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The Eighth Scientific Meeting of The TMJ Association, Ltd.

How Can Precision Medicine Be Applied to Temporomandibular Disorders and Its Comorbidities?

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Preface

The TMJ Association co-sponsored, with entities of the National Institutes of Health, its Eighth Scientific Meeting, titled *How Can Precision Medicine Be Applied to Temporomandibular Disorders and Its Comorbidities*. The meeting was held in September 2016 at the headquarters of the Federation of American Societies for Experimental Biology in Bethesda, MD. It brought together scientific experts, patient advocates, and patients for a three-day brainstorming conference to discuss past, current and future research directions, new technologies, and human studies centered on the use of precision medicine (PM) approaches to address temporomandibular joint disorders and related comorbid chronic pain conditions. Patient advocates highlighted the importance of patient engagement in all aspects of the research arc from basic to clinical studies. Experts from a number of research fields presented results and described future directions that precision medicine will need to follow to improve the diagnosis and treatment of temporomandibular joint disorders. A series of actionable recommendations were generated to guide the scientific research community, TMJ advocates, and meeting sponsors in advancing the research agenda.

Reflecting upon the presentations, it is clear that a broad spectrum of research approaches will be necessary to drive improvements in the individual treatment of chronic pain conditions. Further, patient input in designing the delivery of precision medicine treatments for TMD will be a critical factor in shaping the research agenda driving discovery of these new diagnostics and treatments. An important concept that emerged from this conference was the need to describe TMD and other chronic overlapping pain conditions, in the context of a biopsychosocial model, in order to develop and advance therapies for these conditions. Funding agencies, foundations, and the private sector will play a pivotal role in improving diagnosis and treatment of TMD and overlapping pain conditions by continuing to support research and research training across a broad array of scientific fields and fostering research partnerships to leverage respective strengths of each partner. The effective treatment of TMD and other chronic pain conditions is a complex problem that will be solved with the application of new knowledge gained from interdisciplinary research endeavors and its application to improving the lives of TMD patients.

Martha J. Somerman, DDS, PhD
Director, National Institute of Dental and Craniofacial Research, NIH

John W. Kusiak, PhD
Acting Deputy Director, National Institute of Dental and Craniofacial Research, NIH

The theme of The TMJ Association's Eighth Scientific Meeting is how methods of precision medicine can improve the treatment of temporomandibular disorders (TMD) and associated comorbid conditions. These conditions include vulvodynia, chronic tension-type and migraine headaches, myalgic encephalomyelitis/chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, interstitial cystitis/bladder pain syndrome, chronic low back pain and endometriosis. Current diagnostics and treatments do not reflect the current state of science in this field and fall short in meeting the needs of patients. Successful application of precision medicine technology to TMD and its comorbidities will enable providers to classify TMD patients into subgroups according to specific diagnostic criteria which will lead to more tailored, safe and effective treatments to relieve patients' pain and suffering.

The TMJ Association Scientific Advisory Board

The Eighth Scientific Meeting of The TMJ Association

How Can Precision Medicine Be Applied to Temporomandibular Disorders and Its Comorbidities?

Sunday, September 11, 2016

2:00 – 2:30 p.m. **Welcome and remarks**
Terrie Cowley, President and Co-founder, The TMJ Association
Milwaukee, WI

Allen W. Cowley, Jr., PhD, Program Committee Chairman,
The TMJ Association and Professor and Chairman, Department of Physiology, Medical College of Wisconsin, Milwaukee, WI

National Institutes of Health welcome and remarks
Lawrence A. Tabak, DDS, PhD, Principal Deputy Director, Office of the Director, National Institutes of Health, Bethesda, MD

Patricia A. Grady, PhD, RN, Director, National Institute of Nursing Research, National Institutes of Health, Bethesda, MD

Session 1: **Defining Precision Medicine**
Session Co-Chairs: Christopher Mullins, PhD and William Maixner, PhD

Christopher Mullins, PhD, 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

2:30 – 3:00 p.m. **The U.S. precision medicine initiative**
Eric D. Green, MD, PhD, Director, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD

3:00 – 3:30 p.m. **Key issues for advancing precision medicine for TMD and chronic overlapping pain conditions: Case definitions and phenotypic measures**
William Maixner, DDS, PhD, Center for Translational Pain Medicine, Department of Anesthesiology, Duke University School of Medicine, Durham, NC

3:30 – 4:00 p.m. **Longitudinal gene-brain mapping to guide diagnosis and treatment of mechanistically distinct types of chronic pain**
Apkar Vania Apkarian, PhD, Department of Physiology and Departments of Anesthesia and Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL

4:00 – 4:30 p.m. **N-of-1 trials for personalized decision-making**
Christopher H. Schmid, PhD, Center for Evidence Based Medicine, Brown University School of Public Health, Providence, RI

4:30 – 4:45 p.m. **Break and poster sessions**

4:45 – 5:15 p.m. **In silico approach to developing TMD-related precision medicine applications: Narrowing the gap between currently available and urgently needed**
Yelizaveta Torosyan, MD, PhD, Division of Epidemiology of the Center for Devices and Radiological Health, Food and Drug Administration, Silver Spring, MD

5:15 – 5:45 p.m. **Effective engagement with patient groups around clinical trials**
Jamie Roberts, MPH, MA, CCRP, Clinical Trials Transformation Initiative Duke Clinical Research Institute, Durham, NC

5:45 – 6:15 p.m. **TMD treatment and precision medicine—The past, present and future**
Co-Chairs: Jamie Roberts and Terrie Cowley
Michele Kaseta, Patient Advisory Committee Chair, The TMJ Association
Danica Marinac-Dabic, MD, PhD, Director, Division of Epidemiology of the Center for Devices and Radiological Health, Food and Drug Administration, Silver Spring, MD
Christin Veasley, Director and Co-Founder, Chronic Pain Research Alliance

6:15 – 6:45 p.m. **Session discussion and recommendations**

6:45 p.m. **Dinner and poster sessions at FASEB**

Monday, September 12, 2016

8:00 – 8:15 a.m. **Welcome and remarks**
Martin Frank, PhD, Executive Director, American Physiological Society Bethesda, MD

National Institutes of Health welcome and remarks
Stephen I. Katz, MD, PhD, Director, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD

Session 2: **Examples of Progress in Precision Medicine**
Session Co-Chairs: Lisa Begg, DrPH, RN and James Bibb, PhD

8:15 – 8:40 a.m. **Precision medicine strategies to selectively alter intracellular signaling mechanisms: A new generation of targets**
James A. Bibb, PhD, Departments of Psychiatry and Neurology and Neurotherapeutics, Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX

8:40 – 9:05 a.m. **Targeting chemokine and protease signaling for the control of neuroinflammation and chronic pain**
Ru-Rong Ji, PhD, Department of Anesthesiology and Neurobiology Duke University School of Medicine, Durham, NC

9:05 – 9:30 a.m. **Human chronic pain conditions: Genome-wide analysis and pathways of vulnerabilities**
Luda Diatchenko, MD, PhD, The Alan Edwards Centre for Research on Pain Faculty of Dentistry, McGill University, Montreal, Quebec, Canada

9:30 – 10:00 a.m. **Session discussion and recommendations**

10:00 – 10:15 a.m. **Break and poster sessions**

Session 3: **Pharmacogenomics - Models Targeting Human Disease "In a Dish"**
Session Co-Chairs: Wen Chen, PhD and John Wikswo, PhD
Wen G. Chen, PhD, Program Director, Basic and Mechanistic Research in Complementary and Integrative Health, Division of Extramural Research National Center for Complementary and Integrative Health, Bethesda, MD

10:15 – 10:40 a.m. **Intranasal mesenchymal stem cell transplantation for the repair of neuronal damage in chemobrain**
Cobi J. Heijnen, PhD, Laboratory of Neuroimmunology, Department of Symptom Research, Division of Internal Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX

10:40 – 11:05 a.m. **Probing the complexities of biology and medicine: Closing the hermeneutic circle with in vitro models to study nerve pain and neural responses to pain medication**
John P. Wikswo, PhD, Department of Biomedical Engineering, Department of Molecular Physiology and Biophysics, Department of Physics and Astronomy, and Vanderbilt Institute for Integrative Biosystems Research and Education, Vanderbilt University, Nashville, TN

11:05 – 11:30 a.m. **Induced pluripotent stem cells for disease modeling**
Ulrich Broeckel, MD, Department of Pediatrics, Medicine and Physiology Medical College of Wisconsin, Milwaukee, WI

11:30 – 12:00 p.m. **Session discussions and recommendations**

12:00 – 1:10 p.m. **Lunch and poster sessions**

1:10 – 1:15 p.m. **Remarks from the Office of Research on Women's Health**
Lisa Begg, DrPH, RN, Research Program Officer, Office of Research on Women's Health, National Institutes of Health, Bethesda, MD

Young Investigator Award Presentations

1:15 – 1:25 p.m. **A prediction error model of placebo analgesia and its extinction**
Luana Colloca, MD, PhD, Department of Pain Translation Symptom Science University of Maryland School of Nursing, Baltimore, MD

1:25 – 1:35 p.m. **Clinical characteristics of TMD at onset and predictors of persistence: Preliminary results**
Carolina Beraldo Meloto, DDS, PhD, The Alan Edwards Centre for Research on Pain, Faculty of Dentistry, McGill University, Montreal, Quebec, Canada

1:35 – 1:45 p.m. **The behavioral and neural effects of multipotent stromal cells in rodent models of persistent pain**
Ke Ren, PhD, Department of Neural and Pain Sciences, School of Dentistry and Program in Neuroscience, University of Maryland, Baltimore, MD

1:45 – 1:55 p.m. **Structural and functional abnormalities in chronic orofacial pain disorders: A meta-analytic study**
Massieh Moayedi, PhD, Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada

1:55 – 2:05 p.m. **Lack of evidence for ectopic sprouting of genetically labeled Aβ touch afferents in inflammatory and neuropathic trigeminal pain**
Yong Chen, PhD, Departments of Neurology and Neurobiology, Duke University Durham, NC

Session 4: **Nervous System and Immune/Inflammatory Interactions**
Session Co-Chairs: Yolanda Vallejo-Estrada, PhD and Howard Gendelman, MD
Yolanda F. Vallejo-Estrada, PhD, Director, Neuroscience of Orofacial Pain and Temporomandibular Disorders Program, Integrative Biology and Infectious Diseases Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD

