EIGHTH SCIENTIFIC MEETING
OF THE TMJ ASSOCIATION

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

Meeting Abstracts

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The U.S. precision medicine initiative

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Starting with the launch of the Human Genome Project in 1990, the past quarter-century has brought spectacular achievements in genomics that dramatically empower the study of human biology and disease. Augmenting the advances in human genomics have been innovations in electronic health records, data science, and technologies for capturing environmental, physiological, and lifestyle information. Together, these provide opportunities of unprecedented scale and scope for investigating the underpinnings of health and disease.

To capitalize on these developments, the United States recently launched a major new research endeavor—the Precision Medicine Initiative. This bold effort is framed around several key aims, which include accelerating the use of genomically informed approaches to cancer care, making important policy and regulatory changes, and establishing a large research cohort of >1 million volunteers to facilitate precision medicine research. The latter will include making the partnership with all participants a centerpiece of the cohort's design and development. The Precision Medicine Initiative represents a broad-based research program that will allow new approaches for individualized medical care to be tested in a rigorous fashion, so as to establish a new evidence base for advancing clinical practice and, eventually, human health.

Key issues for advancing precision medicine for TMD and chronic overlapping pain conditions: case definitions and phenotypic measures

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There is increasing recognition that many, if not most, common chronic pain conditions are heterogeneous with a high degree of overlap or co-prevalence.

There is an increased recognition that we should begin to think of common chronic pain conditions as Chronic Overlapping Pain Conditions (COPCs).[1] There are at least two features of COPCs that will be presented by Dr. Maixner: 1) That the etiology/mechanisms of COPCs are multifactorial and 2) that the clinical manifestations of COPCs are diverse, common and shared across COPCs.[2-4]
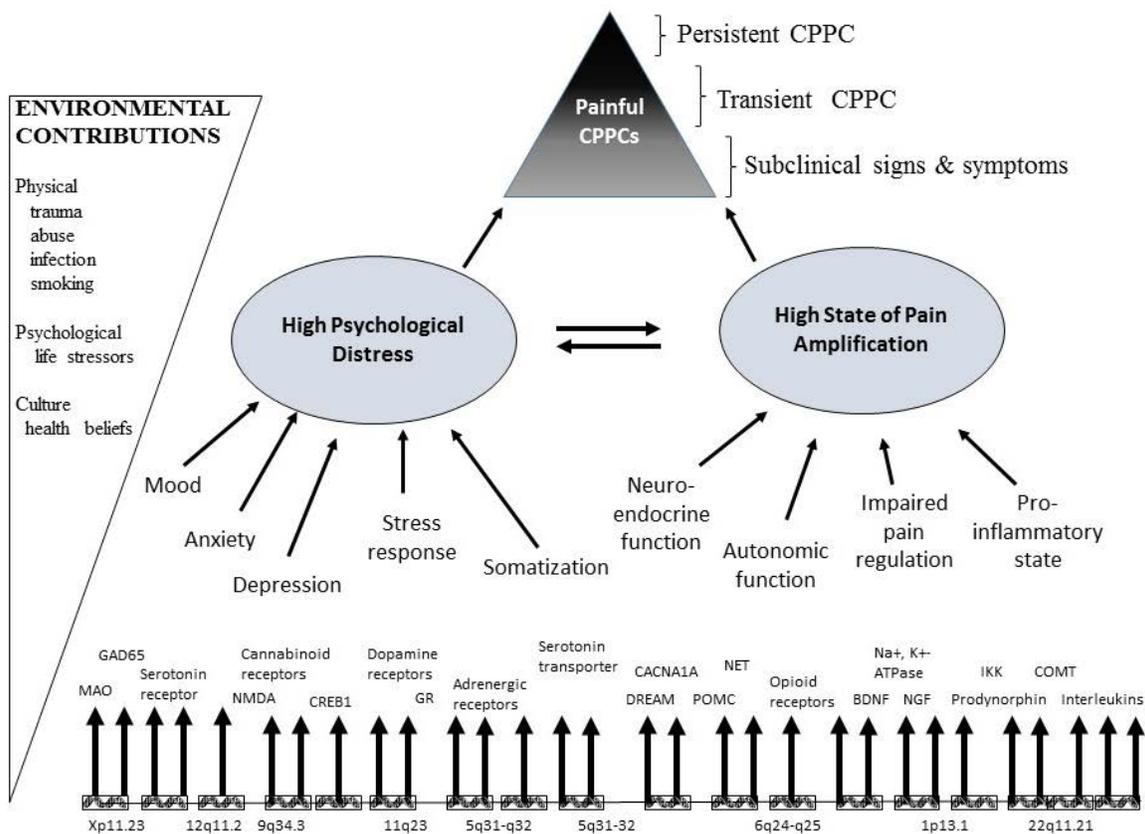
Diatchenko et al, Pain 123: 226-30, 2006 & Maixner et al, Journal of Pain 12, Suppl 3, 4-11, 2011

