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TMJ Science

Joan Wilentz, Editor

Terrie Cowley, Contributing Editor

Steven Pusztai, Karen McQuestion,
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Carolyn Noel, Layout and Design

Deanne Clare, Production Manager
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Send correspondence to:
Editor, *TMJ Science*
P.O. Box 26770
Milwaukee, WI 53226-0770
Phone: 262-432-0350
Fax: 262-432-0375
Website: www.tmj.org
Email: info@tmj.org

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The Seventh Scientific Meeting of The TMJ Association, Ltd.

Genetic, Epigenetic, and Mechanistic Studies of Temporomandibular Disorders and Overlapping Pain Conditions

In This Issue

Preface

The TMJ Association held its Seventh Scientific Meeting in September 2014, bringing together patients, patient advocates and scientific experts to discuss the genetics, epigenetics and mechanisms of Temporomandibular Disorders (TMD) and overlapping pain conditions. The participants were excited to learn about the many advances at the omics level toward understanding TMD and overlapping pain conditions and yet, they also recognized the need for additional research in this area in order to aid in the development of more precision-based, targeted treatment approaches.

A continuing and successful aspect of this meeting was the opportunity for individuals with TMD and other chronic pain conditions to connect with both early investigators and senior scientists in the field. A mutual appreciation of the concerns and challenges facing us will improve our chances as we move forward in developing effective treatments for these conditions.

While there is still much to learn about the factors underlying TMD and associated pain conditions, we believe the ongoing research efforts supported by the National Institute of Dental and Craniofacial Research and the National Institutes of Health Pain Consortium to elucidate the genetic, epigenetic and molecular events underlying TMD and other chronic pain conditions will prove invaluable for defining acute and chronic pain and developing new treatments. The complexity of chronic pain conditions will require research partnerships as well as diverse multidisciplinary efforts and approaches to alleviate the physical, psychological and economic burdens that individuals with TMD and chronic pain face on a daily basis.

Martha J. Somerman, D.D.S., Ph.D.
Director, National Institute of Dental and Craniofacial Research
National Institutes of Health

John W. Kusiak, Ph.D.
Acting Deputy Director, National Institute of Dental and Craniofacial Research
National Institues of Health

The theme of the seventh scientific meeting builds upon evidence from the six previous meetings demonstrating that Temporomandibular Disorders (TMD) are a complex family of conditions influenced by genetics, sex, environmental and behavioral factors. These mediate the vulnerability of patients to TMD many of whom will manifest other chronic pain conditions beyond their jaw and muscle problems. The seventh meeting focuses on epigenetic and genetic factors in TMD and their overlapping pain conditions. These include chronic headache, endometriosis, fibromyalgia, interstitial cystitis/bladder pain syndrome, irritable bowel syndrome, low back pain, myalgic encephalomyelitis/chronic fatigue syndrome, and vulvodynia.

The TMJ Association Scientific Advisory Board

The Seventh Scientific Meeting of The TMJ Association

Genetic, Epigenetic, and Mechanistic Studies of Temporomandibular Disorders and Overlapping Pain Conditions

Sunday, September 7, 2014

3:00 – 3:30 p.m. **Welcome and remarks**
Terrie Cowley, President and Co-founder, The TMJ Association, Milwaukee, WI
Co-founder, Chronic Pain Research Alliance

Allen W. Cowley, Jr., Ph.D., Program Committee Chairman
Medical College of Wisconsin, Milwaukee, WI

National Institutes of Health welcome and remarks
Lawrence A. Tabak, D.D.S., Ph.D., Principal Deputy Director, Office of the Director National Institutes of Health, Bethesda, MD

Martha J. Somerman, D.D.S., Ph.D., Director, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD

Josephine P. Briggs, M.D., Director of the National Center for Complementary and Alternative Medicine, National Institutes of Health, Bethesda, MD

Christopher Mullins, Ph.D., Director of Basic Cell Biology Programs, Division of Kidney, Urologic and Hematologic Diseases, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

Session 1: **Epidemiology And Genetic Signatures Of Temporomandibular Disorders, Overlapping Conditions, And Chronic Pain**
Session Co-Chairs: Susan Maier, Ph.D., Deputy Director, Office of Research on Women's Health, National Institutes of Health, Bethesda, MD and David A. Williams, Ph.D., University of Michigan, Ann Arbor, MI

3:30 – 4:00 p.m. **Epidemiology of temporomandibular joint disorders and related painful conditions**
Gary D. Slade, B.D.Sc., D.D.P.H., Ph.D., University of North Carolina at Chapel Hill, Chapel Hill, NC

4:00 – 4:30 p.m. **Characterization of individuals with chronic pain: pheonotyping approaches used in MAPP**
David A. Williams, Ph.D., University of Michigan, Ann Arbor, MI

4:30 – 5:00 p.m. **Translational research in the genomic era: OPPERA study**
Luda Diatchenko, M.D., Ph.D., McGill University, Montreal, Quebec, Canada

5:00 – 5:30 p.m. **Mechanisms of chronic pain**
Joachim Scholz, M.D., Columbia University Medical Center, New York, NY

5:30 – 6:00 p.m. **Travel awardee poster presentations**

Association between INADL genetic variant and the subgroup with high risk for TMD in the OPPERA study
Shad B. Smith, Ph.D., University of North Carolina at Chapel Hill
Chapel Hill, NC

Case-control analysis in resting and evoked inflammatory profiles
Christopher D. King, Ph.D., University of Florida, Gainesville, FL

6:00 – 7:00 p.m. **Dinner and poster sessions**

7:00 – 7:45 p.m. **Comorbid chronic pain advocates round table**
Round Table Chair: Terrie Cowley, President and Co-founder,
The TMJ Association, Milwaukee, WI,
Co-founder, Chronic Pain Research Alliance

Temporomandibular Disorders
Terrie Cowley, President and Co-founder, The TMJ Association, Milwaukee, WI
Co-founder, Chronic Pain Research Alliance

Interstial Cystitis/Painful Bladder Syndrome
Lee Bryan Claassen, CAE, Executive Director, Interstitial Cystitis Association
McLean, VA

Fibromyalgia
Janet F. Chambers, President, National Fibromyalgia & Chronic Pain Association,
Logan, UT

Myalgic Encephalomyelitis/Chronic Fatigue Syndomre
Suzanne D. Vernon, Ph.D., Scientific Director, Solve ME/CFS Initiative
Los Angeles, CA, Co-founder, Chronic Pain Research Alliance

7:45 – 8:15 p.m. Discussion and recommendations

Monday, September 8, 2014

8:00 – 8:30 a.m. **Welcome and remarks**
Martin Frank, Ph.D., Executive Director, American Physiological Society
Bethesda, MD

National Institutes of Health welcome and remarks
Story C. Landis, Ph.D., Director, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD

Susan Maier, Ph.D., Deputy Director, Office of Research on Women's Health
National Institutes of Health, Bethesda, MD

Session 2: **Mechanisms Of Chronic Pain - Basic And Clinical Studies**
Session Co-Chairs: John Kusiak, Ph.D., Director, Molecular and Cellular Neuroscience Program, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD and Anne Louise Oaklander, M.D., Ph.D., Massachusetts General Hospital, Harvard Medical School, Boston, MA

8:30 – 9:00 a.m. **Study of chronic orofacial pain with preclinical models**
Jianguo Gu, M.B., Ph.D., University of Cincinnati College of Medicine
Cincinnati, OH

9:00 – 9:30 a.m. **Modeling TMJD pain in the laboratory mouse: role of TRP ion channels**
Wolfgang Liedtke, M.D., Ph.D., Duke University Medical Center, Durham, NC

9:30 – 10:00 a.m. **The neurobiology of oral cancer pain**
Brian Lee Schmidt, D.D.S., M.D., Ph.D., New York University College of Dentistry
New York, NY

10:00 – 10:15 a.m. Break

10:15 – 10:45 a.m. **Functional interactions between glutamate receptors and TRPV1 in trigeminal sensory neurons**
Jin Y. Ro, Ph.D., University of Maryland School of Dentistry, Baltimore, MD

10:45 – 11:15 a.m. **Small-fiber polyneuropathy (SFPN), a common underlying diagnosis in syndromes involving unexplained chronic pain and multi-system symptoms**
Anne Louise Oaklander, M.D., Ph.D., Massachusetts General Hospital,
Harvard Medical School, Boston, MA

11:15 – 11:45 a.m. **Travel awardee poster presentations**

Confocal microscopy reveals nerve fiber similarities in fibromyalgia and patients with dry eyes with a normal ophthalmic exam
Daniel E. Harper, Ph.D., University of Michigan, Ann Arbor, MI

TRPV4-mediated trigeminal pain: behavior assessments and mechanisms
Yong Chen, Ph.D., Duke University, Durham, NC