2:05 – 2:30 p.m. **Sexual dimorphism in microglia-neuron signaling in pain neuroplasticity**
Michael W. Salter, MD, PhD, Program in Neuroscience and Mental Health, Hospital for Sick Children, Toronto, Ontario, Canada and Department of Physiology, University of Toronto, Toronto, Ontario, Canada

2:30 – 2:55 p.m. **Role of the immune system in resolution of pain**
Annamieke Kavelaars, PhD, Laboratory of Neuroimmunology Department of Symptom Research, The University of Texas MD Anderson Cancer Center, Houston, TX

2:55 – 3:10 p.m. **Break and poster sessions**

3:10 – 3:35 p.m. **Bridge between neuroimmunity and traumatic brain injury; immunopharmacology approaches for diagnosis/treatment of neurodegenerative diseases**
Howard E. Gendelman, MD, Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE

3:35 – 4:00 p.m. **Specification and maturation of nociceptive neurons from human pluripotent stem cells**
Xue-Jun Li, PhD, Department of Biomedical Sciences
University of Illinois College of Medicine at Rockford, Rockford, IL, and Department of Bioengineering, University of Illinois at Chicago, Chicago, IL

4:00 – 4:25 p.m. **Pain begets pain: Towards a mechanistic understanding of chronic overlapping pain**
Richard J. Traub, PhD, Department of Neural and Pain Sciences
University of Maryland School of Dentistry, Baltimore, MD, and University of Maryland Center to Advance Chronic Pain Research, Baltimore, MD

4:25 – 4:50 p.m. **Comparing experimental pain sensitivity and endogenous pain modulatory processes in men and women**
Hailey W. Bulls, MA, Department of Psychology, University of Alabama at Birmingham, Birmingham, AL

4:50 – 5:20 p.m. **Session discussion and recommendations**

6:30 p.m. **Young investigators and senior scientists pairing dinner at the Marriott Bethesda**

Tuesday, September 13, 2016

8:15 – 8:30 a.m. **National Institutes of Health welcome and remarks**
Martha J. Somerman, DDS, PhD, Director, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD

Session 5: **How an Understanding of Molecular Mechanisms and Model Systems can Guide Precision Medicine in the Field of Chronic Pain**
Session Co-Chairs: Mark Hoon, PhD and Allen W. Cowley, Jr., PhD

8:30 – 8:55 a.m. **Epigenetic regulation of neuropathic pain**
Lingli Liang, PhD, Department of Anesthesiology, New Jersey Medical School Rutgers, State University of New Jersey, Newark, NJ

8:55 – 9:20 a.m. **Exploring the epigenetic mechanisms for individual pain vulnerability**
Zhizhong Z. Pan, PhD, Department of Pain Medicine, Division of Anesthesiology and Critical Care, The University of Texas MD Anderson Cancer Center, Houston, TX

9:20 – 9:45 a.m. **Peripheral nociceptive inputs**
Mark A. Hoon, PhD, Chief, Molecular Genetics Unit, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD

9:45 – 10:10 a.m. **Mechanisms underlying the sense of touch in orofacial regions of rodents – Implications for treating and preventing disease states that affect touch sensation, such as TMD**
Jianguo Gu, PhD, Department of Anesthesiology and Perioperative Medicine, School of Medicine, University of Alabama at Birmingham, Birmingham, AL

10:10 – 10:25 a.m. **Break and poster sessions**

10:25 – 10:50 a.m. **Exploiting the immune response to illuminate host microbiota interactions**
Noah W. Palm, PhD, Human and Translational Immunology Program Yale University School of Medicine, New Haven, CT

10:50 – 11:05 a.m. **Arc of research**
Terrie Cowley, President and Co-Founder, The TMJ Association, Milwaukee, WI
John W. Kusiak, PhD, Acting Deputy Director, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD
William Maixner, DDS, PhD, Center for Translational Pain Medicine, Department of Anesthesiology, Duke University School of Medicine, Durham, NC

11:05 – 11:30 a.m.	**The impact of genetic testing for pain perception in the clinical management of chronic non-cancer pain** Ashley Brenton, PhD, Associate Director, Research and Development Proove Biosciences, Inc., Irvine, CA
11:30 – 12:00 p.m.	**Session discussion and recommendations**
12:00 – 1:00 p.m.	**Lunch and poster sessions**
1:00 – 2:15 p.m.	**Consolidation of meeting recommendations and closing remarks** Session Co-Chairs: Allen W. Cowley, Jr., PhD and John W. Kusiak, PhD

Meeting Summary

by Joan Wilentz

Since 2000, The TMJ Association (TMJA) has held biennial science meetings to advance research and understanding of the complex conditions of pain and dysfunction of the jaw known as temporomandibular disorders (TMD). The meetings are co-sponsored by the Association with components of the National Institutes of Health (NIH), principally the National Institute of Dental and Craniofacial Research (NIDCR), along with funding from private sector donors. The eighth meeting, held September 11 to 13, 2016, at the headquarters of the Federation of American Societies for Experimental Biology (FASEB), in Bethesda Maryland, focused on an exciting new approach to disease treatment and prevention, asking in the title of the meeting, *How Can Precision Medicine Be Applied to Temporomandibular Disorders and Its Comorbidities?*

Out of the Black Hole

Terrie Cowley, Co-Founder and President of the TMJA, welcomed attendees noting that the organizers of the programs have always invited speakers outside the field of TMD research. Not only can this augment the pool of TMJ researchers, but the expertise they bring in their specialty is often directly applicable to TMD studies. Ms. Cowley described how TMD research has evolved since the first meeting, emerging out of a "black hole" where little was understood and treatments were hit or miss, to today's understanding of TMD as a complex family of conditions in which patients frequently report having other chronic pain conditions. She attributed much of the credit for these findings to the research initiatives promoted under the leadership of Lawrence Tabak, DDS, PhD, former Director of the NIDCR and current Principal Deputy Director of the National Institutes of Health.

Dr. Tabak remarked on the unique quality of the TMJA meetings. In addition to the diverse background of speakers, TMD patients are always in attendance, affording opportunities for formal presentations of their stories as well as informal interactions with scientists over the course of the meeting. He also alluded to the dismal state of TMD science when the meetings began and how this led to a plea to broaden the perspective, for example, exploring other chronic pain conditions, and engaging other scientists to cross-fertilize the field. In this way, TMD moved from a cottage industry to inclusion in mainstream biomedical research. A glance at the program of the 2016 meeting confirms that such status has been reached, he said.

In his current position, he said that he is often asked what it is like to interact with patients and patient advocates. "It is not without tension," he commented. But with Terrie leading the way, he, like many other scientists or administrators, has become much better informed and much better educated. Conversations with advocates can be conducted where both parties listen and learn.

Ms. Cowley then introduced her husband, Allen Cowley, Jr., PhD, a past president of the American Physiological Society, chair of the Department of Physiology at the Medical College of Wisconsin, and a distinguished research investigator in the study of hypertension. It was in 1982, following Terrie Cowley's jaw surgery, that the Cowleys discovered how little was known about TMD and how much needed to be done. Dr. Cowley chairs the Association's Scientific Advisory Board and has led the program committee for each of the past scientific meetings.

Dr. Cowley remarked that an important characteristic of the Association's meetings was the mix of senior and young investigators who could submit posters eligible for an award. He saw the meeting as an opportunity for all to engage in a promising area of research which might be new to many and he encouraged lively discussions. He then introduced Patricia Grady, PhD, RN, Director of the National Institute of Nursing Research (NINR), one of the four institutes heading the NIH Pain Consortium.

"The goal of NINR is to relieve symptoms and improve the quality of life of patients," Dr. Grady said. She added her thanks to Ms. Cowley, acknowledging her contributions to such groups as the Interagency Pain Research Coordinating Committee and the Trans-NIH Chronic Overlapping Pain Conditions Working Group. "NINR is a patient-centered institute, with research directed at the relief of symptoms, prominently pain, fatigue and mobility problems," she said. That means exploring the genetic, epigenetic, neuroimaging and other findings associated with symptoms and how they relate—or not—to the patient's own report. Rather than focus on a single symptom, she emphasized the need to explore clusters of symptoms that form a part of the complex whole of the patient's experience. The goal is to come up with the best ways to treat diverse populations across diverse settings.

Dr. Cowley next outlined the agenda for the meeting, reminding attendees that at the end they would be asked to formulate research recommendations to forward to the NIH and other agencies to further progress. He noted that the eighth meeting's focus on the new high-profile area of precision medicine is testament to the progress that TMJ science had made over the decade. Dr. Cowley then quoted from an editorial appearing in the *Journal of Dental Research* by Ronald Dubner, DDS, PhD, a member of TMJA's Scientific Advisory Board:

> *TMD is now considered a multi-dimensional biopsychosocial disorder that has a genetic and immunologic basis that shares common features, not only with other musculoskeletal conditions, such as fibromyalgia, low back pain, and headache, but also with idiopathic inflammatory conditions, such as joint pain, irritable bowel syndrome, vulvodynia, and other somatic and visceral deep tissue injuries.*

That characterization of TMD qualifies it as a prime candidate for applications of

precision medicine, as defined by the National Research Council:

> *Precision medicine refers to the tailoring of medical treatment to the individual characteristics of each patient. It does not literally mean the creation of drugs or medical devices that are unique to a patient, but rather the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease, in the biology and/or prognosis of those diseases that may develop, or in their response to a specific treatment. Preventive or therapeutic interventions can then be concentrated on those who will benefit, sparing the expense and side effects for those who will not.*

The U.S. Precision Medicine Initiative

The first presentation was by Eric Greene, MD, PhD, Director of the National Human Genome Research Institute at NIH, who provided the context and history for what is now the U.S. Precision Medicine Initiative (PMI), a national program announced by former President Obama in his 2015 State of the Union speech. Prior to that, the president had assembled leading scientists in his office seeking their ideas for a bold new initiative the government could undertake comparable to the mapping and sequencing of the human genome 25 years ago.

Enormous strides in genomics have been made since then, Dr. Greene observed. While the initial genome sequencing was costly and tedious, technology has cut the cost a million-fold so that tens of thousands of human and animal genomes have now been sequenced. These data are enabling a better understanding of how genes function, what differences in sequences mean, and at least for some rare diseases, disclosed the genetic defects with the potential for reversing them. The key observation that has emerged is that, while there is a genetic component in most human disease, it is rarely the whole story. Behavior, diet, lifestyle, the environment, and other factors contribute to individual variability in the risk for, or resistance to, disease.