Dr. Maixner will discuss findings that support the view that there are shared biological mechanisms that contribute to the manifestation of COPCs. A unifying theme of his presentation is that multiple biological processes, when coupled with environmental exposures (e.g., injury, infections, physical and psychological stress), contribute to the signs and symptoms that underlie COPCs. Concepts and methods that can be used to identify subpopulations of COPCs patients that contribute to advancing precision pain medicine will be presented.[5]

(Continued on next page)

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Disclosures

Longitudinal gene-brain mapping to guide diagnosis and treatment of mechanistically distinct types of chronic pain

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The pursuit of longitudinal gene-brain mapping has immense potential to guide diagnosis and treatment of mechanistically distinct types of chronic pain. Our work has established that the interaction between the brain and chronic pain gives rise to distinct brain states, characterized by specific anatomical and physiological features, for different clinical pain conditions. Specifically, neocortical grey matter dynamically changes with chronic pain and this reorganization is pain-type specific. In the only longitudinal study of the transition to chronic pain, we used brain imaging to identify functional and structure properties of the limbic brain that predict which chronic back pain patients would transition to chronicity one year later. Findings reveal that risk for chronicity is conferred by subcortical-limbic functional connections between key regions and parallel the transition to the chronic pain state, as well as stable subcortical anatomical circuits that likely reflect predisposition for developing chronic pain. These observations dovetail with longitudinal brain imaging results from the Multidisciplinary Approach to the Study of Chronic Pelvic Pain Research Network, wherein baseline resting state functional connectivity of frontoparietal regions predicts future reductions in urologic pelvic pain 3 months later. Collectively, these results emphasize the potential of multimodal brain imaging to parse brain properties that induce risk (predisposition), their interaction with injury (transition), and the resulting new brain state (maintenance) in clinically undifferentiated chronic pain populations. Our more recent rodent brain imaging and electrophysiological studies that closely parallel the human studies are beginning to provide complementary mechanistic insights regarding cellular, synaptic, and circuit reorganization with transition to and maintenance of chronic pain.

N-of-1 trials for personalized decision-making

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N-of-1 trials are a promising tool to enhance decision-making and improve outcomes. These trials are single-participant multiple-crossover studies for determining the relative comparative effectiveness of two or more treatments for one individual. The individual selects treatments and outcomes of interest and, possibly working with a health care provider, carries out the trial, before making a final treatment decision based on its results. This talk will discuss the advantages and challenges in conducting N-of-1 trials, along with some of the design and analytic considerations. A study to test the effectiveness of the N-of-1 trial as a clinical decision tool comparing patients randomized to N-of-1 vs. usual care is ongoing. The challenges of implementing the decision strategy in such a context and the pros and cons of combining information from different patients in order to provide a better estimate of each individual's effect than from his or her own data alone will be discussed.