11:45 – 12:15 p.m. **Discussions and recommendations**

12:15 – 1:15 p.m. **Lunch and poster sessions**

Session 3: **Genetic And Epigenetic Basis Of TMJ Disorders And Related Chronic Overlapping Conditions**
Session Co-Chairs: Emily Harris, Ph.D., M.P.H., Chief, Translational Genomics Research Branch, Division of Extramural Research Director, National Institute of Dental and Craniofacial Research, National Insitutes of Health, Bethesda, MD and Keji Zhao, Ph.D., National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD

1:15 – 1:45 p.m. **A cellular mechanism of interactions between pain and depression**
Jianren Mao, M.D., Ph.D., Massachusetts General Hospital
Harvard Medical School, Boston, MA

1:45 – 2:15 p.m. **Combined genetic polymorphisms and environmental factors in the etiology of a chronic TMJD murine model**
Karin N. Westlund High, Ph.D., University of Kentucky, Lexington, KY

2:15 – 2:45 p.m. **Roles of COMT, NPY and GCH1 in acute and chronic pain/stress response**
David Goldman, M.D., National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD

2:45 – 3:15 p.m. **Molecular correlates of localized versus co-occurring chronic pain conditions**
Andrea G. Nackley, Ph.D., University of North Carolina at Chapel Hill, Chapel Hill, NC

3:15 – 3:30 p.m. Break

3:30 – 4:00 p.m. **The epigenetic signature of chronic pain in the mouse brain**
Maral Tajerian, M.Sc., Ph.D., Stanford University, Palo Alto, CA

4:00 – 4:30 p.m. **Epigenetic regulation of gene expression and cellular differentiation**
Keji Zhao, Ph.D., National Heart, Lung, and Blood Institute
National Institutes of Health, Bethesda, MD

4:30 – 5:00 p.m. **Integrating epigenetic data into molecular casual networks**
Jun Zhu, Ph.D., Icahn School of Medicine at Mount Sinai, New York, NY

5:00 – 5:15 p.m. **Travel awardee poster presentation**

Associations between ACTN3 and OPPERA pain-related genes in malocclusion
James Sciote, D.D.S, M.S., Ph.D., Temple University, Philadelphia, PA

5:15 – 5:45 p.m. **Discussion and recommendations**

6:30 p.m. **Dinner - A look back at TMJA advocacy and it's impact on scientific research, Bethesda Marriott, 5151 Pooks Hill Road, Bethesda, MD**
Manuel Bonilla, M.S., Chief Advocacy Officer, American Society of Anesthesiologists, Washington, DC, Peter Reinecke, President, Reinecke Strategic Solutions, Inc., Arlington, VA, and Diana Zuckerman, Ph.D., President, National Center for Health Research, Cancer Prevention and Treatment Fund, Washington, DC

Tuesday, September 9, 2014

8:30 – 8:45 a.m. **National Institutes of Health welcome and remarks**
Patricia A. Grady, Ph.D., R.N., F.A.A.N., Director, National Institute of Nursing Research, National Institutes of Health, Bethesda, MD

Session 4: **Functional Genomics Of Pain In Analgesic Drug Development And Therapeutics**
Session Co-Chairs: Jason Wan, Ph.D., Program Director, Mineralized Tissue Physiology Program Integrative Biology and Infectious Diseases Branch, Division of Extramural Research, National Institute of Dental and Craniofacial Research, Bethesda, National Institutes of Health, MD and Roy C. Levitt, M.D., University of Miami Miller School of Medicine, Miami, FL

8:45 – 9:15 a.m. **Pain in the network of genetic and epigenetic control**
Jörn Lötsch, M.D., Goethe – University, Frankfurt am Main, Germany

9:15 – 9:45 a.m. **Targeted genome and epigenome editing using engineered TALE and CRISPR/Cas9 technologies**
Charles A. Gersbach, Ph.D., Duke University, Durham, NC

9:45 – 10:15 a.m. **Imaging orofacial pain in mice**
Xinzhong Dong, Ph.D., Johns Hopkins University School of Medicine, Baltimore, MD

10:15 – 10:30 a.m. Break

10:30 – 11:00 a.m. **Carbonic anhydrase-8 gene therapy inhibits the ITPR1-cytosolic free calcium pathway producing analgesia and anti-hyperalgesia**
Roy C. Levitt, M.D., University of Miami Miller School of Medicine, Miami, FL

11:00 – 11:30 a.m. **Preclinical and translational studies of fenobam, an mGlu5 NAM, for the treatment of pain**
Robert W. Gereau, Ph.D., Washington University School of Medicine, St. Louis, MO

11:30 – 11:45 a.m. **Travel awardee poster presentation**

Repetitive transcranial magnetic stimulation (rTMS) of the primary motor cortex for treating facial neuropathic pain - preliminary results of a randomized, sham-controlled, cross-over study
Roi Treister, Ph.D., Massachusetts General Hospital & Harvard Medical School, Boston, MA

11:45 – 12:15 p.m. **Discussion and recommendations**

12:15 – 1:15 p.m. **Lunch and poster sessions**

1:15 – 2:15 p.m. **Consolidation of meeting recommendations and closing remarks**
Drs. Allen W. Cowley, Jr. and John W. Kusiak

Meeting Summary

by Joan Wilentz

The theme of the 7th Scientific Meeting of The TMJ Association, *Genetic, Epigenetic, and Mechanistic Studies of Temporomandibular Disorders and Overlapping Pain Conditions*, was chosen to highlight how research in cutting-edge fields of science like epigenetics, in combination with genetic and mechanistic studies (explanations of what happens at the cell and molecular level) can deepen our understanding of Temporomandibular Disorders (TMD) and explain why TMD patients frequently experience other painful disorders. While it is now recognized that there is a genetic component that makes some individuals more sensitive and others less sensitive to pain, the field of epigenetics adds another level of influence. If the genes in an individual's genome are likened to instruments in an orchestra, the role of epigenetics is to modulate their performance, turning some instruments on or muting others. Epigenetics research studies the biochemical processes that effect these changes at the level of the genome and seeks to explain why they happen: what circumstances in an individual's environment or life experience can, without changing the make-up of the genes themselves, alter their expression.

The meeting, held September 7–9, 2014, at the headquarters of The Federation of American Societies of Experimental Biology in Bethesda, MD, brought an international gathering of scientists and a number of young investigators who presented posters together with administrators and staff of the National Institutes of Health (NIH), which co-sponsored the meeting. Importantly, as in all previous TMJ Association (TMJA) scientific meetings, chronic pain patients themselves were invited and described their experiences as part of the program.

The meeting opened with welcoming remarks beginning with Terrie Cowley, President of The TMJ Association. She reviewed her personal history of TMD and provided a brief summary of the progress made since the formation of the Association in 1986. While understanding of TMD and overlapping pain conditions has increased since TMJA's first scientific meeting in 2000, she said the research has yet to be translated to patient care. The goal must be more interdisciplinary research, which should then be translated to multidisciplinary clinics. She then introduced her husband, Allen Cowley, Ph.D., Chair of the Department of Physiology at the Medical College of Wisconsin, who also chaired the Program Committee organizing the meeting.

Dr. Cowley thanked colleagues for their help in putting the program together and then singled out for special mention the contributions of John Kusiak, Ph.D., Director of the Molecular and Cellular Neuroscience Program of the National Institute of Dental and Craniofacial Research (NIDCR), NIH and Lawrence Tabak, D.D.S., Ph.D., Principal Deputy Director, Office of the Director, NIH, and former NIDCR Director. He credited them for redirecting the institute's focus on TMD

away from studies of behavior and oral habits to today's extensive basic and clinical research on pain and the recognition that TMD is a complex disorder frequently occurring with other pain conditions.

Dr. Cowley introduced Dr. Tabak who spoke of his education on TMD and the valuable role played by health advocates like the Cowleys in that regard. He stressed the need for an interdisciplinary team approach to TMD and overlapping conditions given their complexity and because advances "so often occur at the interface of disciplines." While there is still much to learn, he affirmed that there has been considerable evolution in the field, evidenced by the titles of the previous six scientific meetings.

In her welcoming remarks, Martha Somerman, D.D.S., Ph.D., the present Director of NIDCR, NIH, also paid tribute to TMJA for her education on TMD. Her arrival at NIH in 2011 coincided with the release of the report of the Institute of Medicine, *Relieving Pain in America*, with many recommendations for research. She said that NIDCR is a leader in the NIH Pain Consortium and also involved in activities of the Federal Interagency Pain Research Coordinating Committee. Pain research also figures in the new NIDCR strategic plan and she invited the audience to view the plan online.

Josephine Briggs, M.D., Director of the National Center for Complementary and Alternative Medicine (NCCAM), NIH, spoke next, remarking that over a third of NCCAM's funding is devoted to chronic pain. She said, "This is largely because traditional medicine has failed to come up with safe and effective treatments for chronic pain. As a result many patients turn to alternative medicine." NCCAM is also one of several NIH Institutes that lead the NIH Pain Consortium and participates in other collaborative research.