The difference today is that we are now not only able to technologically collect genome data on an individual economically and rapidly, but we can also determine in detail the contribution of non-genetic factors in the development and course of disease. For example, there are now wearable monitors to track human physiology (such as movement, heart rate and blood pressure) along with other sensors to record health data. These, combined with electronic health records and genomic information, create a wealth of personalized information.

The intent of the national initiative is to collect this mine of information, not for a few score of Americans, but for at least a million people representing the diversity of the population (age, sex, ethnicity, socioeconomic status, etc.), and do this over decades. The program will enlist volunteers who will actively contribute their ideas and insights into the process,

creating partnerships in research. Needless to say, the result will be masses of information—so-called "Big Data" databases—enabling extensive analyses. The process and the product are described in greater detail on the program's website, www.NIH.gov/precisionmedicine and explains how the data will be applied to precision medicine.

Stratifying Pain Patients

How TMD research has been progressing toward a precision medicine approach was explained by William Maixner, DDS, PhD, Duke University School of Medicine, Durham, North Carolina. Dr. Maixner has been the principal investigator directing OPPERA (Orofacial Pain: Prospective Evaluation and Risk Assessment), a multi-center study of an initial TMD-free group of 3,200 men and women, who have given genetic and biological samples, as well as submitted to various tests and periodic questionnaires. Over a period averaging 5 years, with quarterly follow-ups, 260 of these individuals developed TMD. Using a technique of cluster analysis, the OPPERA investigators were able to define three distinct subpopulations or clusters of the new TMD patients. Cluster 1, which they call the "adaptive" cluster, consisted of patients, more male than female, with relatively mild TMD symptoms and little psychological stress. Cluster 2, with equal numbers of men and women, had more severe symptoms and more psychological stress. Cluster 3 were patients with the most severe and extensive symptoms, of longest duration, with a greater number of psychological indicators, often accompanied by widespread pain or overlapping pain conditions.

The challenge will be to elucidate the molecular pathways that distinguish each of these clusters in order to develop appropriate precision medicine (PM) treatments. Already the investigators have been able to reduce the initial 202 different data elements that had been measured for each volunteer to four key indicators that enable them to assign patients to specific clusters. The next step is to operationalize the findings, and Dr. Maixner described plans underway for a new Integrated Pain Therapy Unit being established at Duke University, as well as an International Data Collection System, which will involve collaboration and data sharing with investigators in Canada, Singapore, China and other international sites.

A similar desire to determine factors that could predict which patients are apt to progress to more severe and chronic pain, compared to those who recover from an acute condition, has governed the research of Apkar Vania Apkarian, PhD, Northwestern University, Chicago, Illinois. In what he described as the first longitudinal brain imaging study of chronic pain, his team scanned the brains of several hundred patients at the outset of back pain (the acute stage) and followed them with repeated scans over several years. Patients either recovered or else developed chronic back pain.

Initially, the brain scans of all patients were alike, showing typical pain circuitry activity. A year later, the recovered patients' scans had returned to normal, with no evidence of pain activity. In contrast, the scans of the chronic pain patients showed a robust reorganization within the central nervous system evident in the limbic area, which is associated with emotional states, rewards and addiction, and in turn, limbic connections to the cortex. It is as though some individuals have a predisposition to, or "an addiction" to, pain. Dr. Apkarian said that following an acute injury their brains are primed to a reorganization of the emotional areas and the establishment of novel connections to the cortex, which is maintained over time. What's more, when the researchers explored the structural and functional organization of the limbic areas in the brains of recovered vs. chronic pain patients, they found intrinsic differences that would allow them to cluster patients according to risk for chronic pain. For example, one difference they found was a reduction in the sizes of the amygdala and hippocampus in the brains of the chronic pain patients compared to their counterparts in the brains of those who recovered.

New Genetic Factors

Luda Diatchenko, MD, PhD, at The Alan Edwards Centre for Research on Pain, Faculty of Dentistry, McGill University, Montreal, Quebec, Canada, is one of the leading geneticists studying TMD as part of the long-running OPPERA project. She has used Genome-Wide Association Studies (GWAS) comparing the genomes of TMD patients with normal controls. These studies make use of the fact that genomes differ in many ways, including at single sites in the DNA sequence where one or another of the four nucleotides constituting the genetic code, abbreviated A, C, G, and T, is substituted for another. These sites are called "single nucleotide polymorphisms" (SNPs, pronounced snips), and there are millions of them in the human genome.

In Dr. Diatchenko's GWA studies, she was able to identify a number of SNPs that were more common in TMD patients than in controls, suggesting their role as biomarkers of risk for TMD. As it happens, it appears that SNPs that are associated with risk for a specific trait, in this case the trait is having TMD, are often located in or near a disease-related gene or involved in its regulation in tissues where the gene is expressed. Such genes are described as "expression Quantitative Trait Loci," eQTLs, where "loci" refers to the gene's location in the genome (on which chromosome, etc.), and the other terms are measures of how richly (quantitatively) the gene is being expressed in the tissue of interest. "Expression" itself is measured by the amount of ribonucleic acid (RNA) expressed in the cell, which is often (although not necessarily) indicative of the amount of protein that is being transcribed by that gene.

The "tissues of interest" that Dr. Diatchenko examined were dorsal root ganglions. These are the nerve cell bodies of sensory neurons that include pain neurons (nociceptors) that lie in clusters adjacent to the brain and spinal cord. The cells send branches to the periphery of the body (such as the jaw) to respond to sensory stimuli, including pain, and transmit signals via the axons that extend from the cell bodies into the brain or spinal cord. Using databases that catalogue which genes are expressed in specific tissues, along with sophisticated analytic techniques, Dr. Diatchenko has been able to identify three new SNP-associated eQTLs involved in TMD pathology. Interestingly, the newly discovered genes establish a connection between TMD pathology and the immune system since they include genes that activate classes of immune cells. These studies have thereby revealed an important genetic relationship between the immune system and TMD pain.

Individualized "N-of-1" Trials

The emphasis on the uniqueness of the individual and the goal of PM to capitalize on those distinctive qualities to develop more personalized diagnostics and treatments was one of the major themes of the TMJA meeting. The presentation of Christopher Schmid, PhD, Brown University School of Public Health, Providence, Rhode Island, on N-of-1 trials was a case in point. Typically, in a clinical trial to evaluate a new drug or diagnostic aid, the investigator enrolls enough people (say n=100 or 100 participants) to obtain statistically meaningful results at the end of the trial, stated in terms of averages. ("75 percent reported improved pain scores"). But "averages" can conceal findings that some patients were made worse by the experimental treatment, while others scored better than average.

In an N-of-1 trial, a single individual is the subject and tries different treatments for a specific disorder switching from one treatment for a fixed length of time to another. Each switch is called a crossover, and the trial may consist of half a dozen or more crossovers of different treatments or a placebo, tested in random order, with results recorded at the end of each treatment. For this to work, the treatments must be relatively fast-acting and not linger on to interfere with the next treatment. To eliminate this problem, a washout period will need to be added before the next treatment starts. Also, the disorder being studied (say back pain) must revert to the baseline condition to ensure comparability of results. Dr. Schmid went on to discuss ways to analyze and combine results of multiple N-of-1 trials and described several trials underway.

An important advantage of N-of-1 trials is the active engagement of the patient in deciding what treatments to test and which outcome measures are judged to be most important. This "patient-centric" approach is invaluable in itself as a means of promoting scientific literacy, improving patient-provider communication, and

enabling patients to make decisions on what treatments to adopt, which could assure greater compliance. Another advantage is that these trials do not require access to a major medical center, but can be conducted in a community setting, out of a doctor's office, and can be facilitated in some cases by using a smart phone app for recording outcome measures.

Another approach to enhance information gathering and analysis for PM use was described by Yelizaveta Torosyan, MD, PhD, from the Centers for Devices and Radiological Health in the Food and Drug Administration (FDA), the section that evaluates and regulates products used in *arthroplasty*—joint replacements. Her talk highlighted the potential for *in silico* methodology to enhance understanding of the complexity of TMD and allow stratification of patients into subgroups, particularly in relation to the benefit or risk of joint implant surgery. The term *in silico* refers to the use of computer modeling to simulate and visualize disease processes. Because of the advances in "big data" analysis, where genetic and other data from large numbers of individuals can be amassed and analyzed, *in silico* approaches can yield information on biomarkers for diagnosis, treatment and prevention applicable to different patient subgroups. Dr. Torosyan also described a new public-private initiative called MDEpiNet, which brings together patients, providers, device manufacturers, FDA staff and researchers to exchange information and share data.

Traditional clinical trials remain a vital step in the process of moving promising discoveries in the lab or tested in animal models to determine whether the discovery is safe and effective in human patients. However, clinical trials require recruitment of patients and lengthy protocols subject to review by institutional review boards. They are costly, may last for years, and in the end, may prove to be inconclusive. Jamie Roberts, MPH, MA, CCRP, Duke Clinical Research Institute, Durham, North Carolina, said that the average length of time between the lab and the marketplace for a new product is 12 years. To improve the process and shrink the time it takes, she described the Clinical Trials Transformation Initiative, to improve the quality and efficiency of clinical trials. "The barriers often begin with the protocol itself," she said. It is not always feasible and often requires amendments, which add both time and expense to the trial. Another major barrier is enrollment of volunteers, who may not understand exactly what they are being asked to do and the value that may come from their participation. The answer, of course, is better communication of the science along with the realization that the trial participant should be a well-informed partner in a scientific enterprise whose input needs to be heard—in short, a change in thinking from a concentration on the drug or device and its market potential to a patient-centered approach. Toward that end, Ms. Roberts emphasized the important role that patient advocacy

organizations can play, not only as a source of volunteers, but in actively participating in shaping the protocol at the outset and seeing to it that the input and ideas from trial participants are respected in designing the way the trial is conducted and deciding what outcomes should be measured.