Disclosures
None

In silico approach to developing TMD-related precision medicine applications: Narrowing the gap between currently available and urgently needed

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Temporomandibular Disorders (TMD) are known for clinical heterogeneity and multifactorial etiology, and possible adverse outcomes from treatments such as Temporomandibular Joint (TMJ) arthroplasty can impose an additional layer of complexity on the already complicated sum effect. In the meantime, a proper management of TMD patients requires distinguishing among Patient-Procedure-Device related inputs which ultimately define treatment success or failure. Variations in nociceptive threshold and underlying inflammatory conditions, including osteoarthritis as one of the main TMD causes, can not only define the extent of initial – functional and clinical – presentation of TMD, but can also predetermine the treatment outcome after TMJ arthroplasty. Both of these patient-related characteristics – tolerance to pain and susceptibility to inflammation – are prone to inter-individual variations due to environmental factors (e.g., health habits) and demographic/genetic factors (e.g., sex, race/ethnicity, SNPs). Genome Wide Association Studies (GWAS) suggested that a number of SNPs in the genes involved in pain perception and inflammation may help identifying patients that are more prone to development of severe TMDs or that have TMDs with certain phenotypic features. However, many of putative biomarkers for the initial TMD are also identified as potential drivers of the adverse treatment outcomes in TMD/TMJ arthroplasty patients. As a result, potential use of these biomarkers is complicated by uncertainty whether the positivity for such a biomarker would indicate the initial inflammatory condition, or whether it would demonstrate the post-implantation inflammatory response that may lead to an implant-elicited adverse reaction resulting in revision surgery. In addition, many of the currently identified putative TMD biomarkers are influenced by patient's race/ethnicity, which further complicates their potential utility in TMD/TMJ arthroplasty subpopulations with different ethnic backgrounds.

This presentation will outline the current field of TMD/TMJ arthroplasty biomarkers and will discuss the existing challenges impeding development of *actionable* biomarkers. The presentation will particularly highlight the need for developing different types of biomarkers, ranging from diagnostic TMD biomarkers that would be specific for the underlying causal mechanisms (e.g., functional pain versus degenerative process) to prognostic TMJ arthroplasty biomarkers that would be indicative of the implant-specific outcomes (e.g., wound healing versus osteolytic loosening). As possible solutions for developing TMD/TMJ arthroplasty biomarkers that could be applicable in clinical and regulatory settings, the presentation will discuss possibilities for *in silico* research using GWAS and expression profiling data in association with the clinical and epidemiological evidence gathered from observational studies and patient queries.

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No relevant financial or nonfinancial relationships to disclose.

Effective engagement with patient groups around clinical trials

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Background
Patient groups are developing diverse skill sets and assets to provide valuable trial services, funding, and the ability to enhance collaboration, as well as providing the necessary information to ensure that precision-medicine trials become a reality. Research sponsors across the clinical trials enterprise are recognizing the benefits of continuous and meaningful patient group engagement, but all stakeholders need further guidance on operationalizing this new model. CTTI's best practices consolidate actionable recommendations for establishing strong, active patient group engagement during all phases of the research and development lifecycle. Our evidence gathering, along with further consultation with experts, has led to the identification of best practices and tools for patient group engagement in the clinical trial enterprise. Additional work is underway to develop a framework for assessing the value and impact of patient group engagement.

Importance
Patient-centered clinical research demands greater participant engagement as patients become more empowered in their health-care decision-making. To ensure a high level of patient participation and engagement in clinical trials, the medical therapeutic research and development community must elevate the patient voice in research planning. Organizations are beginning to incorporate processes and practices that allow patients to become more actively involved in precision-medicine clinical trials, from design through execution and dissemination of results. However, best practices and a value proposition for engaging with patients and patient groups are necessary to further the cause of deeper, more meaningful levels of patient engagement in the clinical research enterprise. CTTI has developed widely accepted Best Practices and a proposed value model for measuring the impact of various engagement methods.