Dr. Briggs was followed by Christopher Mullins, Ph.D., Director of Basic Cell Biology Programs, Division of Kidney, Urologic and Hematologic Diseases, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), NIH. Dr. Mullens addressed NIDDK's research on conditions of chronic pelvic pain, which overlap with TMD. The Institute currently funds a major epidemiological study, *Multidisciplinary Approach to the Study of Chronic Pelvic Pain* (MAPP), details of which were discussed later in the meeting.

Last to make welcoming remarks at the opening session was Dr. John Kusiak who explained that attendees would be asked to formulate research recommendations at the conclusion of the meeting. He asked them to consider the compelling questions and critical challenges posed during the meeting—ones that need to be addressed in the next 5 to 10 years. He urged that they identify knowledge gaps, areas that require NIH facilitation, barriers hindering progress, and the necessary tools and resources.

Epidemiology of TMD

Gary Slade, Ph.D., University of North Carolina at Chapel Hill, NC, discussed the prevalence of TMD in the United States. Data collected from National Health Interview Surveys from 1989 through 2009, based on yearly interviews with a sample of 100,000 Americans, indicate that the prevalence of TMD has remained fairly stable, with about 5% of the population, or 11.5 million adults, reporting that they live with significant facial pain. The distribution of women was double that of men. Caucasians and African-Americans had similar prevalence, while prevalence among Native Americans was twice as high. There was a significant income gradient with twice the prevalence of facial pain at lower household incomes. There was also considerable overlap with other pain conditions so that people reporting TMD also reported experiencing headache, neck, or low back pain at rates far exceeding what might be expected by chance.

The age distribution in women is an "inverted U" with the highest prevalence seen in women at midlife, ages 35 to 44, increasing from younger ages and tapering off in old age. The distribution in men showed no variation with age. Interestingly, women who were born in the 1960s had a higher incidence of facial pain as measured in 1989 than those born in other decades.

Dr. Slade is also a member of the research team conducting the prospective epidemiological study of TMD known as OPPERA (Orofacial Pain: Prospective Evaluation and Risk Assessment). The aim of the study was to collect data and tissue samples from an initial sample of TMD-free adults between 18 and 44 years of age, follow them for several years and see who developed TMD. The investigators would then review the data and tissue samples to see if there were any measures that were risk factors for TMD distinguishing those who developed TMD from those who did not. The study is an example of "intermediate phenotyping," identifying features of a person that remain stable over time—ones that reflect genes, environment and life experiences—but are associated with the risk of developing a particular disease or disorder.

Dr. Slade reported that 260 of the initial 2,737 adults studied developed TMD over a three-year time period. This works out to an incidence—the rate at which new cases of TMD develop in a fixed period of time—to 3.5% per year. However, the rate was double among those who had initially reported that they had *other* pain conditions such as irritable bowel syndrome or headache; it was also increased among those who had checked conditions such as depression, acid reflux, or sleep apnea on a 20-item checklist of non-specific health conditions.

David Williams, Ph.D., University of Michigan, MI, described a second large-scale pain phenotyping study. This was the MAPP (*Multidisciplinary Approach to the Study of Chronic Pelvic Pain*) that Dr. Mullens had alluded to earlier. The multi-site study is characterizing over a thousand patients

with urologic chronic pain syndromes such as interstitial cystitis, chronic prostatitis, and bladder pain syndrome. Similar to the OPPERA study, extensive data and biospecimens were collected at baseline with periodic follow-up questionnaires and clinical exams. Information is collected on the end-organ specifics and the sensory aspects (intensity and location) of their pelvic pain as well as emotional and cognitive components of their illness experience. Measures include self-reported questionnaires, quantitative sensory testing (e.g., noting pain threshold), neuroimaging studies, and analyses of biospecimens, including tests of urine samples for infection. The self-report questionnaires record information on how the diagnosis was made, the symptoms and their effects on sexual functioning, self-esteem and social relationships. Broader questions concern their pain experience and whether they suffer other pain conditions. Other questions ask about attitudes and beliefs, mood, personality, and whether there was any early life trauma. Dr. Williams said research on pelvic pain can proceed at many different levels, moving up from genes to proteins, cells, body systems and on to the syndrome itself. MAPP has started at the highest level looking at the syndrome in what he called Wave 1. Investigators are now launching Wave 2, which will look at the levels of the genome and proteome. The hope is that the multiple analyses MAPP affords will yield subsets of patients whose chronic pelvic pains fall into distinct pathophysiology patterns, leading to more tailor-made and effective treatments.

Luda Diatchenko, M.D., Ph.D., McGill University, Quebec, Canada, is a collaborator with the OPPERA group conducting genetic studies based on the tissue samples participants provided at the outset. She noted that over 90 percent of the variation in human genomes occurs as a result of substitutions of one nucleotide (the molecule containing one of the four bases, A, C, T, or G, that constitute the genetic code) for another nucleotide at single sites along the genome. She and her team examined the DNA samples of the OPPERA volunteers looking for such "single nucleotide polymorphisms" or "SNPs," particularly in genes known to be involved in pain perception. Their hope was to find that participants at risk for TMD shared a SNP in one or more pain-related genes and no such SNP was found in study participants who did not develop TMD. Their analyses yielded two genes of interest: EREG (epiregulin) and EGFR (epidermal growth factor receptor). The SNPs that they found were also found in the DNA of TMD patients in two other studies. The investigators studied the function of the two genes in animal models, determining the pain pathways involved and confirming that the gene alterations were associated with increased pain. Further, they showed that antagonist drugs (molecules that impede the action of the protein encoded by the EGFR gene) resulted in strong analgesic properties in a mouse pain model, while mice in which the EGFR gene was eliminated altogether (EGFR "knockout" mice) showed reduced pain sensitivity. Dr. Diatchenko concludes that SNP analysis might be a fruitful way

to identify not only new targets for pain treatment, but also a new class of antagonist drugs effective in controlling chronic pain.

Amplifying the Pain Message: Central Sensitization

Joachim Scholz, M.D., Columbia University, NY, explained the pain that living creatures experience is categorized according to its source. Nociceptive pain results when nerve cells responsive to pain, called nociceptors, are stimulated by noxious stimuli such as excessive heat, mechanical force, or toxic chemicals. Nociceptors are found throughout the periphery of the body and in deep tissues. If the noxious stimulus is prolonged or intense, the affected area can become inflamed and flooded by inflammatory chemicals. Under such conditions the nociceptors can become hyperactive, firing repeatedly at lower thresholds and releasing a steady stream of neurotransmitters in a process called peripheral sensitization. In turn, the heightened input from the peripheral nociceptors can lead to heightened excitement of the neurons in the next way-station in the pain pathway, the neurons in the central nervous system. The process can continue in a chain reaction to relay stations ascending higher up in the brain, aided by nervous system chemicals that further sensitize the circuit, a process leading to central sensitization. This can result in long-term changes in pain circuits allowing them to be activated even when the original source of noxious stimulation has abated. It can also result in an exaggeration of pain to a noxious stimulus (hyperalgesia) and also to a feeling of pain in response to a normally non-painful stimulus (allodynia).

In contrast, neuropathic pain occurs when there is injury or disease that damages nerve cells and fibers of the nervous system itself. In this case, the system reacts with abnormal fiber growth at the site of injury and recruitment of nearby immune system cells within the nervous system. These processes also result in central sensitization, yielding the same sort of heightened responses to pain as seen in nociceptive central sensitization. In addition, the processes initiated by the immune cells can result in excessive excitatory pain-signaling pathways ascending into the brain, but a profound loss of descending inhibitory pathways that normally modulate pain. It is these multiple long-term changes in the circuitry of pain pathways and the many neurochemicals involved in central sensitization that make chronic pain so difficult to treat.

The opening session ended with a roundtable of chronic pain patients (see p. 26) and poster presentations by young investigators (p. 30).

The second session began with welcoming remarks from Martin Frank Ph.D., Executive Director, American Physiological Society, Bethesda, MD, Story C. Landis, Ph.D., retiring Director, National Institute of Neurological Disorders and Stroke, NIH, and Susan Maier, Ph.D., Deputy Director, Office of Research on Women's Health, NIH. As in past TMJA scientific

meetings, Dr. Frank welcomed guests as the host representing the Federation of American Societies of Experimental Biology. Drs. Landis and Maier spoke of the growing commitment of their organizations to pain studies, either collaborating in the development of new research initiatives or leading various trans-NIH and interagency committees to facilitate pain studies and planning future interdisciplinary meetings. Dr. Maier emphasized pain as a women's health issue and her office's role in coordinating women's health research at NIH and outreach to the public.

Mechanisms and Models of Chronic Pain

In the absence of effective treatments for chronic pain, two speakers in the second session addressed the importance and utility of animal models for studying pain mechanisms and testing candidate drugs.

