracting the child with games or books has proven especially effective, and parents can be engaged as "coaches" in this process. However, the distraction method is more

effective for some children than for others. Dr. McCarthy and colleagues conducted a study to identify factors that predict which children are at high risk for distress during painful procedures, and which parents are likely to be most effective as coaches. The study included 542 children of four to ten years of age who were undergoing insertion of an intravenous catheter. Predictors examined included child and parental behavioral variables (such as child temperament and coping style, and parental coaching style) and procedural variables (e.g., what kind of topical anesthetic was used). Among these measures, age and temperament was associated with distress. Younger children and children with higher levels of general activity (which may be a measure of impulsivity) were at higher risk for distress.

Dr. McCarthy's group also analyzed the study subjects for common variations in 25 genes previously shown to be associated with pain. Variations in one of these, the gene for endothelin receptor A (EDNRA), was associated with higher levels of distress. Endothelin is a peptide involved in blood vessel constriction and pain, and EDNRA receptors are found on the sensory neurons that mediate pain sensation from the skin. The association between the presence of EDNRA gene variants and higher levels of distress was consistent across several different measures of distress.

Jon-Kar Zubieta, M.D., Ph.D., Phil F. Jenkins Professor of Psychiatry, Research Professor, Molecular and Behavioral Neuroscience Institute, University of Michigan
Genetic Influences on Motivational Networks

Both the sensory and emotional experiences of pain are mediated by brain neurons that release opiate-like peptides (known as “endogenous opioids”). Opioid neurons interact with other brain neurons that release the neurotransmitter dopamine. Dopamine neurons are involved in behavioral responses to reward and stress. Interactions between dopamine and opioid neurons contribute to the pleasurable effects of drugs of abuse and the development of addiction.

Dr. Zubieta and colleagues have shown that brain dopamine neurons also contribute to pain responses. They did brain imaging studies on subjects in whom pain was induced experimentally by infusing saline into a jaw muscle, and found that dopamine neuron activity correlated with both the sensory and emotional experiences of pain. They also found that a variant in the gene for COMT, an enzyme that metabolizes dopamine, is associated with individual differences in the brain opioid neuron activity, pain sensitivity, and emotional responses to pain. A third system of neurons, ones that release the peptide neuropeptide Y (NPY) also appear to play a role in pain responses. NPY neurons are activated by emotionally stressful stimuli (such as images of fearful faces), and are thought to help suppress anxiety during chronic stress. Variants in the NPY gene also contribute to individual differences in opioid neuron activity and pain responses.

PANEL SESSION

Genes: Tools and Models

Moderator: Linda Porter, Ph.D., National Institute for Neurological Disorders and Stroke, NIH

David Fink, M.D., Robert Brear Professor and Chair, Department of Neurology,
University of Michigan; VA Ann Arbor Healthcare System
A Clinical Trial of Gene Therapy for Pain

Neurotransmitters and other molecules mediating pain sensation could serve as targets for analgesic development. However, most of these molecules are distributed widely in the body and are involved in functions other than pain perception. Thus, attempts to block the effects or increase the levels of these molecules are likely to produce serious undesirable side effects. Gene therapy offers a possible solution to this problem. Viruses carrying a gene for a therapeutic protein can be injected into a localized site, and will remain there and generate the protein for weeks or longer.

Dr. Fink and colleagues are using gene therapy techniques to develop new pain therapeutics. They used the Herpes simplex virus as their gene vector (i.e., to carry the therapeutic gene to the desired site). Herpes simplex is taken up specifically by sensory neuron endings after injection into the skin and transported to their cell bodies, where the virus replicates and generates copies of the therapeutic gene. They have used this method to deliver genes for three different candidate therapeutic proteins: two opioid peptides and the small peptide neurotransmitter gamma amino butyric acid (GABA). All three of these reduced pain-related behaviors in rodent models of inflammatory pain, neuropathic pain, and pain caused by cancer. The gene for one of the opioid peptides is now being tested for safety in a clinical trial in cancer patients.