Objectives
1. An introduction to The Clinical Trials Transformation Initiative and the Best Practices for Patient Group Engagement in Clinical Trials Project
2. An understanding of best practices, recommendations, and tools to maximize patient engagement in the development of precision-medicine research and trials
3. A brief introduction to how such engagement might be measured

TMD treatment and precision medicine—The past, present and future

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Danica Marnica-Dabic, M.D., Ph.D., Director, Division of Epidemiology of the Center for Devices and Radiological Health, Food and Drug Administration, Silver Spring, MD, 20993, USA
Christin Veasley, Director and Co-Founder, Chronic Pain Research Alliance

Currently, we know very little about the genetic, environmental, psychophysical and other factors that affect an individual's responsiveness to treatment. This has left health care providers and patients with little to no information on which to base important treatment decisions, and many describe the process of selecting a safe and effective treatment for TMD and overlapping conditions from the myriad that are available as a "roll of the dice." This session will explore the promise that precision medicine holds in transforming this situation. Michele Kaseta will describe her experiences in being diagnosed with TMD and undergoing TMJ implant (and other) surgical procedures and how her treatment outcome may have been improved if advances in precision medicine were available a decade ago. Dr. Danica Marinac-Dabic, Director of the Division of Epidemiology at the Food and Drug Administration (FDA), will provide a summary of a new FDA initiative being undertaken with multiple stakeholder groups to advance individualized, patient-centered diagnosis and treatment of TMD. Specifically, the long-term goals of this initiative are to: i) develop outcome assessment and reporting tools based on patient input; ii) understand the complex interplay of patient biology, anatomy, genetics, and physiology with TMJ medical devices and clinical patient-centered outcomes in order to better target therapies toward the patients most likely to benefit from them; and iii) develop evidence to incorporate patient-centered data into clinical care. Christin Veasley, Co-Founding Director of the Chronic Pain Research Alliance, will conclude the session with an exploration of the complexity of the overlap among TMD and other chronic pain disorders, and how precision medicine techniques will aid in more accurate and adequate diagnostic approaches that are based on underlying pathophysiology, as well as the selection of safe and effective treatment that is individualized, patient-centered, and based on scientific evidence.

Precision medicine strategies to selectively alter intracellular signaling mechanisms: a new generation of targets

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Therapeutic development based on targeting cell surface receptors or the catalytic activity of enzymatic proteins has provided many effective drugs for disease treatments. More recently protein-protein interactions have provided additional targeting strategies. However, lack of specificity, unwanted side effects, and pharmacokinetic limitations have hindered advances. Post-translational regulatory mechanisms present a vast and largely undeveloped pool of targets for which more precise or patient-specific strategies hold promise. In studying protein phosphorylation/dephosphorylation in brain function, we have found that the targeting of phosphorylation sites of the protein kinase Cdk5 can have wide ranging effects including neuroprotection from ischemic and traumatic brain injury, enhancement of cognition, and anti-depression. New data has also revealed Cdk5-dependent pathways as targets for the development of anti-cancer therapies. Relevant to this meeting, Cdk5 dysregulation has been suggested to contribute to pain sensation and craniofacial pain. This presentation will provide an overview of the roles of Cdk5 in these diverse processes and show how these mechanisms may be specifically targeted as a possible way to treat disease. New data on novel systemic Cdk5 inhibitors now being tested will also be presented. From these studies, we hope to show novel ways that intracellular signaling mechanisms may be targeted for more precise and effective treatment strategies.