Milk-drinking rats. Jianguo Gu, Ph.D., University of Cincinnati College of Medicine, OH, described a rat model of chronic neuropathic pain based on constricting the infraorbital nerve, a branch of the trigeminal nerve whose fibers supply sensation to areas of the lower face and mouth. The behavior of these "ION-CCI" (infraorbitlal nerve chronic constriction injury) rats was compared to sham-operated controls when placed in a glass box with a partition near one end containing a window. The rat can gain access through the window to a tube of sweetened milk, a highly desirable reward. Initially, both sham and test animals spent equal time at the window drinking milk but when the temperature of the apparatus was decreased or when the rats were subjected to a mechanical force, the nerve-injured animals spent significantly less time drinking milk compared to controls. The researchers interpret this behavior as the result of allodynia. The rats were experiencing pain when trying to drink because their nerve injury caused normally non-painful stimuli like colder temperatures and mechanical forces to feel painful. Similar results were obtained when rats were injected with a potent cancer drug known to cause neuropathy as a side effect.

Dr. Gu went on to describe the use of the rat model to test the efficacy of a candidate drug to control pain. The drug chosen, Retogamine, is an anti-convulsant that belongs to a class of nervous system neurochemicals Kv7.2, which affects neurons in the trigeminal ganglion, the site containing the cell bodies of the trigeminal nerves. Subsequent tests of the behavior of both the ION-CCI and cancer drug-injected rats showed that the Retogamine effectively blocked the rats' allodynia and restored their milk-drinking habits. The team is currently assessing the effects of a second Kv7.2 agent, CR341, which so far shows effects similar to Retogamine in blocking cold-induced allodynia in the ION-CCI mice.

Chewing behavior in mice. The model developed by Wolfgang Liedtke, M.D., Ph.D., Duke University Medical Center, Durham, NC, and colleagues involved

exposing mice to stimuli causing inflammation in the jaw area. The resulting pain in their chewing muscles led the mice to reduce the biting force they would normally use in eating—a situation comparable to TMD patients who choose soft diets because it hurts to bite into more solid foods. The researchers were able to calibrate how much of a reduction using a bite force-measuring instrument they developed. Before describing the model in detail, Dr. Liedke discussed a molecule that acts as a gatekeeper able to activate the cell bodies of nociceptors in the trigeminal ganglion. The molecule, Transient Receptor Pain Vanilloid 4 (TRPV4) is an ion channel that sits on the membrane of the nerve cell. In response to inflammation, the channel opens to allow calcium ions to enter and excite the cell to fire. Moreover, by comparing wild-type mice with mice genetically lacking the gene that codes for TRPV4 ("knockout" mice), the investigators were able to show that the TRPV4 was critical in generating the pain behavior. Mice lacking the TRPV4 gene and exposed to inflammation showed little reduction in bite force compared to normal mice. Dr. Liedke said that the results were the same whether inflammation was induced by mechanical force or by injecting formalin, a chemical irritant in the mouse whisker pad. In addition, further studies of TRPV4 confirmed that the molecule plays a key role in regulating other Transient Receptor Pain (TRP) channels and pain pathways. Given these results, the researchers believe that TRPV4-antagonist drugs offer a novel source of analgesics to treat inflammatory jaw pain and other trigeminal pain disorders, including headaches and migraines.

The potency of TRP ion channels in exacerbating pain was further illustrated by Jin Y. Ro, Ph.D., University of Maryland School of Dentistry, MD. He described how two systems responsive to pain in jaw muscle tissue and thought to be independent, actually interact to lower the pain threshold at the site of muscle injury and give rise to hyperalgesia. One system uses glutamate, a neurotransmitter in muscle nociceptors activated in response to pain. The other system uses a TRPV channel, TPRV1, which also responds to pain and leads to mechanical sensitivity and causes hyperalgesia. Dr. Ro's experiments showed that the two systems interact through complex biochemical pathways. He echoed Dr. Liedke's belief that these findings could lead to the development of novel drugs to inhibit TRPV1 action.

Oral cancer. The point of the presentation of Brian Schmidt, D.D.S., M.D., Ph.D., New York University College of Dentistry, NY, was that the study of oral cancer pain could illuminate aspects of chronic TMD pain. He noted that overall, oral cancer pain is considered more severe than other cancer pain, that it increases in severity with disease progression and becomes totally debilitating in terminal stages. Yet little is known about its cause. It is not truly chronic pain but a form of acute recurring pain. It is not due to inflammation nor is it due to tumor size and its encroachment on other tissues. Rather it appears to be mechanically induced when using facial

muscles—it is a "functional" pain similar to what TMD patients experience. He suggested the stretch receptors were involved and that the pain resulted from extensive reciprocal interactions between the tumor and elements of the peripheral nervous system. Further analysis of the neural microenvironment of orofacial tissues indicates that in addition to peripheral nociceptors contributing to oral cancer pain, there is evidence for pain-inhibiting mechanisms. It appears that certain cannabinoid receptor-bearing nerve cells can stimulate the release of endogenous opioids.

Small fiber polyneuropathy. That other conditions associated with severe pain can shed light on TMD and its associated chronic overlapping pain conditions was further illustrated by Anne Louise Oaklander, M.D., Ph.D., Massachusetts General Hospital and Harvard Medical School, MA. She described a disease of the nervous system called small fiber polyneuropathy (SFPN). By definition "small fiber" refers to the thinnest nerve fibers, either lacking a myelin insulating sheath altogether or having a very thin sheath. These fibers are among the oldest elements of the nervous system from an evolutionary standpoint and respond to a range of sensory inputs including pain and inflammation. Small fiber polyneuropathy describes a disorder that usually begins in the lower limbs and is associated with the dying back of the small fibers. Blood pools in the legs contributing to low blood pressure, chronic widespread pain (CWP) and multiple organ effects, including gastro-intestinal and oral symptoms like burning mouth, headaches and cognitive deficits, which are due to reduced blood flow to the brain.

The disorder can be readily diagnosed by measuring innervation in skin biopsies from the leg and noting abnormal autonomic nervous system functioning. While SFPN is usually associated with age-related conditions such as diabetes or cancer, Dr. Oaklander's point is that it can explain CWP in children and adolescents and may also be a major factor in the pain of fibromyalgia and other CWP syndromes. Accordingly, she conducted analyses of data from 41 young people diagnosed with CWP, 27 adults with fibromyalgia, and 30 healthy volunteers. She found that 59% of the youngsters had definite SFPN and 17% had probable SFPN and 41% of the fibromyalgia patients had abnormal skin biopsies vs. 3% of controls. Other studies have indicated that early onset SFPN is often found in association with autoimmune conditions in which the immune system mistakenly attacks the body's own tissues and indeed, when immunomodulatory therapies have been initiated in pediatric patients two-thirds experienced some relief in pain and other symptoms.

Following Dr. Oaklander's presentation, Daniel Harper, Ph.D., University of Michigan, MI, one of the young investigators invited to present a poster at the meeting, demonstrated that the use of confocal microscopy to examine innervation in the cornea of the eye can provide a non-invasive way to diagnose

SFPN. In his study he compared corneal innervation in fibromyalgia patients, normal controls, and individuals with dry eyes either with or without normal tear function. His results indicated that both fibromyalgia patients as well as dry eye patients with normal tear function had significantly reduced corneal nerve length compared to controls or to dry eye patients with decreased tear function. Whether the shortened nerve length contributes to the cause of fibromyalgia or is a symptom has yet to be determined.

The use of animal models to elucidate the role of genetic, epigenetic and environmental factors in association with TMD and overlapping pain conditions continued with the presentations of the speakers on the afternoon of day 2 of the meeting.

Genetic, Cellular and Molecular Studies

Pain and depression. Jianro Mao, M.D., Ph.D., of the Center for Translational Pain Research of the Massachusetts General Hospital in Boston, MA, proposed a cellular mechanism that links pain and depression. It is well known that chronic pain patients frequently suffer from depression, a mental state often thought to be the result of experiencing unending pain. But it is possible that people diagnosed with depression may also be more sensitive to pain and perhaps its progression from acute to chronic. At present, patients with both conditions are treated with analgesics and antidepressants as two independent conditions. Dr. Mao's interest has been in determining if there are cellular mechanisms that link the two. His studies used rats that were of a breed genetically predisposed to depression or else animals that became depressed following repeated exposure to the forced swim test. (The test involves measuring how long an animal actively swims in a water tank as opposed to just keeping its head above water—the latter taken as a sign of despair, as though it had given up hope.)