The three presentations that ended the opening day of the meeting were a further object lesson in why a patient-centric orientation with better patient-provider communication is critical. Michele Kaseta is a TMD patient who said that the dentist she first saw diagnosed TMD and advised invasive treatment with a jaw implant. That surgery led to recurrent problems and repeated surgeries over many years at an immense financial burden, while doing nothing to relieve the overlapping condition of migraine headaches that she had also reported, affecting her and other members of her family. Danica Marinac-Dabic, MD, PhD, Director of the Division of Epidemiology in the Office of Surveillance and Biometrics of the Center for Devices and Radiological Health at the FDA, emphasized the Center's current actions to expand its patient registries to enrich what can be a valuable database annotating patients' experiences with implants. She added that it is the intent of the Center to engage all its personnel in one-on-one meetings with patients over the coming year. Christin Veasley, Director and Co-founder of the Chronic Pain Research Alliance, described how patient advocacy organizations were instrumental in promoting research that demonstrated that many chronic pain patients experience more than one pain condition, for example, TMD and irritable bowel syndrome. She showed data from a white paper on chronic pain in women, which charted the increasing amount of research on overlapping pain conditions and the shift of the focus on the source of pain from the end organ or organs where symptoms are experienced to the central nervous system. As this is occurring, the drive to expand data collection to reflect genetic, environmental and lifestyle information will inform precision medicine initiatives and lead to better diagnostic, treatment, and prevention measures.

On the second day of the meeting, Martin Frank, PhD, Executive Director of the American Physiological Society (APS), welcomed attendees on behalf of the Federation of American Societies for Experimental Biology (FASEB), whose tree-lined campus in Bethesda, Maryland, has been the venue for seven of the eight TMJA scientific meetings. "The APS was the founding member of FASEB in 1887," he said. The organization has now grown to represent 30 scientific societies and 125,000 scientists, a collective and forceful voice advocating for government support of research.

Following Dr. Frank, Stephen Katz, MD, PhD, Director of the National Institute of Arthritis, Musculoskeletal and Skin Diseases (NIAMS), one of the NIH

co-sponsors of the meeting, described meeting Terrie Cowley shortly after he became Director, at a time when the horrific effects of failed jaw implant surgeries were in the news. He complimented the Cowleys for all they have done to advance research and educate scientists as well as the public. He mentioned several areas where NIAMS supports research relevant to TMD and precision medicine, mentioning comorbidities, studies of pain in conditions such as fibromyalgia, which often overlaps with TMD, and patient-centered outcomes initiatives.

New Targets for Pain Drugs on Signaling Pathways

When you feel pain, it is because nociceptors are activated and send electrical signals into the central nervous system. There, they are routed along pathways that terminate in higher centers in the brain. Some centers provide information on where you hurt and how bad it is. Other centers interpret your feelings about what has happened. Are you angry or annoyed because the hammer hit your thumb rather than the nail? Or, are you fearful or anxious because the pain might be a sign of serious illness like heart disease or cancer?

But the signal a nociceptor sends doesn't work like a simple switch turned on to send its bad news message up to the brain. The strength of the signal, how long it lasts, and even whether it gets sent at all, are among features that reflect the activities of a large cast of other cells and molecules that can manipulate neuronal signaling, sometimes turning up the volume, sometimes quieting it. Included in that cast are cells and molecules of the immune system that can infiltrate the nervous system, as well as non-neuronal cells that are part of the nervous system itself. There are also descending pain pathways from neurons in the brain that can suppress the incoming signals.

All these manipulations get further complicated in the case of chronic pain, in which the normal pain apparatus and circuitry are dysregulated so that the nervous system itself behaves abnormally.

A number of speakers addressed these issues at the TMJA meeting, each focusing on specific cells and/or molecules that figure in "signaling pathways," the term that describes the cascade of biochemical events that occur in the course of transmission of pain messages.

Cdk5. James Bibb, PhD, Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, Texas, described one such molecule, a very versatile enzyme (a protein) called Cyclin-dependent kinase 5, Cdk5. He reminded the audience that signaling pathways depend on the ability of neurons to generate an electrical signal able to cross the synapse, the junction between nerve cells, to stimulate the next neuron in the pathway. "At birth, there are some 3,000

synapses in a defined microscopic volume of cortical brain tissue," he said. That number grows to 10,000 at age 3, a time when the first memories form and then streamlines to 7,000 synapses in adulthood.

One function of Cdk5 is to form complexes with other proteins that result in changes in the nerve cell membrane that allow the nerve signal to be generated across the synapse. Cdk5 also regulates dopamine transmission—the neurotransmitter associated with reward. It is involved in responses to caffeine; it also plays a role in brain development and cell division. Importantly, it also mediates sensitivity to pain.

Indeed, Dr. Bibb's studies showed that animals lacking Cdk5, because the gene coding for its production had been deleted or "knocked out" of the genome, had distinctly positive effects. Mice were better, faster learners, more curious and resilient, suggesting that a drug that could inhibit Cdk5 might benefit patients with Alzheimer's disease. Other studies suggested that the use of a drug to inhibit Cdk5 might work as an antidepressant. The elimination of Cdk5 in animal studies also helped maintain the viability of brain cells, limiting the number of cells dying as a result of a stroke or traumatic injury. Finally, he reported that Cdk5 has been implicated in neuroendocrine cancers, again suggesting that its elimination might be beneficial. The role of Cdk5 in the development or maintenance of TMD pain is ripe for study.

Glia and Neuroinflammation. Ru-Rong Ji, PhD, Department of Anesthesiology and Neurobiology, Duke University School of Medicine, Durham, North Carolina, described the normally protective role of inflammation in response to an acute injury or infection. The hallmarks of inflammation—swelling, redness, heat and pain—alert the body to harm and the need to guard the affected area, while at the same time reflecting the recruitment of immune and other cells to the site of injury to defend the body and begin the healing process. In the case of acute nerve injury, the process is called *neuroinflammation*.

Many cases of TMD involve neuroinflammation which, if not resolved, can transition to a state of chronic pain with continued infiltration of immune cells into the affected area, along with activation of nearby "glial" cells, the name for a variety of multi-functional non-neuronal cells found throughout the nervous system. Glial cells provide structural support and nutrients to surrounding neurons and appear to possess other novel functions, as proposed at the meeting.

Chemokines. Dr. Ji described the inflammatory immune and glial cells as being able to "talk to and listen to" nociceptors, secreting a variety of signaling molecules that can either amplify or dampen the nociceptors' behavior. In chronic pain, the system is dysregulated, resulting in continued mobilization of immune and glial cells to

amplify pain. Rather than try to resolve the chronic pain by eliminating the excess immune or glial cells, Dr. Ji proposed targeting specific "chemokines." These are small protein molecules that are secreted to attract and guide immune and other cells to the site of injury. He would also target certain proteases, enzymes that promote neuron-glial interactions and neuroinflammation.

Interestingly, he found a variety of other chemokines that had a positive effect. It appears that a chemokine known as CXCL12 attracts a population of stem cells[1] found in bone marrow. In experiments in animals, it was demonstrated that when transplanted, these "bone-derived stem cells" (BMSCs) selectively targeted injured lumbar spinal dorsal root ganglions. The ganglion neurons themselves actually produce CXCL12, which induces BMSCs that express a receptor for CXCL12 on their membranes to move to the ganglions where they secrete a molecule that is very effective in reducing neuroinflammation and pain at the injured site. CXCL12 was found to be necessary for the homing of BMSCs to the injured ganglions.

Microglia. Further evidence that glial cells can contribute to pain was described by Michael Salter, MD, PhD, Hospital for Sick Children and Department of Physiology, University of Toronto, Ontario, Canada. He has studied microglia, a population of glial cells abundant in the brain and spinal cord, in a mouse model of peripheral nerve injury. Microglia are the nervous system equivalents of macrophages (literally, big eaters), a class of immune cells that seeks out, engulfs, and devours infectious agents and other unwanted material in the body.

In the case of nerve injury, microglia can ***cause*** neuropathic pain. This happens because the nerve injury activates a signaling pathway that results in the release of **"brain-derived neurotrophic factor" (BDNF)** from microglial cells. BDNF in turn activates receptors on spinal cord nociceptors that transmit pain signals to the brain by turning up their response rather than inhibiting the signals. Curiously, Dr. Salter discovered that this mechanism of exacerbating pain works in male mice and not in females. Indeed, microglia in female mice show none of the activation apparent in male microglia. Yet female mice are no less susceptible to nerve-injury-induced pain hypersensitivity. In that case, the culprits appear to be a species of T cells of the immune system.[2]

1. Stem cells are primitive cells that, given appropriate stimulation, can develop into a variety of cell types. "Embryonic" stem cells are the source for the development of all cell types in the body as the fertilized human egg develops. There is a ban on federally funded research using human embryonic stem cells because they would have to be extracted from human embryos, which would be destroyed in the process. Other stem cells are found in various organs of the body where they serve as resources for selected cell types. New research (described later) has now obviated the need to use embryonic tissue.
2. The immune system consists of cells and molecules engaged in the defense of the body. T cells are one segment of that population (called T cells because they originate in the thymus gland). They generally have cell surface receptors that distinguish them into subsets such as helper cells, killer cells, effector and regulatory cells.

Chronic inflammation as a source of pain due to immune system stimulation turned up again in a presentation on a seemingly unrelated subject, the microbiome—the population of microorganisms that live in and on us. That population is unique to each individual and is modified by such factors as genetics, diet and medications. The sum total of these organisms vastly outnumbers the number of cells in the human body, with a complement of genes that, in sum, likewise outnumbers the human genome. Noah Palm, PhD, an investigator at the Human and Translational Immunology Program, Yale University School of Medicine, New Haven, Connecticut, has been studying the gut microbiome in relation to inflammatory bowel disease, IBD[3], a chronic pain condition with bowel symptoms. Gut bacteria represent at least 150 bacterial species and hundreds of strains and several species have already been implicated in a number of gastrointestinal disorders.

Most gut bacteria are mutually beneficial "symbionts," or at least do no harm. But there are some among them that, on occasion, may upset the balance, or as Dr. Palm put it, "move the gut from a state of symbiosis to dysbiosis." Using samples of gut bacteria from IBD patients and the latest gene sequencing technology, he was able to profile the species of bacteria in each patient with the hope that there might be some common species that stood out as possible causes. But no pattern showed up, indicating that each patient has a different mix of bacteria and that many combinations of these bacteria could lead to disease. Rather than trying to describe the many combinations of such harmful bacteria, Dr. Palm turned to the immune system, which he was able to use as a tool to identify the responsible culprits. It turns out that, when the immune system recognizes an enemy bug in the gut of nearly any type, it secretes unique antibodies of a class called immunoglobulin A, IgA, into the open space of the intestine or colon to capture and coat the unwanted bugs for removal. Dr. Palm first cultured the species of bacteria found in each patient's sample and then ran them through a "pipeline" of mucosal antibodies. That resulted in subsets of bacterial species for each IBD patient that were coated by IgA antibodies. The next step was to take a mix of the bacteria belonging to the coated species, putative enemy bugs, and transplant them into germ-free mice and compare them with a mix of unmarked species from IBD patients also transplanted into germ-free mice.