Disclosures
None

Targeting chemokine and protease signaling for the control of neuroinflammation and chronic pain

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Current analgesics predominately modulate pain transduction and transmission in neurons and have limited success in controlling disease progression. Accumulating evidence suggests that neuroinflammation, which is characterized by infiltration of immune cells, activation of glial cells (e.g., microglia and astrocytes) and production of inflammatory mediators in the peripheral and central nervous systems, has an important role in the induction and maintenance of chronic pain including TMJ-associated pain.[1] My talk focuses on emerging therapeutic targets such as chemokines and proteases that promote spinal cord neuroinflammation and chronic pain via neuron-glia interactions. In particular, I will discuss how proteases (e.g., caspase-6) and chemokines (e.g., CXCL1) regulate microglial and astroglial signaling and synaptic plasticity in inflammatory and neuropathic pain.[2,3] Targeting excessive neuroinflammation could offer new therapeutic opportunities for chronic pain including TMJ-associated pain and neuroinflammation-related neurological and psychiatric disorders. I will also show the data that bone marrow stem cells can effectively control neuroinflammation and chronic pain.[4]

Acknowledgements

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Disclosures
I have no financial interest in this study.

Human chronic pain conditions: genome-wide analysis and pathways of vulnerabilities

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Background
Genome-wide association studies (GWAS) successfully identified genetic variants that affect risk of a wide range of human psychiatric and neurological conditions, but human chronic pain conditions have only started to be evaluated on the GWAS basis. Functionality of many identified single nucleotide polymorphism (SNP) variants can be assessed and confirmed through their regulation of RNA transcript expression levels, so called expression quantitative trait loci (eQTL). Manifestation of chronic pain conditions largely depend on the functioning of the nervous system, thus the identification of eQTLs in the relevant transcription system is crucial. One of the critical organs in gating pain stimuli is dorsal root ganglions (DRG).

Materials and methods
We performed a genome-wide association study (GWAS) of temporomandibular disorder (TMD) tested on 1,082 chronic TMD cases and 2,144 TMD-free controls enrolled in the OPPERA study (Orofacial Pain: Prospective Evaluation and Risk Assessment). The Omni2.5M platform was used with 1KG phase 3 imputation creating over 30 million genetic markers. The replication of the results has been done in four independent TMD cohorts. We then used genome-wide data from human DRG eQTL dataset obtained from 300 subjects to assess functionality of identified hits.

Results
Our analysis identified three new genome-wide loci that contribute to the risk of TMD. One locus has been replicated and showed association with multiple *tras* DRG eQTLs. Pathway analysis on eQTL hits identified new potentially causative biological pathways for TMD that involves the activation of T and B cells.

Conclusions
Our results suggest that at least a portion of chronic pain patients develop their conditions through immune, rather than neurological, processes. Elucidation of the biological mechanisms by which genetic markers contribute to the perception of pain in chronic pain patients will enlarge our understanding of pathophysiology of chronic pain conditions and enable the development of novel effective drugs and methodologies that permit better diagnoses and approaches to personalized medicine.

Disclosures
Dr. Diatchenko is a Medical Advisory Board member and shareholder at Proove Biosciences, Inc.

Intranasal mesenchymal stem cell transplantation for the repair of neuronal damage in chemobrain

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Background

Progress has been made in the treatment of cancer leading to a sharp increase in the number of survivors. However, cancer treatment poses severe side effects including the consequences of neurotoxicities like pain, fatigue and cognitive deficits which can persist long into survivorship. Currently, there are no pharmacologic treatments that have proven value in the management of cancer treatment-induced neurotoxicities. Cisplatin is a platinum-based drug and is widely used for many cancer types. Over the past years, mesenchymal stem cells (MSCs) have become a potentially attractive therapeutic option for peripheral and cerebral neuronal damage.

Methods

C57/Bl6 mice were treated with 2 cycles of 2.3mg/kg of cisplatin (5 daily doses followed by 5 days rest per cycle). Cognition was determined by the novel object and place recognition task (NOPRT) and the Puzzle box test. Neuronal arborization was measured in Golgi-stained brains and MBP staining was used as a measure of white matter damage. Mitochondrial function was analyzed by Seahorse technology. Chemotherapy-induced peripheral neuropathy (CIPN) was measured as mechanical hyperalgesia using von Frey hairs.