Dr. Mao's interest was in an enzyme (**the enzyme is indoleamine 2, 3-dioxygenase**) in the metabolism of **trytophan**, a chemical in the nervous system that contributes to depression. He wondered if production of the enzyme was increased ("up-regulated") in the depressed rats and if depressed rats would also demonstrate exaggerated pain behaviors. The answer to both questions was yes. His experiments showed that the expression of the enzyme was increased (as measured in the hippocampus) in the depressed rats and rats with increased enzyme expression had lower pain thresholds in response to both mechanical and heat stimuli. In turn, inhibiting the action of the enzyme resulted in attenuating both the depression and pain behaviors, suggesting the potential for a single drug targeting the enzyme as a means of treating patients with both pain and depression.

A "double-hit" model of chronic TMD. Karen Westland High, Ph.D., University of Kentucky, KY, described a mouse model for chronic TMD that reflects both genetic and environmental factors. She and

colleagues compared normal wild type mice with mice lacking the genes for two cell surface receptors for forms of tumor necrosis factor alpha (TNF alpha). TNF alpha is a cytokine, one of a large group of immune system chemical messengers that act like hormones to stimulate inflammatory and immune responses. To model TMD, the jaws of the mice were injected unilaterally with Complete Freund's Adjuvant (CFA). She described this as "the first hit" (an injury) since it resulted in inflammatory damage measured by hypersensitivity to both mechanical and heat stimuli applied to the affected jaw in both the wild type and knockout mice. The second hit came three weeks later, after the effects of the first hit with CFA had healed. Then both the wild type and knockout mice had their colons infused with a small amount of mustard oil, an irritant causing pain and sensitivity in the area. After several weeks the responses to heat and mechanical stimuli returned to normal in the wild type mice but not in the knockout mice, which now experienced renewed jaw hypersensitivity and inflammation lasting for at least 18 weeks. Serum samples comparing the wild and knockout mice showed distinctly different cytokine profiles both at 2 weeks (after the initial jaw inflammation had resolved) and at 18 weeks when the knockout mice were exhibiting chronic TMD symptoms. Levels of TNF alpha and other cytokines significantly increased in the knockouts compared to controls. The investigators explored a variety of agents to counter the chronic TMD symptoms. These met with varying degrees of success suggesting that further tests with other cytokine-modifying agents would be fruitful.

Gene cross-talk modulates pain and stress responses. David Goldman, Ph.D., National Institute on Alcohol Abuse and Alcoholism, NIH, described research that builds on findings that functional genetic polymorphisms—those changes in a nucleotide at a single place in the genetic code called a SNP—can alter an individual's response to pain or stress for better or worse. Earlier studies showed this to be the case for the catechol-O-methyltransferase (COMT) gene, providing evidence that the SNP variant in question lowered the threshold for pain and was more common in TMD patients than controls. Dr. Goldman said that the COMT variant was associated with anxiety and responses to emotional stimuli and that the function of the gene is subject to the actions of other genes that modify how the gene is translated into messenger RNA. Dr. Goldman went on to describe two other genes affecting pain, GTP cyclohydrolase (GCH1) and neuropeptide Y (NPY). It appears that high expression of a GCH1 polymorphism is associated with leg pain following post lumbar (lower back) surgery in human patients, and to high levels of experimental pain in volunteers. With regard to NPY, he emphasized that the gene links pain with emotion, noting that a polymorphism found in the promoter region of the gene (where transcription of the genetic code for NPY begins) affects the amygdala, a key emotional area of the brain, and also the hippocampus, associated with memory.

Isolated vs. comorbid pain conditions. The point that Andrea G. Nackley, Ph.D., a member of the OPPERA team at the University of North Carolina, Chapel Hill, NC, made in her talk is that there are essential differences in the molecular correlates associated with a chronic pain condition that occurs in isolation compared to the correlates found when that condition is accompanied by another pain condition. She proposed these differences can then serve as biomarkers—chemical entities that could be measured in the bloodstream or in the genome and used in making diagnoses. As well, they could lead to differential treatments based on whether the pain is local or occurs as a comorbid pain condition. These local vs. comorbid differences are consistent with the concept that isolated pain conditions reflect peripheral sensitization while comorbid conditions are indications of central sensitization.

As evidence, Dr. Nackley presented data from self-reported questionnaires, clinical exams, and analyses of tissue samples from two clinical studies. One study compared findings from 103 patients with TMD alone with 66 patients with TMD and widespread bodily pain (WBP), in which patients report pain at 6 or more body sites. The second study compared 33 patients with vestibulodynia (VBD), 23 with both VBD and irritable bowel syndrome (IBS), and 22 healthy controls. In general, patients with the comorbid conditions reported greater decreases in general and physical heath, lower thresholds and greater intensities of pain in experimentally applied mechanical or heat stimuli, greater disruptions in daily life, and more somatization (a measure of one's sensitivity and perceptual response to internal and external sensory stimuli) compared to those with isolated pain conditions or healthy controls. Serum and genetic analyses also showed differences in cytokine profiles that distinguished the isolated from the comorbid pain cases as well as different sets of "microRNAs" (miRNAS) involved in pain processing. (MicroRNAs are small non-coding RNA molecules that affect gene expression.) Interestingly, the isolated cases of TMD and VBD shared certain cytokine features not found in the comorbid cases, suggesting that there may be commonalities across isolated pain conditions no matter what body organ or tissue is involved. In summary, Dr. Nackley believes that differential findings of molecular correlates can be used as valuable tools to distinguish among subgroups of chronic pain patients and help to define each subgroup's unique pathology.

Epigenetics Mechanisms Can Change the Brain in Chronic Pain

Epigenetics as a mechanism underlying long-term deleterious changes in brain structure and function in response to chronic pain was the theme of a number of speakers following Dr. Nackley's presentation. As noted earlier, epigenetics is the science that studies the ways and means by which genes can be turned on or off without changing the genes themselves (which happens with mutations). Epigenetic changes occur through biochemical

modifications of some part of the DNA sequence for a gene. An important way this can happen is through the attachment of a methyl group (a molecule consisting of one carbon and three hydrogen atoms) to the promoter region of a gene. This effectively silences the gene—prevents it from being expressed. Removal of a methyl group on the other hand will have the opposite effect, allowing the gene to be turned on.

Changes in the prefrontal cortex. Maral Tajerian, Ph.D., Stanford University, CA, began her presentation noting that patients —and animals—suffering chronic pain often show signs of depression, anxiety, and cognitive impairment, characteristics associated with brain activity in the prefrontal cortex and the amygdala. She proposed that pain induces these long-term changes via epigenetic mechanisms, altering the expression of genes at the highest levels of the brain.

In support of this hypothesis, she and coworkers developed a mouse model. Surgery was used to create a peripheral nerve injury in male mice by constricting a branch of the sciatic nerve, which innervates the leg. Sham surgery (without injury) was performed in comparable mice as controls. At 6 months all test animals exhibited hypersensitivity to mechanical and cold stimuli, limited movement ability and anxiety as measured by standard field tests. To document epigenetic changes the levels of DNA methylation in their brains were measured. In contrast to the control brains, the brains of the injured animals showed hypomethylation—less methylation—in the prefrontal cortex and the amygdala, indications that some genes that had been silenced could now be expressed. Other parts of the brain were not affected. The investigators noted that hypomethylation affected several genes involved in pain circuitry. These genes were overexpressed in the brains of injured mice compared to those genes in control mice.

In a separate experiment a second set of mice had the same nerve injury or sham surgery. After three months the injured mice were removed from standard cages and placed either in an enriched or an impoverished environment. A similar environmental manipulation was applied to the control mice. After two months in the enriched environment the injured mice showed less hypersensitivities to cold and mechanical stimuli than injured mice in the impoverished environment as well as a return to a level of DNA methylation in the prefrontal cortex close to the levels in normal control mice, suggesting that an environmental manipulation can reverse epigenetic changes. Many questions remain about how these changes come about, which genes are affected, and what kinds of interventions can reverse epigenetic changes, but the results of this study suggests that pursuing ways to reverse epigenetic changes is yet another approach to alleviate chronic pain.

Epigenetics research is being applied to enhance understanding of the cause of many complex conditions including obesity, cancer, neurodegenerative diseases, high blood pressure and immune disorders.

Equally important are studies to determine how epigenetic factors affect the normal growth and development of the body's cells and tissues. Two speakers following Dr. Tajerian's discussion, Ken Zhao Ph.D., National Heart, Lung, and Blood Institute, NIH, and Jun Zhu, Ph.D., Icahn School of Medicine at Mt. Sinai, New York, NY, described their work on these aspects of epigenetics research, suggesting that the techniques employed could be applied to the study of chronic pain.