The results were striking. All the germ-free mice with the species that had been IgA marked developed severe colitis, and all the mice with the unmarked bacteria transplants stayed heathy. How this will play out in terms of treatment and precision medicine is not clear. However, Dr. Palm suspects that probably it will not mean that each IBD patient will need a totally personalized approach to combat the particular set of enemy bugs found,

3. IBD includes ulcerative colitis and Crohn's disease and is not the same as Irritable Bowel Syndrome, a chronic pain disorder with similar bowel symptoms that overlaps with TMD.

but there may be some commonalities that could lead to treatment, perhaps vaccination, for a wider group of patients. In any case, he has concluded that at least for IBD patients, the gut is exposed to the "constant tickling of the immune system by specific bacterial species," probably because they are able to penetrate normally sterile parts of the gut, and that this is at the root of their chronic pain and IBD symptoms.

Good and Bad Immune Cells?
One might think that the immune system's role in chronic pain, as revealed in the studies of Drs. Bibb, Ji, Salter, and Palm, is all bad. But that view got turned on its head in the presentation of Annemieke Kavelaars, PhD, from the Laboratory of Neuroimmunology of the University of Texas MD Anderson Cancer Center in Houston, Texas. She has been studying animal models of "chemobrain," the devastating fatigue, neuropathy and brain deficits affecting cognition, memory and other higher mental faculties in human cancer patients treated with potent anti-cancer drugs. In her lab, mice exposed to the cancer drug paclitaxel develop the mouse version of chemobrain, which includes peripheral neuropathy and inflammatory pain.

She has found that certain types of T cells can be protective and work to reduce chemotherapy-induced pain and inflammation via the production and release of interleukin 10 (IL-10). There are many types of T cells in the immune system classified according to their surface receptors as CD 3+T cells, CD 4+T cells, CD 8+T cells and others. Dr. Kavelaars demonstrated the positive effects of the T cells by noting that both male and female mice, whose genes for these T cells had been knocked out, suffered prolonged inflammatory pain and mechanical allodynia (a light touch experienced as pain) in comparison with normal or "wild type" mice. When CD 3+ or CD 8+ specifically, but not CD 4+ T cells were transferred to the gene-deficient mice, they resolved their chemically induced pain in times comparable to the normal mice. Dr. Kavelaars also noted that the number of CD 8+ cells in dorsal root ganglions increased in her chemotherapy model. The positive effects of IL-10 were similarly shown by subjecting mice to an anti-IL-10 treatment and finding that their inflammatory pain was prolonged. Conversely, a normal resolution time could be restored by injecting IL-10 into the spinal cord.

Dr. Kavelaars hypothesizes that, when nerve injury (as from chemotherapy) results in inflammatory pain and neuropathy, there needs to be an active regulatory process involving "endogenous resolution pathways." If these processes are disrupted, the acute stage of inflammation and neuropathy can transition to chronic pain.

Chemobrain Repair. Cobi Heijnen, PhD, from the same Laboratory of Neuroimmunology at The University of Texas MD Anderson Cancer Center in

Houston, Texas, as Dr. Kavelaars, has also been studying chemobrain in mice treated with the powerful cancer drug, cisplatin. Her research shows promise for a treatment and a way of applying that treatment that has worked dramatically to repair the damage. The remedy is the intranasal infusion into the brain of "mesenchymal stem cells," MSCs. These are a type of stem cell found in bone marrow that are normally the source of selected connective tissue cells.

Dr. Heijnen's cisplatin-treated mice showed failings in standard mouse learning and memory tasks compared with control mice who were infused with saline. They also suffered hyperalgesia (excessive pain), peripheral neuropathy and showed signs of anxiety and depression. Images of the chemo mouse brain showed losses in nerve connectivity and disorganized white matter. When the experimental animals were treated with MSCs, however, they showed remarkable recoveries, evidenced by the behavioral tests. Microscopic studies of mouse brains affected by cisplatin and later supplied with MSCs showed new growth of neural elements and other signs of repair and restoration. The infused MSC cells do not survive for long in the brain, so Dr. Heijnen believes that in some way they transfer information that enable neurons to repair themselves. There is also evidence that MSCs may be the source of new mitochondria to replace those damaged in brain cells. Mitochondria are called the powerhouses of cells, supplying the energy needed for normal operations. Remarkably, the MSC treatment not only restored brain function in the treated mice but also relieved their peripheral hyperalgesia and neuropathy. MSCs are easily obtainable and, since they do not take up residence in the brain where they might present other problems, offer an attractive remedy to an all too frequent aftermath of cancer treatment.

The possibility of repair of the damage that might be wrought by "bad" immune cells also figured in the research of another speaker at the TMJA meeting, whose field of research concerns neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. Howard Gendelman, MD, Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, Nebraska, argues that a type of infectious agent released by the injured nervous system triggers a destructive immune system response. He provided experimental evidence that in neurodegenerative diseases, such as Parkinson's disease and post-traumatic brain syndromes, there are increased numbers of neurodestructive (T-effector cells) and dysfunction of anti-inflammatory (T-regulatory cells) leading to neurodegeneration.

In studies carried out in mice, the neuroinflammation and neurodegeneration of Parkinson's disease was attenuated by treatment with T cells obtained from normal donor mice or by inducing normal T-regulatory cell production chemically. He proposes that such

immunopharmacological strategies could lead to new opportunities to curtail many devastating nervous system disorders, including chronic pain.

Organs on a Chip and iPSCs

Ingenious inventions in recent years have led to new microscopic devices that simulate how whole organs of the body behave in health and in disease. They are called "organs-on-a-chip." Some of these devices can, in the interest of precision medicine, incorporate cells that are derived from individual patients with a disease of interest. The patient's cells are replicated by using the patient's own stem cells as a source, which have been stimulated to develop into the type of cells found in the organ affected by the disease. This is now possible as a result of Nobel Prize-winning research in 2006, which showed that a mature human cell in the body can be reprogrammed to a more primitive stage to become a stem cell that can develop into all cell types. These new types of stem cells are called "induced Pluripotent Stem Cells" (iPSCs). Speakers at the TMJA meeting had much to say about how these advances are being used today and how, when applied to studies of TMD, they could greatly facilitate development of precision medicine methods of TMD diagnosis, treatment, and prevention.

John Wikswo, PhD, Vanderbilt Institute for Integrative Biosystems Research and Education, Vanderbilt University, Nashville, Tennessee, described the basic plan of an organ-on-a-chip, using the word "chip" to convey the microscopic size of the device. The "organ" consists of several narrow channels which permit the delivery of fluids and drugs to the cells on the chip. One model of a human lung on a chip, for example, consists of a channel of lung cells from the small airways of the lung on one side of a porous membrane (the middle channel) with capillary blood vessel cells on the other side. Mechanical forces applied to the membrane can stretch and contract it to simulate breathing.

Important to the field of pain and brain research, Dr. Wikso's team has also created a chip containing cells equivalent to those that make up the blood-brain barrier, a fine network of cells lining the brain which blocks passage into the brain of any elements in the blood supply too large to make their way through the barrier to reach neurons on the other side of the barrier.

He said that one of the current challenges in organ chip research is to couple together one or more organs to create a "homunculus" (a miniature human being) to study the interaction of organs, using as an example the case in which one organ metabolizes drugs and, in the process, creates by-products toxic to other organs. A personalized homunculus can also be created using an individual's own iPSCs. Dr. Wikso doubted that organs-on-a-chip could exactly replicate the TM joint, which is made up of several different cell types, but saw their potential, for example in the blood-brain

barrier chip studies, as a way to determine individual differences in how effectively (or not) pain drugs are able to enter and exit the brain.

The use of iPSCs as a means of modeling complex diseases was discussed by Ulrich Broeckel, MD, Department of Pediatrics, Medicine and Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin. In a study of heart failure in subjects with high blood pressure (hypertension) for example, he described creating 250 iPS cell lines of cardiomyocytes (heart muscle cells) from an epidemiological study of African American and Caucasian hypertension patients—enough of a sample to demonstrate the diversity of the cell lines generated for this group. Then, using genetic and other data that had been obtained from the patients, the researchers conducted genome-wide association studies (GWAS) to relate single nucleotide polymorphisms (SNPs) and genomic loci (eQTLs) that contribute to variation in the expression levels of genes. In this way, they were able to identify SNP-eQTL related genes and use that data to guide hypertension drug testing, develop biomarkers predicting drug effects, and facilitate toxicology testing. The potential for using iPSC lines to regenerate disease-damaged or destroyed cells related to chronic pain and TMD dysfunction could be on the horizon.

Males vs. Females in Pain

Time and time again in the course of the meeting, speakers noted sex differences in pain responses or in underlying nervous or immune system mechanisms involved in pain perception and control. Further highlighting the importance of such differences was Lisa Begg, DrPH, RN, Research Program Officer, Office of Research on Women's Health at the NIH, an office that has supported all of the TMJA's scientific meetings. Dr. Begg described the Office as one that works collaboratively and cooperatively on trans-federal initiatives, especially emphasizing the need for the inclusion of patients in these activities. The Office works across the board with all components of the private and public sectors, from scientists and their societies to industry, the media, the general public and non-profits like the TMJA and other advocacy groups. She was pleased to report that, beginning in 2016, investigators submitting research grant proposals to the NIH must now incorporate sex as a biological variable to be evaluated in their pre-clinical and clinical research studies. If that is not included, strong justification as to why not must be provided. She summed up this approach in terms of "the four C's": Consider (design appropriate studies), Collect (the data), Characterize (analyze the data) and Communicate (publish the findings).

Modeling COPCs—A Woman's Issue

Animal models for complex diseases are challenging and will always fall short of the mark, given that no non-human species can truly simulate the human animal. Nevertheless—and clearly from

the research reported at the meeting—*rodents* may be the pain researchers' best friends. Also, since the investigators tend to use the same kinds of tests to measure changes in behavior in rodents following a painful event, their results gain strength by allowing comparisons of findings.