Results

Cisplatin treatment induced persistent mechanical hyperalgesia and a decrease in cognitive function long after cessation of treatment. Cisplatin also induced a neuronal mitochondrial dysfunction such as a decrease in mitochondrial oxygen consumption rate in the peripheral as well as central nervous system. In the brain, cisplatin treatment was associated with a decrease in complexity of white matter and a decrease in neurogenesis as shown by the number of doublecortin-positive precursors aligning the subventricular zone. Two intranasal administrations of two million MSCs after completion of cisplatin treatment reversed the cognitive impairment and the associated structural and functional defects.

In search of a mechanism we observed that intranasally administered MSCs can be traced in the brain 12-24 hours after nasal administration. MSC did not survive long term in the brain and were not incorporated into the network. We propose that MSC act by transferring intracellular information to damaged neurons leading to repair of adult neurons and neuronal stem cells.

Conclusion

Intranasal administration of MSC is an attractive non-invasive option for treatment of neurotoxic side effects of chemotherapy.

Disclosures

None

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

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In vitro organ-on-chip (OoC) models can recapitulate human physiology more realistically than planar monocultures on plastic. Multiple groups are working to couple together two or more OoCs, populated with human cells, to create a homunculus – a miniature, *in vitro* representation of key organs in a human.[1,2] Homunculi are of particular interest for studying how one organ metabolizes drugs and environmental toxins and creates metabolites that are toxic to other organs. Constructing OoCs with cells derived from human induced pluripotent stem cells (hiPSCs) that in turn are derived from a specific person, including patients with genetic or acquired diseases, will lead to personalized homunculi that serve as patient-specific *in vitro* disease models. OoCs could thereby help realize several of the potential long-term benefits of NIH's Precision Medicine Initiative,[3,4] including matching a treatment to the disease of a specific patient, elucidating the underlying mechanisms of a disease, and designing better treatments.

OoCs and other 3D cultures and their associated technologies and hardware present opportunities to advance our understanding and treatment of nerve pain and chronic overlapping pain conditions (COPC). While it is unlikely that OoCs will recreate a temporomandibular joint or recapitulate its dysfunction, these tools are immediately applicable for studying the response of human neurons, the blood-brain barrier, and the neurovascular unit to drugs[5] and to study genetic differences and environmental factors that influence drug influx and efflux for pain treatment. Mass spectrometry for untargeted neuroimmune metabolomics[6] will enable exploration of the metabolic aspects of COPC. The ability to grow human central or peripheral nerves in perfused 3D microenvironments will be critical for understanding neuroimmune interactions. OoCs could recapitulate other operational components of nociception, including the neuromuscular junction, the dorsal root ganglion (DRG), and spinal sensory neurons. Critical to understanding nerve pain and COPC is the ready availability of human neurons. The differentiation of DRG neurons from human embryonic pluripotent stem cells (hePSCs) is an important first step.[7,8] The shift from hePSCs to hiPSCs is critical for personalized

nociception models, and a MicroFormulator being developed to formulate aliquots of customized cocktails of small molecules and growth factors that are delivered to and removed from each well of a 96-well plate should improve the yield and phenotypic purity of hiPSC-derived cells. Neuroelectric recording arrays will allow measurement of neural activity in a DRG-on-a-chip. Mass spectrometric metabolomics will provide information about the COPC metabolic and signaling pathways. These tools should help remediate COPC.

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Disclosures
None

Induced pluripotent stem cells for disease modeling

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The development of novel therapeutics is often hampered by the availability of relevant disease models. In particular the identification of novel drugs through high-throughput screening relies on the availability of cells or tissues resembling a disease phenotype. Recent technological advances in stem cell technologies, the availability of induced pluripotent stem cells (iPSCs) and the development of protocols to differentiate these cells into a broad spectrum of tissue types provides now an unprecedented resource for disease modeling. Human induced pluripotent stem cells and derived cell types have been shown to not only resemble various disease phenotypes but also reflect the genetic risk and disease susceptibility.