Epigenetics and T cell development. Dr. Zhao began by describing how the genetic material of a cell is arranged in the nucleus when not organized into chromosomes (which happens when the cell is poised to divide). In the non-dividing state the genetic material remains highly organized in long chains of DNA but is entangled with other molecules, including histones, which are proteins that can affect gene expression. However, only a small portion of the DNA in the nucleus constitutes the human genome: the 20,000 or more genes that code for proteins used in the body. The remaining DNA sequences constitute "non-coding DNA," meaning that they do not code for proteins. Nevertheless, these non-coding sequences are transcribed into RNA and have become the focus of intense interest since particular sets of non-coding RNAs have been found to play a regulatory role in gene expression. In particular, Dr. Zhu has focused on long chains of non-coding RNA molecules transcribed from DNA sequences positioned between protein-coding genes. There are tens of thousands of these "lincRNAs," (long non-coding intergenic RNAs) throughout the genome, and Dr. Zhao and his colleagues wanted to know what their function was and how they are controlled. A series of clues, such as finding that expression of lincRNAs correlated with the expression of immune response genes led, through many complex steps, to the team's conclusion that lincRNAs are expressed during the development and differentiation of a major type of immune cell, the T cell, that they have important functions in T cells, and that their expression is regulated by several transcription factors.

Epigenetics and disease. Dr. Zhu's study emphasized the value of integrating large databases of genomic and epigenetic information in combination with other large databases such as the "transcriptome," in studying disease. The transcriptome is the set of all RNA molecules that are transcribed in a particular cell or population of cells. This includes the messenger and transfer RNAs used when a gene is actively being translated into a protein, as well as any transcribed non-coding RNAs. This is a dynamic set since it reflects any genes that are being actively expressed in the cell at a given time. In turn, it reflects environmental factors. Dr. Zhu illustrated the use of these large databases along with probability theory in studies of obesity, diabetes, neurodegenerative diseases and chronic obstructive lung disease (COPD). In the case of COPD, their approach led to the discovery of candidate genes associated with the risk for COPD and a "molecular causal network" in which specific epigenetic regulations and altered transcripts could explain the onset of COPD symptoms as well as disease progression.

Functional Genomics of Pain in Analgesic Drug Development and Therapeutics

The session began with the introduction of Patricia A. Grady, Ph.D., R.N., F.A.A.N., Director of the National Institute of Nursing Research, NIH. Given the multidisciplinary nature of nursing research, Dr. Grady said, the Nursing Institute has been a major player in trans-NIH and trans-professional activities. The Institute is one of the co-leaders of the Pain Consortium at NIH and also participates in the Federal Interagency Pain Research Coordinating Committee. A commitment to advance nursing skills to reduce major symptoms such as pain and fatigue and improving quality of life has engaged the Institute in studies of personalized medicine, tools for measuring symptoms and the development of patient-controlled monitoring devices. Dr. Grady also highlighted research focusing on pediatric and other special populations as well as the importance of addressing the needs of families and caregivers and improving the patient-provider communication. Echoing what other NIH representatives had said, she emphasized the importance of partnerships and collaboration in research to advance understanding and treatment of chronic pain.

The last group of speakers at the meeting explored pain drugs and therapeutics in light of genomic and epigenetic research.

Pain in the network of genetic-epigenetic control. Jorn Lötsch, M.D. Goethe–University, Frankfurt am Main, Germany, discussed two broad themes in his presentation. The first concerned interactions between genetic and epigenetic mechanisms involving pain-associated genes. A prime example he cited was a SNP variant of a gene that codes for one of several nerve cell membrane receptors important in pain control, the mu-opioid receptor. Many opioid drugs target the mu receptor exciting the cell to action to inhibit pain. However, the SNP variant Dr. Lötsch described results in fewer mu receptors being established on nerve cells and thus fewer targets for the drugs and less pain relief. The reason? Dr. Lötsch explained that the change in the DNA sequence of the variant form allowed methylation of an additional silencing site on the DNA sequence for the gene and in this way decreased gene expression. Fortunately, the down-regulation of the gene was minor and not clinically significant for patients.

Dr. Lötsch went on to say that many common drugs including pain medications, anticonvulsants and others, have epigenetic effects. In particular, chronic use of opioid drugs has been shown to lead to significant DNA methylation of the mu receptor gene, which explains the paradoxical effect that chronic opioid use can lead to increased pain.

The second half of Dr. Lötsch's presentation described a sophisticated methodology incorporating machine learning to tie complex genetic information concerning pain genes to the signs and symptoms of

specific subgroups of pain patients (pain phenotypes). Applications of this technology could lead to improved diagnoses and personalized treatments.

Genome and epigenome editing. The mapping and sequencing of the human genome was accomplished in 2001, noted Charles A. Gersbach, Ph.D., Duke University, NC. The next steps have been directed toward determining which parts of the genome represent genes that encode proteins, which are non-coding sequences, and finding out what all these various elements of the genome do—a process called genome annotation. Once mutations of genes associated with disease were discovered, scientists also sought ways to eliminate the defective gene and replace it with its normal counterpart. Dr. Gersbach reviewed past methods of genetic engineering to do this, noting problems that have made these efforts less than ideal. The situation has changed dramatically and recently with the advent of new technology that promises more precise and efficient means of editing genomes and achieving this at low cost.

The new technology is based on studies of immunity in bacteria. These microorganisms incorporate into their DNA portions of viral DNA and the DNA of other foreign invaders that the bacteria have successfully fought. When examining bacterial genomes scientists discovered that these foreign sequences were the interspaces between "clustered regularly interspaced short palindromic repeats" (CRISPR). The interspaces with the foreign DNA allowed the bacterium to recognize and cut up the DNA of the foreigner should it invade again. A set of genes associated with the clustered repeats was also discovered and it is this combination of CRISPR and CRISPR-associated genes (called Cas) that forms the basis of the new technology. Using CRISPR/Cas methodology investigators can create synthetic enzymes that can break the DNA of an organism's genome at a specific site in order to repair, excise, replace or in other ways manipulate the genome.

Rather than detail the complexities of the CRISPR/Cas system in practice, Dr. Gersbach addressed how it has been applied. As an example he described how the technology enabled the development of synthetic enzymes targeted to the mutated gene that causes the muscle-wasting disease Duchenne muscular dystrophy. The enzymes were able to remove a defective sequence in the code for the gene that stopped its translation too early to allow for its complete transcription into protein. The CRISPR technology restored the normal function of the gene, which codes for the protein dystrophin. While most of the examples Dr. Gersbach mentioned related to editing the genome, he said that CRISPR technology would be invaluable in epigenome editing, enabling the silencing of selected genes, or alternatively, providing a means of highly specific gene expression.

Following Dr. Gersbach's presentation of revolutionary developments in the broad field of genetic and epigenetic engineering, the perspective changed to focus on TMD with descriptions of new imaging techniques and promising new drugs.

In vivo imaging of jaw pain. Xinzhong Dong, Ph.D., Johns Hopkins School of Medicine, MD, reminded attendees of the essential role of the small diameter nociceptor neurons in the trigeminal ganglion in transmitting signals from the jaw to the brain in response to painful stimuli. If there is continued inflammation or nerve injury, the pain message can be amplified and spread to other nerve cells, including large diameter neurons normally not responsive to pain stimuli, in the process of central sensitization. However, these changes have not been amenable to visualization. Dr. Dong and colleagues have resolved this issue by using a model of neuropathic pain in a specially bred mouse model in which selected nerve cells fluoresce a shade of green when excited. This technique allowed observation of intense activity of cell bodies and terminals of nociceptors in various brain tissue samples. The neurons detected were TRPV1 nociceptors, which Dr. Dong and earlier speakers had described as key components in the body's system of pain detection and modulation. The team also discovered a descending pathway, one using the neurotransmitter serotonin, which helps maintain central sensitization. Excitingly, the new technology is now being applied to visualize how the brain changes in response to a variety of sensory signals in living mice using functional neuroimaging.

Promising New Drugs

Calcium is critical to the normal functioning of the nervous system, said Roy C. Levitt, M.D., University of Miami Miller School of Medicine, FL. Calcium atoms inside neurons are essential to neuronal excitability and synaptic transmission among other vital functions. Not surprisingly, any disruption in the normal functioning of calcium is serious, Dr. Levitt noted, and occurs in a number of neurological diseases, including spinal cerebellar ataxia. This is a genetic disease causing spinal deformities and movement disability, but one in which patients also suffer severe chronic pain. It is that symptom that prompted Dr. Levitt and colleagues to study the genetics of the condition. The problem is a mutation in the gene for carbonic anhydrase 8 (Car8), an enzyme that inhibits a receptor on nerve cell membranes, inositol triphosphate receptor (ITPR1), which regulates intracellular calcium release. The mutated gene causes a deficit in Car8 and increased levels (up-regulation) of ITPR1. Particularly affected by the deficit are the cell bodies of small neurons (such as nociceptors) that respond to sensory stimuli.