One speaker at the TMJ meeting, Richard Traub, PhD, Department of Neural and Pain Sciences, University of Maryland School of Dentistry, Baltimore, and University of Maryland Center to Advance Chronic Pain Research, Baltimore, Maryland, has risen to the animal model challenge. In a talk called *Pain Begets Pain*, he not only described a rat model of chronic pain, but one that models two chronic pain conditions occurring in the same individual. These two conditions are part of the group of disorders, the so-called "chronic overlapping pain conditions" (COPCs) that occur in chronic pain patients at frequencies higher than chance would predict:

> *chronic fatigue syndrome, chronic low back pain, chronic tension-type headache, chronic migraine, endometriosis, fibromyalgia, interstitial cystitis, irritable bowel syndrome, temporomandibular disorders, and vulvodynia.*

These conditions predominantly or exclusively occur in women in the childbearing years. Stress generally exacerbates the pain, which is often accompanied by depression or anxiety.

These findings have been instrumental in producing a paradigm shift in thinking, away from looking at the symptoms and signs at the end organ where pain is felt, e.g., the jaw, the bowel or the joints, and toward "shared altered neural, immune and endocrine mechanisms, leading to dysregulation of the normal central nervous system [and] driving pain hypersensitivity."

Dr. Traub's rat model attempts to replicate the co-existence of TMD and one of its more common overlapping conditions—irritable bowel syndrome (IBS). To simulate TMD, he used either an injection of the inflammatory irritant Complete Freund's Adjuvant (CFA) into the jaw of male and female rats, or a chronic constriction injury by tying off the infraorbital nerve to the masseter muscle. These reliably generated jaw pain, which was generally greater in female rats than in males. The rats were then subjected to the stress of a repeated forced swim test. This is a well-known measure of anxiety and/or depression in rodents, based on how soon the test animal gives up swimming and just treads water when placed in a tank with no dry landing spots to escape to. Animals in pain give up sooner than healthy animals.

What Dr. Traub found was that the combination of jaw pain and the forced swim stress led to chronic visceral hypersensitivity, simulating IBS. This is measured by noting how soon and to what degree abdominal muscles contract, producing cramps (called the

visceromotor response, or VMR) in response to the insertion of a balloon attached to tubing into the anus and gradually inflated. Non-jaw-injured (healthy) animals stressed by the swim test normally react with a transient visceral hypersensitivity that lasts for a few days. But the jaw pain and stressed animals showed a prolonged visceral hypersensitivity lasting for weeks to months, simulating chronic visceral hypersensitivity. In addition, these animals demonstrated referred pain, experiencing pain from simply touching the skin area over the colorectal area.

In variations of his test model, Dr. Traub showed that the order of events was important. If the jaw injury occurred *after* the forced swim, it did not generate chronic visceral hypersensitivity. Responses of female rats were always greater than in male rats and were estrogen dependent, showing the greatest VMR responses at the time in the rat estrus cycle when estrogen levels are highest. In contrast, female rats whose ovaries were removed showed decreased sensitivity.

Dr. Traub and his team have been using their COPC model to explore central mechanisms that may be contributing to chronic comorbid pain and what therapies might counter them. While their model is not a perfect match for the human situation, they have been able to show that their results distinguish between pure stress-induced pain and their comorbid model. In particular, they note that stress itself can stimulate the release of hormones from the adrenal gland through stimulation of the hypothalamic-pituitary-adrenal (HPA) pathway. These hormones can aid survival, but if stress is prolonged they can contribute to pain. However, the investigators believe their comorbid model establishes other mechanisms besides stress at work. In particular, they have used brain scans and gene expression studies showing more intense activation of pain centers and pathways, resulting in "central sensitization," the term used to describe the dysfunction of the central nervous system that leads to pain hypersensitivity.

Fundamental Sex Differences

Following Dr. Traub's presentation, Hailey Bulls, MA, Department of Psychology, University of Alabama at Birmingham, Alabama, enlarged on the theme of sex differences in pain experience not in rats, but in humans. She reviewed epidemiological data and experimental tests that point to greater prevalence of chronic pain in women and their greater intrinsic pain sensitivity. But first she alluded to the 2011 Institute of Medicine report, calling attention to the high costs of chronic pain in America (from 560 to 635 billion dollars annually) and global studies that showed significantly higher female prevalence of chronic pain in a range of countries (Sweden, France, Norway, United Kingdom and the Netherlands, among others). Females also outnumber males in suffering back pain, widespread pain,

migraine, osteoarthritis, musculoskeletal, neuropathic, and oral pain. In the case of TMD, the prevalence of women to men increases with the severity of the condition, reaching a ratio of nine women to one man in the most severe cases.

She then described two studies that sought explanations for these differences through quantitative sensory testing, which measures pain thresholds, intensities, and unpleasantness in response to sensory stimuli such as heat, cold, or pressure applied to various parts of the body. The first experiment compared 48 young, healthy adults (24 men and 24 women) in two different tests. The first test evoked pain in the forearm when a blood pressure cuff is applied to the upper arm, depressing blood supply to the forearm. The test measured the time the subject could tolerate the pain resulting from this "ischemic pain task." Women could tolerate the test for much shorter times than men.

The second test involved applying increasing pressure to the back of the forearm to determine the threshold pressure that produces pain. That threshold can be increased before causing pain by means of a conditioning procedure involving the hand of the other arm. The hand is immersed in successively colder ice water until it generates pain. Once that temperature is determined for each subject, the pressure experiment can be repeated while the opposite hand is placed in the pain-producing ice water. This results in an inhibition of pain in the other arm and greater tolerance to pressure. Again, women showed less conditioned inhibition and therefore less efficient pain control than men.

Ms. Bulls went on to describe a second larger, unpublished study of men and women, ranging in age from 19 to 76. The results continued to affirm sex differences. Women overall showed lower thresholds and less tolerance in tests of heat and pressure applied at various surfaces of the body, as well as greater temporal summation, which measures the extent to which pain is facilitated following repeated applications of the stimulus at a particular site.

The final day of the meeting continued discussions of molecular mechanisms and systems involved in initiating or controlling chronic pain, and how an understanding of their actions can guide precision medicine approaches.

The morning began with welcoming remarks from Martha Somerman, DDS, PhD, the Director of the National Institute of Dental and Craniofacial Research at the NIH. Dr. Somerman remarked that much of the agenda of the TMJ meeting perfectly matched current thinking and initiatives of the Institute. Thus, they are inviting research to develop organs-on-a-chip that would represent dental and craniofacial tissues. They are concerned with sex differences, not only in relation to pain, but also to

other conditions and in normal growth and development. And, as a participant in the **TMJ Patient RoundTable**, she said that NIDCR was fully on board with a patient-centered approach.

Dr. Somerman went on to describe an ambitious program of the institute, *NIDCR 2030, A Vision for the Future*. This is a strategic plan to determine where the Institute wants to be by that time, with oral health providing the window to assess total health and a breaking down of the research siloes that have been so divisive in the past. The vision will entail being able to recognize emerging science and move it forward. In that regard, she saw the TMJA meeting and attendees as important resources, inviting all to participate in what will be an interactive process. She especially looked forward to the recommendations that would emerge from the meeting as being instrumental toward those ends.

Epigenetics and Chronic Pain

While there are genetic factors that render some people inherently more sensitive to pain, it is evident that there are also epigenetic factors that can influence pain sensitivity. Epigenetics is the study of molecular changes (epigenetic "marks" or "mediators") and associated phenotypes (the actual physical make-up of an individual) that are heritable but do not involve changes in the DNA sequence. The epigenetic marks, or mediators, contribute to biological regulation by influencing gene expression. Epigenetic modifications may result in changes in the protein products of genes and can be transmitted through normal cell division or germline transmission (affecting eggs or sperm) without altering the DNA sequence. These changes in expression occur as a result of removing or adding molecular groups to specific parts of the DNA in the nucleus of cells that either free up a gene so it can be expressed or else silence it. These chemical processes involve DNA methylation, histone modification, or the use of non-coding ribonucleic acid (RNA).

An individual's "epigenome" is that individual's genome with all the epigenetic marks indicated, in this way capturing the biological influences of environmental and lifestyle factors in a quantifiable way. Epigenetic dysregulation has emerged as a hallmark of several complex pathologies, including hypertension, cardiovascular disease and cardiovascular risk factors, such as smoking, diabetes, and aging. Relevant to the long-term goal of the Precision Medicine Initiative, meeting attendees considered epigenetic profiling of individual patients to be a meaningful complementary strategy to physiological profiling and DNA sequencing in developing TMD and chronic pain prevention and treatment strategies.

Silencing Pain Relief Genes. Lingli Liang, PhD, Department of Anesthesiology, New Jersey Medical School, Rutgers State University of New Jersey, Newark, New Jersey, has been studying neuropathic pain that follows a

nerve injury, noting that earlier studies suggested that epigenetic silencing of genes related to pain relief might be at work.

Her studies used a mouse model of a nerve injury induced by tying off a spinal nerve. The dorsal root ganglions in the affected area showed increased messenger RNA and protein expression of an enzyme, G9a, which is a histone methyltransferase. This is an enzyme that catalyzes the addition of certain molecular groups to histones, which are proteins bound to the DNA in a cell's nucleus (and thus a form of epigenetic modification). Indeed, Dr. Liang found that the enzyme is *required* for the transfer of the molecular complex to histones and that it represses gene expression for selected potassium channels and opioid receptors important in ameliorating pain. The G9a was overexpressed in the spinal dorsal root ganglion (DRG) and led to increased excitability of the neurons and pain hypersensitivities. When she used a G9a inhibitor or conducted studies of injured mice in which the G9a gene had been knocked out, the animals showed recovery from mechanical and cold allodynia and thermal hyperalgesia. She concluded from these studies that the G9a enzyme is a potential target for neuropathic pain therapy.

No Two Rats are Alike!

Animal models of chronic pain have traditionally used some type of spinal nerve injury such as partial nerve ligation or inflammatory pain induced by the injection of an irritant such as Complete Freund's Adjuvant into soft tissue. Investigators then typically compare the injured animals with normal controls using a range of sensory tests, such as the paw withdrawal test (how soon does the injured animal withdraw a paw when exposed to an increasing heat stimulus) or emotional tests, such as an open field test, which measures anxiety by observing how willing an injured animal is to explore a new unknown area or, as a measure of depression, the forced swim test. Results are then usually presented in terms of averages for the animals in each group.