In this presentation we will describe the conceptual framework, biological mechanisms and potential applications for using iPSC-derived cells in precision medicine. Incorporating genetic and phenotypic diversity will require the development of a large number of iPSCs. We will present results from our recent study, which generated 250 iPSC lines from participants in a large biracial epidemiological cohort. Building on extensive phenotypic and genetic data available, we discuss approaches for disease modeling, disease gene identification, and methodology for complex tissue interaction analysis between different cell types or materials. Finally, we will demonstrate how this approach can be used to identify underlying genetic factors in vivo in order to guide drug and biomarker development as well as toxicology testing.

Disclosures
None

Role of the immune system in resolution of pain

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Chronic pain and its comorbidities, including depression and anxiety are among the most disabling and costly disorders. Transient pain commonly develops in response to chemotherapeutic treatment, tissue damage and inflammation. The associated behavioral responses such as reduced activity, guarding of damaged tissue, and social withdrawal serve an adaptive purpose, and pain should resolve after tissues heal and inflammation resolves. We hypothesize that the resolution of pain depends on an active regulatory process involving endogenous resolution pathways. Dysregulation of these resolution processes results in transition into maladaptive chronic pain.

We investigated the contribution of T cells and endogenous interleukin (IL)-10 signaling to chemotherapy-induced peripheral neuropathy and inflammatory pain. The results show that chemotherapy-induced mechanical allodynia was prolonged in T cell-deficient (Rag1-/- or Rag2-/-) male and female mice compared with wild-type (WT) mice. Similarly, inflammatory pain was prolonged in T cell-deficient mice. There were no differences between WT and T cell deficient mice in onset or severity of mechanical allodynia. Adoptive transfer of either CD3+ or CD8+, but not CD4+, T cells to Rag1-/- mice normalized resolution of pain. In the model of paclitaxel-induced neuropathic pain, the number of T cells in lumbar dorsal root ganglia (DRG) increased and CD8+ T cells were the major subset. Inhibition of endogenous IL-10 signaling by intrathecal injection of anti-IL-10 to WT mice or Rag1-/- mice reconstituted with CD8+ T cells delayed recovery of paclitaxel-induced mechanical allodynia. Intrathecal anti-IL-10 treatment also prolonged inflammatory pain. In addition, recovery was also delayed in IL-10 knock-out mice. Conversely, administration of exogenous IL-10 attenuated paclitaxel-induced allodynia and promoted resolution of inflammatory pain. In vitro, IL-10 suppressed abnormal paclitaxel-induced spontaneous discharges in DRG neurons. Paclitaxel increased IL-10 receptor expression in the DRG but only in the presence of CD8+ T cells. In conclusion, we identified a novel mechanism for resolution of pain that requires CD8+ T cells and endogenous IL-10. We propose that CD8+ T cells increase DRG IL-10 receptor expression and that IL-10 suppresses the abnormal paclitaxel-induced spontaneous discharges by DRG neurons to promote resolution. Clinically, peripheral blood CD8+ T cell function and/or the capacity of individuals to produce IL-10 may represent biomarkers of risk for developing persistent peripheral neuropathy after completion of cancer treatment.

Disclosures
None.

Bridge between neuroimmunity and traumatic brain injury; immunopharmacology approaches for diagnosis/treatment of neurodegenerative diseases

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Aberrant innate and adaptive immune responses are effectors of neurodegenerative, neuroinfectious and TMJ disorders. Disease is heralded by a generalized, but subtle immune activation orchestrated by the release of extracellular prion-like aggregated and oxidized or otherwise modified proteins. These are responsible for an inflammatory neurotoxic cascade. The perpetrators of such events include effector T cells and activated microglia. What ensues are, for example, Alzheimer's and Parkinson's disease, amyotrophic lateral sclerosis and stroke with changed frequencies of effector T cell and reduced numbers or function of regulatory lymphocytes. The control of such immune responses could lead to new therapeutic strategies and the means to effectively combat a composite of diseases that currently have quite limited therapeutic options.