Gene therapy. Interestingly, there is a mouse model, called "waddles," of a mutated Car8 gene, which manifests as a movement disorder in the animals. Dr. Levitt and associates wondered whether the mice also experienced pain, and showed that yes, the mice were hypersensitive when tested, showing allodynia to mechanical stimuli and hyperalgesia to thermal stimuli. Next they considered whether some form of gene therapy might relieve the animals' pain. This required the technically complex constructions of the correct form of the mutated gene and incorporating it into a

viral vector to transfer the gene into the diseased animals. They also replicated the mutated form of the gene to use with a viral vector as a negative control. The experiment worked. The diseased animals with the normal gene no longer showed mechanical allodynia and thermal hyperalgesia while the control animals remained hypersensitive.

In further studies Dr. Levitt was able to demonstrate that inflammation in and of itself may figure in down-regulation of Car8 in relation to ITPR1 and thus may be a factor in hypersensitivity and calcium dysregulation. He also noted that there is considerable variability in the human Car8 gene, which could explain variability in susceptibility to chronic pain.

Fenobam. Throughout the meeting, speakers identified neural pathways, transmitters, cell surface receptors, and brain or spinal cord areas that play a large, and in some cases, exclusive, role in the perception and response to pain. So it was with the report of Robert W. Gereau, Ph.D., Washington University School of Medicine, St. Louis, MO. The drug he described targets one of a large family of metabotropic glutamate receptors (mGluRs). These receptors span nerve cell membranes throughout the nervous system, especially in pain pathways. When activated by glutamate, a major excitatory neurotransmitter, mGluRs can modulate the level of excitability of the cell and synaptic transmission. They do this indirectly through interactions with molecules within the nerve cell that trigger a chain of biochemical events that modulate the cell's firing. (The word "metabotropic describes this characteristic.)

What made a particular mGlu receptor, mGlu5, interesting, Dr. Gereau observed, was not only the abundance of neurons with this receptor in key parts of the pain network, but also findings from previous studies in mice suggesting that the receptor would be a good target for analgesic drugs. Moreover, they did not have to develop such drugs. As it turns out, an mGlu5 receptor targeted drug called Fenobam had been developed in the 1970s to treat anxiety in human patients. Fenobam acts specifically and exclusively to block the actions of mGlu5 receptors.

Following up with new pain tests in mice, Dr. Gereau's team showed that Fenobam was successful in blocking inflammatory, neuropathic and visceral pain. Furthermore, there were no adverse effects on behavior, blood chemistries, and liver enzymes and no indication that repeated doses would lead to tolerance. Tests in human volunteers have also been promising, with no serious adverse events reported in multiple human trials. Research will continue with clinical trials to test Fenobam's utility in pain patients. In particular, it is hoped that the drug will be effective in reversing the central sensitization common to many chronic pain conditions.

The Comorbid Chronic Pain Roundtable

The scientific meetings sponsored by The TMJA have, since their inception, included a roundtable session in which patients and patient advocates describe the conditions they represent, the outstanding problems patients face, and what research is sorely needed that will ultimately lead to the relief of the pain and suffering their constituents experience. Career scientists and young investigators in attendance have valued these sessions which, for some, may constitute their first encounter with individuals with the very diseases they are studying in the lab.

Roundtable panelists at the seventh scientific meeting included Terrie Cowley, President of The TMJ Association, Milwaukee, WI; Janet Chambers, President, National Fibromyalgia and Chronic Pain Association, Logan, UT; Lee Bryon Classen, CAE, Executive Director, Interstitial Cystitis Association, McLean, VA; and Suzanne Vernon, PhD, Scientific Director, Solve ME/CFS Initiative, CA.

In brief opening remarks, the panelists emphasized the time and financial costs of obtaining an accurate diagnosis of their condition—an average of 5 years in the case of interstitial cystitis, Ms. Classen said, a bladder disorder characterized by frequency, urgency and pain. Ms. Chambers remarked on the disbelief of providers that a fibromyalgia patient could have so many symptoms and even questioned whether the condition existed. That disbelief also pertains to ME/CFS. Overall, the representatives affirmed that pain was the overwhelming symptom of their condition and that all lacked standard diagnostic criteria as well as safe and effective treatments. Stigma was also an issue. Ms. Chambers commented on the contempt fibromyalgia patients experience and the reluctance on the part of physicians to make the diagnosis or prescribe drugs. As a result, many fibromyalgia patients have been unable to obtain prescription pain medications, such as opioids, and some have contemplated suicide.

The group was then asked to address four questions posed by John Kusiak, Director of the Molecular and Cellular Neuroscience Program, NIDCR, who had earlier explained to attendees that at the conclusion of the meeting they would be asked to develop recommendations for research, addressing "compelling questions and critical challenges."

The Questions:

1. What value do you see in research to address genetics and epigenetics in the risk to developing your specific condition or that of our constituents?

Ms. Cowley expressed her belief that knowing genes that are involved in TMD and overlapping pain conditions would provide clues leading to better diagnostics and risk assessment. The predictive value alone would be important, while epigenetic information might explain how changes in behavior or the environment might trigger the onset of TMD. Furthermore, knowing

epigenetic factors might also offer the possibility of reversing genomic changes and ameliorating TMD. Others agreed. Ms. Classon noted that at present there are no diagnostic tests for interstitial cystitis; the diagnosis is arrived at by a process of elimination, a length of time in which symptoms can worsen. If new genetic or epigenetic research leads to an accurate IC test it would shorten the time from onset of symptoms to diagnosis and result in less pain and suffering. Dr. Vernon summarized the current state of research on ME/CFS noting that there are clear genetic patterns that distinguish CFS patients from controls so both genetics and epigenetics research is valuable and should be applied to overlapping pain conditions as well.

2. Describe your experiences or that of your constituents with the diagnosis and currently recommended treatments for your or any overlapping conditions.

The experiences of roundtable members and their constituents were universally negative in relation to achieving timely and accurate diagnoses while safe and effective treatments for their conditions were either non-existent or, in the case of a single FDA-approved treatment for IC is only effective in half the patients.

3. What value do you see in research which will lead to treatments for overlapping pain conditions?

"It would be a miracle drug!" said Ms. Cowley. And it would reduce health care costs, because right now people with overlapping pain conditions see a variety of specialists each prescribing a different treatment according to the end organ affected. Ms. Chambers commented that recognizing that many fibromyalgia patients are part of a larger chronic pain population that could be treated with a single drug would help eliminate the stigma her constituents suffer. Dr. Vernon praised the OPPERA and MAPP research studies for pioneering the collection of data on overlapping pain conditions, recognizing the complexity of this patient population, and proposing a new taxonomy of disease based not on end-organs but on molecular correlates.

4. Which areas of research on chronic overlapping pain conditions need emphasis in the second decade of this century?

The group made a number of suggestions for moving forward. Ms. Cowley said that the epigenetics and genetics areas were in their infancy insofar as TMD is concerned, and a strategic plan should be developed. Epidemiology is also a vital area. We don't really know how many people have TMD, much less how many have TMD and fibromyalgia or TMD and IC or ME/CFS. Ms. Chambers commented that we need to know more about any complementary and alternative treatments chronic pain patients use, often because mainstream medicine hasn't worked. Studies of diet and nutrition are also needed since some patients report that their symptoms abate with changes in diet. Along those lines Ms. Classon suggested that more research is

needed on what variables worsen symptoms. Are allergies associated with flare-ups of IC, for example? Dr. Vernon made a strong case, not for any research area in particular, but for promoting and facilitating research partnerships, not only across scientific disciplines and institutes at the NIH, but between the research establishment and patient advocacy organizations themselves. This would also be a way of ensuring that NIH support is not wholly concentrated on basic research, but moves into translational and clinical research, and in this way accelerating the path leading to the ultimate goal: restoring the health and well-being of chronic pain patients.

Dr. Kusiak thanked the panel for their advice and turned to the audience for questions. There were two interesting suggestions for research. Dr. William Maixner, who is the lead investigator in the OPPERA study, said it would be really valuable to find out which pain patients benefit from the chronic use of opioids and which patients do not. And Dr. Allan Basbaum, University of California, San Francisco, wondered whether any drugs approved for treating symptoms relating to an end-organ (e.g., the bladder in IC) have any positive effect on symptoms of any overlapping pain condition that the IC patient may be experiencing.

Summary Overview of Recommendations

There is a critical need for resources to support research to advance understanding of the causes and mechanisms underlying Temporomandibular Disorders (TMD) and overlapping chronic pain conditions, including chronic headache, endometriosis, fibromyalgia, interstitial cystitis/bladder pain syndrome, irritable bowel syndrome, low back pain, myalgic encephalomyelitis/chronic fatigue syndrome, and vulvodynia. Case definitions of these disorders should be developed that conform to current scientific findings. Also of highest priority is research to develop novel treatments for these and other chronic pain conditions and the translation of new discoveries into clinical practice. Toward those ends, workshops, training programs and other venues should be implemented to help current investigators and those new to the field to acquire breakthrough technologies, such as next generation sequencing, CRISPR-based gene editing and therapy, including the application of engineered DNA-binding proteins for therapeutic use.