In light of the goal of precision medicine to personalize treatment based on genetics, epigenetics, and other factors unique to the individual, Zhizhong Pan, PhD, Department of Pain Medicine, University of Texas MD Anderson Cancer Center, Houston, Texas, has studied individual differences in animal models. He used partial ligation of the sciatic nerve in a study of pain in rats, and noted that the injured animals were generally similar in their responses in the **sensory** aspects of pain, as measured by how soon they withdrew their paws in the paw withdrawal test compared to the control rats. But in tests of the **emotional** responses to pain, such as anxiety and depression, they ranged widely, from very vulnerable to very resilient.

How to account for these differences? Dr. Pan's approach employed a method that has been described as "the breakthrough

technique of the decade"—optico-genetics. The technique involves genetically altering specific cells in the body so that the genome in the nucleus of the targeted cells contains a new light-sensitive gene. This can be done by incorporating the light gene into the genome of a virus (rendered harmless) and having the virus infect the targeted cells, and in this way insert the light gene into the cell's nucleus. When exposed to light of the appropriate wavelength, the cells can be activated and their behavior monitored. The exciting feature of the technique is that it allows the behavior of cells to be observed in real time in living animals.

Dr. Pan used this method to study neurons in a part of the rat brain called the parabrachial area. This area is a relay station along a pain pathway that transmits incoming pain signals from the spinal cord to the central part of the amygdala, one of the brain areas associated with emotion. He found that chronic pain significantly altered the levels of the enzyme involved in silencing genes, DNA methyltransferase, in the amygdalas of the injured rats, and that the rats with the highest levels of the enzyme were those found to be the most resilient. DNA methyltransferase is a family of key enzymes that catalyze the methylation of DNA and hence the expression levels of genes. Since he had earlier found that stimulation of the parabrachial → amygdala pain pathway leads to anxiety behavior in normal rats, he concludes that at least one explanation for the differences between vulnerable and resilient rats in his model lies in the more potent epigenetic controls exercised by the resilient animals.

Some New Anatomy Lessons

Medullary Pain Control. The history of pain research is one in which successive generations of researchers have discovered new varieties of neurons, neurotransmitters and pathways involved in pain perception and control. Mark Hoon, PhD, Molecular Genetics Unit, National Institute of Dental and Craniofacial Research, at the NIH, described the latest revelation. He has discovered a small population of pain-modulating neurons in the medulla. The medulla lies at the base of the brain stem, the part of the brain that lies atop the spinal cord and under the cerebral hemispheres. Nerve centers in the medulla have long been associated with control of breathing and other vital functions. A clue that something unusual involving pain was happening in the medulla was reported 20 years ago, when researchers found that electrode stimulation in the area produced a modest amount of analgesia. Dr. Hoon's research has enabled him to pin down exactly which neurons are specialized to exert analgesic effects in response to a noxious stimulus and where they are in the medulla.

Using a model in which capsaicin, a potent pain-causing molecule (which is also the ingredient of hot peppers) is injected in the hind paw of a mouse, he

found a small population of neurons in the ventrolateral medulla, abbreviated as VLM[4]. One unusual feature of these cells is that they responded to noxious stimuli from the hind paw located on the same side of the body as the VLM cells. (Most ascending pathways cross over from the right side of the spinal cord to the left side and vice versa when they reach the brain.) Subsequent tests showed that when the VLM cells themselves were directly stimulated, they exerted potent analgesic effects, which could be blocked by naloxone, the chemical that blocks opioid analgesia. Interestingly, the analgesic effects are widely distributed so that stimulation of the VLM cells on one side of the medulla led to analgesia in both hind paws. When the VLM cells were inhibited, pain responses worsened, and when the cells were surgically removed, the result was increased baseline pain sensitivity. Dr. Hoon conjectures that one of the possible mechanisms contributing to chronic pain may involve some dysregulation of the newly discovered VLM neurons.

Feeling a Gentle Touch. The skin of an animal is endowed with many sensory nerve endings that relay information on touch, pressure, pain and temperature. But there remain many unknowns about how these different modalities are translated (technically, "transduced") into the electrical signals used by the nervous system to relay information to the brain. Jianguo Gu, PhD, of the University of Alabama at Birmingham, Birmingham, Alabama, has been studying the sense of touch. In particular, he is interested in how humans normally perceive a gentle touch in the orofacial area, a highly sensitive area. Unfortunately, for many patients with TMD and other orofacial pain conditions, a gentle touch can feel intensely painful, the condition pain specialists call mechanical allodynia.

Dr. Gu has focused on a very sensitive tactile area in a rodent's snout, the whisker. In his studies in mice, he has found that at the base of the hair follicles of the whiskers in the epidermis are saucer-shaped oval cells called Merkel's discs, which are in contact with nearby sensory nerve fibers. While Merkel's discs have been known about for many years, only recently has the element in the disc responsible for transducing the mechanical force been discovered. It is a trans-membrane protein called a Piezo2 ion channel. "Piezo" is Greek for pressure, and when the protein is activated by a mechanical force on the whisker, it opens channels on the Merkel disc membrane that results in the release of the neurotransmitter serotonin. Serotonin crosses the synaptic gap to excite the nearby nerve ending and start the nerve signal carrying touch information on its path to the brain.

The Making of a Nociceptor. One of the outstanding developments of the decade was the discovery that the insertion of a few key genes into a mature human cell could convert it to a stem cell capable of

4. "Ventrolateral" describes the location of the cells in the underside of the medulla that faces forward (anteriorly) and on the side of the medulla. Since VLM cells are paired on either side of the medulla, there is a right VLM and a left VLM.

developing into any of the specialized cells of the body. Speakers at the meeting saw the potential of using the technique in connection with precision medicine to study disease, using the unique cells derived from the stem cells of a patient with the disease in question. But there are countless other applications. Testing of candidate new drugs, for example, might be facilitated by having a ready supply of cells that would be the target for the drug. That was one of the goals discussed by Xue-Jun Li, PhD, Department of Biomedical Sciences, University of Illinois College at Rockford and Department of Bioengineering at Chicago, Chicago, Illinois. His talk was essentially a step-by-step recipe for turning an induced Human Pluripotent Stem Cells into a nociceptor.

Prior to the last presentation on Tuesday morning, there was an informal discussion of the "Arc of Research," a reprise of TMD research from the beginning of The TMJ Association in 1986 to where the field stands today. Allen Cowley noted Terrie's many treks to Capitol Hill in the early 90s urging Congress to respond to the grave harms that TMJ implants were causing patients. Three weeks after she met with the Chief Investigator of the Human Resources and Intergovernmental Relations Subcommittee of the Committee on Government Operations in the House of Representatives, a hearing was scheduled, titled *Are FDA and NIH Ignoring the Dangers of TMJ (Jaw) Implants?* Twenty-four years of Congressional Report Language have followed, advising NIH to address TM disorders and expand research. Eight scientific meetings co-sponsored by the Association and NIH agencies have now taken place along with many one-on-one meetings with NIH institute and center directors. These occasions enable Terrie to narrate compelling stories of patients relayed to the Association while Allen describes the science needed.

Terrie continued the story by introducing the "new" science that began in 2006, when NIDCR Director, Dr. Larry Tabak, allocated funds for the OPPERA study—research that has taken TMJ research into the 21st century. She turned to Dr. John Kusiak, PhD, Acting Deputy Director of the NIDCR, to trace the origin of the study, now in its second decade.

Dr. Kusiak made two points. One was that, as a small institute with limited funding, NIDCR followed a policy of making relatively small research grants for limited time periods to single investigators who were free to conduct their project on their own. But, under Dr. Tabak, they changed policy to gamble on a major investment in a long-term research project to explore risk factors for TMD. Because of the degree of risk that would be involved, the research mechanism employed was a cooperative agreement, an arrangement that allows for considerable staff input during the course of the research. The second point he wanted to make was that, although the staff made demands on how the project

should be managed and the kinds of expertise they expected in the research team, there was no mention anywhere in the announcement of the project of seeking genetic risk factors, or indeed any research that would involve genes and genomics.

William Maixner, OPPERA's principal investigator, picked up the story, explaining that at the time of the announcement, his group had been conducting some preliminary studies of women with TMD, collecting blood samples. This was happening at a time when it had been discovered that common polymorphisms of the catechol methyltransferase (COMT) gene were associated with low, medium or high sensitivities to pain. Coincidentally, he was able to add new expertise to his group. In short order, it was clear that he had the wherewithal to apply for the new NIDCR cooperative agreement.

He then summed up some of the discoveries over the decade. "Today we are at a stage where we can move from the 'arc of research' to the 'arc of translation'—to go from the lab into the clinic." This takes different skills and expertise and marks a readiness to move to the private sector as a means to operationalize discoveries.

How this might play out was described by the last speaker of the day, Ashley Brenton, PhD, representing a diagnostic company in California. She described how the company has been collecting samples for a biobank and also developing a pain perception sensitivity score, incorporating genetic information and measures of selected neurotransmitters. The company has also conducted Continuing Medical Education programs and is currently working on a TMD diagnostic questionnaire and patient profile. These are works in progress, with the expectation that they will yield a personalized medicine approach to optimize treatment for each TMD patient.

* * *

The meeting next turned to a general discussion on how best to move forward on ways to incorporate precision medicine in research to improve outcomes for the treatment, diagnosis, and prevention of TMD and its overlapping pain conditions.

Research Recommendations to Further Progress on How Precision Medicine Can Be Applied to Temporomandibular Disorders and Its Comorbidities

The TMJ Association's Eighth Scientific Meeting explored the role that "precision medicine" can play in improving the treatment of temporomandibular disorders (TMD) and comorbid chronic pain conditions. Past research has affirmed that TMD represents a family of complex pain conditions, in which environmental, behavioral and genetic factors interact to generate psychological stress and pain hypersensitivity. The result can lead to a worsening of TMD symptoms and the development of chronic pain conditions elsewhere in the body. Current diagnostic methods and treatments fail to reflect the new state of science and fail to meet the needs of patients. The presentations and discussions at the meeting led to recommendations which could significantly advance the diagnosis, treatment and prevention of TMD and associated overlapping pain conditions, using precision medicine approaches.

Next-generation sequencing (NGS) using low cost technology can now be used to sequence an individual's genome. The genome can then be scanned with the goal of identifying those variants in sequence segments, which could provide relevant information about the state of the individual's health, risk of disease, and appropriate treatment choices. At this time, most diagnostic tests follow a one-test–one-disease paradigm, despite the ability of NGS to produce a wealth of data about a patient. While NGS may lead to the identification of disease-causing DNA variants or associated susceptibility genes, such results provide only a one-dimensional starting point towards understanding the multiple dimensions of the pathological process. Expanding studies to a more comprehensive precision medicine approach, as emphasized below, will enable more subtle delineations of the patient population into subgroups amenable to different types of treatment.