Ze'ev Seltzer, D.M.D., Canada Research Chair (tier 1), Professor of Genetics and Physiology, Center for the Study of Pain, Faculties of Dentistry and Medicine, University of Toronto

Comparative Approaches to the Genomics and Phenomics of Neuropathic Pain: Advantages and Limitations

A critical issue in human genetics studies is to identify pain phenotypes that will be most useful for genetic studies. (A “phenotype” is a specific characteristic of a physiological or disease state that can be analyzed quantitatively. An example of a pain phenotype would be patient report of average pain severity during a specific time period.) Currently, no standardized phenotypes have been agreed on in the genetics research community. However, possible criteria for choosing such phenotypes include clinical relevance, knowledge of biological mechanism underlying the phenotype, and availability of an animal model in which the same phenotype can be measured.

Dr. Seltzer's group has been studying the genetics of post-traumatic pain in humans and in a rodent model. The rodent model is one in which two nerves in one limb are cut, which results in the animal licking and biting that limb. This behavior was used as a phenotype for genetic studies. Seltzer and colleagues identified a gene that might be involved in this pain response, which they called *Pain1*. Subsequent clinical studies showed that this gene is also linked to post-amputation and post-mastectomy pain in

human patients. *Pain1* encodes a protein called colony stimulating factor 2 receptor beta (Csfrb) that is expressed by activated microglia. Microglia are immune cells that patrol the nervous system to identify infectious agents and other foreign proteins and help mount inflammatory responses. Thus, *Pain1* seems likely to be involved in inflammatory pain.

Erin Milligan, Ph.D., Assistant Professor, Department of Neurosciences, Health Sciences Center, University of New Mexico
A Novel Mechanism Underlying Nonviral Gene Therapy To Control Neuropathic Pain

Pain signaling involves not only neurons but also glia, the non-neuronal cells of the nervous system. One class of glial cells important in mediating neuropathic pain are microglia, small cells that perform immune functions in the nervous system. Dr. Milligan and colleagues studied the role of microglia in an animal model of neuropathic pain, chronic constriction injury of the sciatic nerve. Following such an injury, animals show heightened pain sensitivity, and exhibit pain responses to non-painful stimuli such as light touch. The development of this abnormal pain response is associated with activation of spinal cord microglia and their release of pro-inflammatory cytokines (small protein signaling molecules). The effects of these cytokines are inhibited by the anti-inflammatory cytokine interleukin-10 (IL-10), suggesting that IL-10 could be used to treat neuropathic pain.

Because IL-10 does not penetrate the spinal cord from the bloodstream, Dr. Milligan and colleagues explored gene therapy approaches for administering the peptide. They delivered an IL-10 transgene as part of a “naked” DNA package (i.e., without using the viral protein coat typically used to deliver therapeutic genes). Using this approach, they were able to obtain both short- and long-term pain relief in the neuropathic pain model, and also in an animal model of chemotherapy pain. The concentrations of DNA required for pain relief were too high for use in humans, but the investigators were able to reduce the necessary concentration 150-fold by encapsulating the DNA in FDA-approved polymers (long-chain molecules). Microglia appear to play a key role in the therapeutic effects of both the naked and encapsulated DNA: activated microglia migrate to the site of the DNA injection, where they engulf the DNA particles and may help distribute them to surrounding tissue.

W. Dan Tracey, Ph.D., Director, Molecular Genetics of Pain Signaling Laboratory and Departments of Anesthesiology, Cell Biology, and Neurobiology, Duke University Medical Center

Genetic Analysis of Nociception in Drosophila

The fruitfly (*Drosophila*) is an extremely useful animal model for studying the genetics of pain perception. Like humans and other animals, fruitflies have sensory neurons that are activated by painful stimuli; they also show behavioral responses to pain, such as escape behavior. The fruitfly also offers a special advantage in that one can easily mutate large numbers of different genes in this organism, and thereby identify genes involved in different biological pathways. Using such a genetic screen, Dr. Tracey and colleagues

discovered a gene called *painless* whose mutation leads to reduced thermal and mechanical pain sensitivity in fruitfly larvae. *Painless* encodes a protein very similar to the human protein TRPA1, a receptor that is expressed on sensory neurons and activated by cold and noxious chemicals. In the fruitfly, *painless* is expressed in a special subpopulation of sensory neurons, called Class IV neurons, which have highly branched endings within the body wall. Dr. Tracey and colleagues have identified two other genes in Class IV neurons also required for pain perception: *pickpocket* and *dTRPA1*. By isolating Class IV neurons and using molecular profiling techniques to determine what other genes these neurons express, it should be possible to discover additional genes involved in pain perception.

Closing Remarks and Adjournment

Linda Porter, Ph.D., National Institute for Neurological Disorders and Stroke, National Institutes of Health