The pain research community could meaningfully advance their field by embracing the technologies of genomic scale analytical approaches in order to explore genetic and epigenetic markers that are predictive of risk for chronic pain. Such efforts include the need to access, manage, and analyze "big data" sets which are comprised of large patient databases that include longitudinally collected clinical data, patient surveys, human and animal phenotypes, imaging data, and genomic scale DNA/RNA sequence data. Training of additional computational biologists and bringing their expertise to the research community is critical.

To expedite progress in the pain field, scientists and clinicians must work with private and government entities to explore ways to build the infrastructure required for the recommended research and for its translation into clinical medicine. Attendees proposed that the National Institutes of Health (NIH) conduct a feasibility/cost analysis study to determine the funding and resources needed to support proposed epidemiological, mechanistic, and genomic/epigenetic discovery research, together with new translational initiatives. This information could serve as a blueprint for patient advocacy groups, individual scientists and their professional organizations to alert elected officials to the imperative to expand support of research on chronic pain, given the enormity of its burden in the United States.

Recommendations

Epidemiology and diagnostics. Both patient advocates and scientist attendees emphasized the importance of developing more scientifically based case definitions for TMD and overlapping pain conditions. They noted that diagnostic criteria and case definitions used by the practitioner community have not meaningfully progressed over the past 20 years. Advancements in this area are long overdue and need to move beyond past diagnostic criteria that have largely emphasized end organ symptoms.

- Well-powered epidemiological studies are needed to obtain data on the extent and varieties of pain conditions reported in the U.S. population, such as pain of unknown origin, female-related medical conditions, post-surgical pain, and iatrogenic pain.

- □ Patient experience and input are critical in developing these criteria.
- □ Innovative ways must be developed to simplify and streamline data gathering for these studies.
- □ Large cohort databases related to subjects with TMD and overlapping pain conditions should be coordinated and linked. These databases should only include those verifiably diagnosed according to newly developed case definitions.

• Expand research to develop novel prognostic markers and mechanistically based criteria for the diagnosis of these disorders.

• Include chronic pain as a priority in broad public health initiatives. This effort will move this issue into the national spotlight.

• Evaluate and prioritize current treatments for TMD and related overlapping pain conditions, so as to determine what is evidence-based and what is anecdotal.

Basic and clinical mechanistic studies in neuroscience and genomics. The need to advance understanding of the mechanisms leading to the chronicity of pain was emphasized with a strong recommendation to broaden research in this area. Given the complexity of TMD and overlapping pain conditions, it was recommended that the NIH better integrate and coordinate research in this field. To facilitate progress, investigators should utilize the latest tools and technologies, developing and expanding model systems, and exploring genomic and epigenomic mechanisms. Specifically:

• Develop methods to evaluate more productively the sensory, affective and cognitive components of chronic pain utilizing the most recent advances in neurosciences and brain imaging.

• Advance studies of the general illness aspects of chronic pain conditions, such as fatigue, sleep disorders, and depression. Determine how these characteristics contribute to the initiation and to the persistence of chronic pain.

• Develop novel non-surgical, non-injury-based animal models for the study of chronic TMD pain and comorbid conditions.

Genetics, epigenetics and functional genomics of TMD and overlapping pain conditions. Attendees recommended that research be expanded to obtain molecular correlates of localized versus co-occurring generalized chronic pain and depression. Genomic DNA and RNA deep sequencing are needed to identify sequence variants and DNA methylation patterns that are associated with risk for overlapping pain conditions. There is a compelling need for the development of novel analytical tools to advance discovery in this field. Researchers need to acquire and apply cutting-edge tools that are emerging for targeted genome and epigenome editing. Specifically:

• Exon and whole genome exon sequencing as well as RNA sequencing technologies need to be more widely applied to the field of chronic generalized pain.

- □ Genetic and epigenetic signatures are needed to determine underlying mechanisms and predictors of disease risk.
- □ Greater genomic scale research is needed to move the field beyond the current focus on one gene, one pathway research.

- □ Genetic and epigenetic biomarkers that predict risk for chronic pain and identification of new therapeutic targets should be accelerated.
- □ Epigenetic markers driven by environmental factors, such as diet, phytohormones, stress, surgery, dental procedures, and pharmacologics are needed. Studies directed at deep sequencing of large pedigrees with unique pain conditions are called for, as are more twin studies.

• Novel methods need to be developed for experimental and therapeutic delivery of engineered DNA-binding proteins. These are considered among the most important challenges in the field in the coming years.

Training needs. A list of workshops and programs to facilitate progress in genetics, epigenetics and functional genomics follows. These workshop and program needs should be circulated to all NIH Institutes and Centers through the NIH Pain Consortium, which can lead to inter-institutional coordinated actions for implementation of these programs.

• Workshops should be organized to enable investigators to utilize and mine national resources and websites. These resources contain a wealth of phenotype and DNA sequence data from mouse, rat, human, and other model organisms.

• Avenues should be explored to help investigators acquire and apply exciting new technologies for gene editing, such as ZFN, TALE and CRISPR/Cas9 methods to engineer both protein-based and RNA-guided transcriptional activators and repressors targeted to human genes that are already linked to pain pathways.

• An urgent need exists to train more computational biostatisticians and bioinformaticians who can manage and analyze large data sets and advance systems approaches for the discovery of mechanistic pathways, novel therapeutic targets, and diagnostic approaches for chronic pain.

• Sensitive to research involving genome editing and other novel therapeutic approaches, workshops to address ethical issues are essential. These workshops should include patients, scientists, clinicians, legal experts, and ethicists. This workshop will guide the design and conduct of clinical studies and trials that will use such approaches.

Development of therapeutics. Ranking among the highest priorities to emerge from the meeting was a recommendation for the rapid development of novel, non-opioid, mechanistically based therapeutic agents for the treatment of these overlapping pain conditions.

• Broaden efforts to identify novel molecular pathways and therapeutic targets that are amenable to manipulation and consequently can alter chronic pain treatment.

• Collaborate with the Food and Drug Administration to facilitate the conduct of randomized controlled clinical trials based on repurposed molecules as well as other novel treatments, such as pharmacologics and devices.

• Public and private organizations should explore opportunities for more interactions with the Patient-Centered Outcomes Research Institute (PCORI).

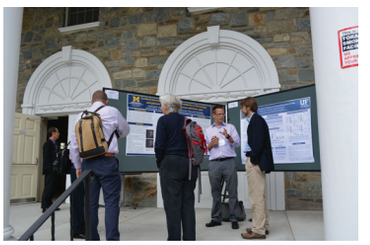
TMJA Young Investigator Awards

At the TMJ Association's Seventh Scientific Meeting, *Genetic, Epigenetic, and Mechanistic Studies of Temporomandibular Disorders and Overlapping Pain Conditions*, September 7–9, 2014, the Association recognized the following young investigators for their research interests in the area of TMJ disorders and overlapping pain conditions. The following young investigator listed below received a travel grant award to attend our meeting with the opportunity to meet and learn from prominent research scientists, government officials and TMJ patients.

The TMJ Association would like to thank the National Institutes of Health for making these travel awards possible.

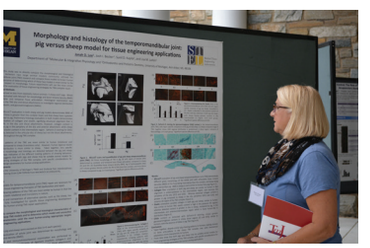
Presenter: Yong Chen, Ph.D., Duke University, Durham, NC
Poster: TRPV4-mediated trigeminal pain: behavior assessments and mechanisms
Mentor: Dr. Wolfgang Liedtke

Presenter: Daniel E. Harper, Ph.D., University of Michigan, Ann Arbor, MI
Poster: Confocal microscopy reveals nerve fiber similarities in fibromyalgia and patients with dry eyes with a normal ophthalmic exam
Mentors: Drs. Daniel Clauw and Richard Harris



Presenter: Christopher D. King, Ph.D., University of Florida, Gainesville, FL
Poster: Case-control analysis in resting and evoked inflammatory profiles
Mentors: Drs. Roger Fillingim and Joe Riley III

Presenter: James Sciote, D.D.S, M.S., Ph.D., Temple University, Philadelphia, PA
Poster: Associations between ACTN3 and OPPERA pain-related genes in malocclusion



Presenter: Shad B. Smith, Ph.D., University of North Carolina at Chapel Hill, Chapel Hill, NC
Poster: Association between INADL genetic variant and the subgroup with high risk for TMD in the OPPERA study
Mentors: Drs. Luda Diatchenko and William Maixner

Presenter: Roi Treister, Ph.D., Massachusetts General Hospital & Harvard Medical School, Boston, MA
Poster: Repetitive transcranial magnetic stimulation (rTMS) of the primary motor cortex for treating facial neuropathic pain—preliminary results of a randomized, sham-controlled, cross-over study
Mentor: Dr. Anne Louise Oaklander

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