At this time, NGS and precision medicine approaches are only just beginning to be considered for TMD and its associated comorbidities—and for the chronic pain field in general. Progress has been slow, in part as a consequence of the limited availability of:

- Appropriate animal models of these complex conditions;
- Molecular and cellular tools to identify and functionally annotate susceptibility or disease genes;
- Physiological approaches to determine the signaling pathways which lead to the progressive development of TMD;
- Analytical tools to analyze big data in efficient ways; and
- Appropriately trained physicians/dentists and basic/translational scientists who can assemble and integrate NGS data with data related to the regulation of gene function and the expanding knowledge of the cell and molecular physiology of chronic pain.

Despite these challenges, there are now exciting scientific opportunities to identify new genes and effector pathways that contribute to the pathogenesis of TMD and associated chronic pain conditions. Application of technologies which can produce an abundance of data about a patient should yield a deeper understanding of the biological basis of TMD and help identify new drug targets to delay or reverse the onset or the progression of these disorders. Importantly, the research recommendations below have been driven by patient needs, as reflected by their involvement in the planning and attendance at the meeting. The recommendations thus reflect a patient-centric approach that will help advance more meaningful and personalized diagnostic and therapeutic measures, which take into account individual variability in genes, family history, environment and lifestyle.

Recommendation 1. Bring TMD and Chronic Overlapping Pain Conditions stakeholders together to develop and implement precision medicine approaches for diagnosis and treatment.

There is a need to form integrated research groups to coordinate basic research, human data collection and sharing, and translation of basic and clinical science into therapeutic advances. These integrated groups should reflect patient needs, population diversity (race and sex) and recognition that the majority of patients are female. The key stakeholders should be patients and their advocates, academic units, government agencies and the private sector. Strategies of 'implementation science' must be employed so that patients, families and other stakeholders can interact with scientists and physicians in the development of scientific protocols and studies.

Physicians, researchers and patients should come together in academic medical centers to form Regional Centers of Excellence. To encourage this approach, NIH could provide competitive planning grants for centers that involve participation by medical, dental, and other professional schools in a region.

External advisory committees could then guide and coordinate center activities, as is the case with NIH cooperative agreements. The centers should represent different areas of expertise and disease focus related to TMD and its comorbidities, the most prominent area being chronic pain. The centers should be sufficiently flexible to respond to local needs to focus on specific areas of research, enable coordination of local resources, develop pilot grant support for preliminary data, and be able to link to other centers to develop national data coordinating centers.

As the Regional Centers of Excellence are formed, their research and training initiatives should be supported by NIH research, research training grants and cooperative agreements, as well as grants and contracts from other government agencies, including the United States

Department of Veterans Affairs, the Department of Defense, and private sector foundations and corporations.

Examples of several regional Research Centers are emerging at Duke University, the University of Toronto, and the University of Alabama, each of which is currently developing focus areas of research on TMD and comorbid conditions.

A specific model of the collaborative and integrative research needed in this field is one currently being supported by the VA: The VA Spinal Cord Injury (SCI) Consortium represents a cooperative effort involving UC San Francisco, Palo Alto VAMC, UC Irvine, San Diego VA/UCSD and UC Davis. The consortium brings together patients, clinical trials and rehabilitation experts, neurosurgeons, stem cell biologists, cell therapists, informatics experts and experimental rodent biologists. Similar planning and integration of diverse scientific and clinical expertise, working in a patient-centric manner, should be developed and supported by NIH or interagency initiatives.

Recommendation 2. Advance understanding of the molecular, genetic and neural mechanisms that mediate persistent pain conditions in individuals with TMD.

To implement a precision medicine approach toward this end, the following areas should be addressed:

- Expand cohorts in prospective studies to obtain richer phenotypic and genomic databases. These data will enable more detailed elucidation of molecular pathways, molecular markers, and pathophysiological mechanisms to characterize risk factors that predispose to TMD and chronic pain.
- Advance opportunities to apply NGS approaches, in order to obtain more personalized diagnostic and therapeutic measures, with the goal of accounting for individual variability in genes, family history, environment and lifestyle.
- Support studies to assess the epigenomic impact of both natural environmental exposures and those related to implant devices that can lead to great harm to genetically susceptible individuals.
- Encourage the affordable use of sequencing and informatics applications to carry out whole genome methylation and histone analysis studies.
- Support research on how the oral microbiome affects the development of TMD and related comorbidities such as chronic pain conditions.
- Encourage the development and application of phenotyping tools for collection of environmental exposure data as a part of the medical history.

Recommendation 3. Integrate the brain imaging structure-function database together with studies related to TMD and chronic overlapping pain conditions.

Recommendation 4. Study the role of the immune system in the development of chronic overlapping pain conditions.

- Support research on immunopharmacological approaches for diagnosis and treatment of chronic pain.
- Support research on the role of the peripheral immune system in TMD and related chronic pain conditions.

Recommendation 5. Develop strategies/methodologies/protocols for disease modeling to advance precision medicine.

Research is needed to develop guidelines for identifying subgroups of patients with distinct mechanisms of disease and particular responses to treatments. The research design of a precision medicine approach should incorporate the following:

- Expansion of existing patient cohorts to enable collection of more detailed patient phenotypes with longitudinal follow-up and collection of associated biological specimens for precision medicine.
- Emphasis on the importance of shared decision-making between the clinician and patient to assess the patient's potential for benefit or harm from proposed treatment.
- Establishment of additional cohorts to increase power, replicate findings and extend the findings to more diverse patient populations. Replication is of critical importance for genetic studies, and support in this area will strengthen the efforts in other target areas, such as biomarker identification and the development of more relevant disease models.
- Increase efforts towards developing synergies with other NIH initiatives, such as the Precision Medicine Initiative cohorts. Study designs for the above mentioned cohorts should build and utilize the infrastructure of these other initiatives.
- Develop animal models which better recapitulate the disease phenotypes.

Recommendation 6. Develop Induced Pluripotent Stem Cell (iPSC) technologies to provide a tool for basic and translational investigators studying chronic pain.

Strategies should be developed to encourage the development, validation, and application of iPSC cell lines from chronic pain patients to discover new mechanisms of disease and new therapeutic targets.

- Support investigators in the field to develop a collection of iPSC models and leverage genome editing technologies (e.g. CRISPR-cas9) and use these cell models from pain patients in conjunction with other emerging technologies, such as 3D culture systems (organs-on-chips) and xenografts.

• Support careful characterization of disease iPSC lines (maintenance, authentication, and distribution), coupled with support for a dedicated repository.

• Encourage research utilizing iPSCs for candidate drug testing, compound screening, drug repurposing and toxicity testing (clinical trials in a dish), in order to assess effectiveness of therapeutic compounds on an individual's own genetic background.

• Emphasize the development of novel drug treatments. Building on the concepts of precision medicine, these efforts should incorporate the above outlined platforms, integration of genetic findings in patient cohorts, biomarker development based on samples collected from various patient cohorts as well as leveraging the Precision Medicine Initiative cohorts, when integrated with iPSC cell technology and animal models, has a high likelihood to yield more targeted, disease relevant compounds.

• Develop the infrastructure for support of these recommendations by requiring significant administrative oversight and guidance based on the achievement of milestones in each area.

Recommendation 7. Address the dire lack of appropriately trained physicians and dentists at medical, dental and other professional schools in the U.S.

• Increase the numbers of appropriately trained physicians/dentists and basic/translational scientists who understand the technologies required to carry out precision medicine.

• Encourage the development of educational materials and guidelines for students, healthcare providers, patients and the public.

• Encourage workshops that include medical and dental students/fellows, practicing physicians and allied health nurses and assistants, patients and administrators.

• Encourage the implementation of "patient-centric" educational processes. Medical treatments and diagnostics for TMD are currently designed for the average patient as a "one-size-fits-all approach." A precision medicine approach will require an individual patient's data regarding health, lifestyle, and environment.

TMJA Young Investigator Awards

At The TMJ Association's Eighth Scientific Meeting, ***How Can Precision Medicine Be Applied to Temporomandibular Disorders and Its Comorbities?***, September 11-13, 2016, the Association recognized the following young investigators for their research interests in the area of TMJ disorders and overlapping pain conditions. The following young investigators 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 American Association for Dental Research for making the following travel awards possible.

Presenter: **Luana Colloca, MD, PhD,** Department of Pain Translation Symptom Science, University of Maryland School of Nursing, Baltimore, MD
Poster: A prediction error model of placebo analgesia and its extinction
Mentor: Dr. Susan Dorsey

Presenter: **Carolina Beraldo Meloto, DDS, PhD,** Alan Edwards Centre for Research on Pain, Faculty of Dentistry, McGill University, Montreal, Quebec, Canada
Poster: Clinical characteristics of TMD at onset and predictors of persistence: Preliminary results
Mentor: Dr. Luda Diatchenko

Presenter: **Ke Ren, PhD,** Department of Neural and Pain Sciences, School of Dentistry, & Program in Neuroscience, University of Maryland, Baltimore, MD
Poster: The behavioral and neural effects of multipotent stromal cells in rodent models of persistent pain
Mentor: Dr. Ronald Dubner

Presenter: **Massieh Moayedi, PhD,** Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada
Poster: Structural and functional abnormalities in chronic orofacial pain disorders: A meta-analytic study

Presenter: **Crystal Stinson, DDS,** Texas A&M University, Baylor College of Dentistry, Dallas Texas
Poster: Lateral thalamic control of nociceptive response after whisker pad injection of varicella zoster virus
Mentor: Dr. Phillip Kramer

Presenter: **Andrew E. Bertagna, DMD Candidate,** College of Dentistry, University of Illinois at Chicago, Chicago, IL
Poster: Mechanical and inflammatory mediated degeneration of mandibular cartilage is associated with altered NG2-type VI collagen colocalization and cytosolic NG2 residues in articular chondrocytes
Mentor: Dr. David Reed

Presenter: **Yong Chen, PhD,** Department of Neurology, Duke University, Durham, NC
Poster: Lack of evidence for ectopic sprouting of genetically labeled Aβ touch afferents in inflammatory and neuropathic trigeminal pain
Mentor: Dr. Wolfgang Liedkte

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