Disclosures
None

Specification and maturation of nociceptive neurons from human pluripotent stem cells

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Background
Chronic pain is a debilitating condition, which directly affects about one-fifth of the global population. Nociceptive neurons play an essential role in pain sensation by transmitting painful stimuli to the central nervous system. However, investigations of nociceptive neuron biology have been hampered by the lack of accessibility of human nociceptive neurons.

Materials and methods
Based on our successful generation of neural lineage cells and spinal progenitors from human pluripotent stem cells (hPSCs), we investigated the specification and maturation of nociceptive neurons from hPSCs by establishing a chemically defined system. The mRNA and protein expressions of various neural markers at different time points after differentiation were examined by RT-PCR and immunostaining, respectively. The function of sensory neurons was evaluated using calcium imaging.

Results
We established a system for efficiently guiding human pluripotent stem cells into nociceptive neurons by first inducing these cells to the neural lineage. Subsequent addition of retinoic acid and BMP4 at specific time points and concentrations yielded a high population of neural crest progenitor cells (AP2α+, P75+), which further differentiated into nociceptive neurons (TRKA+, Nav1.7+, P2X3+). The overexpression of Neurogenin 1 (Neurog1) promoted the neurons to express genes related to sensory neurons (Peripherin, TrkA) and to further mature into TRPV1+ nociceptive neurons. Importantly, the overexpression of Neurog1 increased the response of these neurons to capsaicin stimulation, a hallmark of mature functional nociceptive neurons.

Conclusions
Taken together, this study reveals the important role that Neurog1 plays in generating functional human nociceptive neurons and provides a potential tool for high throughput screening of therapeutic agents for pain.

Disclosures
None

Pain begets pain: towards a mechanistic understanding of chronic overlapping pain

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Background
Pain and stress have a reciprocal interaction and are potentially modifiable risk factors for poor health outcomes including chronic pain. However, the interaction between pain and stress to generate de novo pain is not fully understood. Many chronic pain conditions have minimal identifiable origins in organic disease, tend to overlap in presentation and are referred to as Chronic Overlapping Pain Conditions (COPCs). Generally these conditions are more prevalent or exclusive to women and symptoms can be exacerbated or triggered by stress. Current thinking focuses on modifications to shared neural, immune and endocrine mechanisms underlying altered CNS function contributing to pain hypersensitivity. Our studies look at the effects of orofacial pain and stress to induce de novo visceral hypersensitivity.

Materials and methods
Intact male and female rats and ovariectomized rats with E2 replacement were stressed using a 3 day forced swim paradigm. Direct (visceromotor response to colorectal distention (VMR)) and indirect (referred pain) visceral sensitivity and orofacial mechanosensitivity were measured. Orofacial pain was produced by either intramuscular CFA or infraorbital nerve injury.

Results
Stress induced visceral hypersensitivity with longer duration in female rats. The hypersensitivity was estrogen dependent, independent of sex and tempered by testosterone. The presence of orofacial pain followed by stress further increased the duration of visceral hypersensitivity (comorbid pain). Multiple mechanisms contribute to the stress and comorbid-induced pain. Peripherally restricted CRF receptor antagonists and mast cell stabilizers prevented, but did not reverse, the stress and comorbid induced visceral hypersensitivity. Colonic lidocaine attenuated the VMR but referred pain persisted, indicating that central sensitization contributes to the visceral hypersensitivity. fMRI revealed changes in supraspinal processing of noxious visceral stimuli during stress-induced visceral hypersensitivity. This likely reflects changes in descending 5-HT function following orofacial pain and stress. Finally, RNA deep sequencing revealed significant changes in gene expression in the spinal cord during stress-induced and comorbid pain-induced visceral hypersensitivity.

Conclusions
Stress induces visceral hypersensitivity that is more robust in females, likely due to action by estradiol. However, the duration of stress-induced visceral hypersensitivity is relatively short- days to weeks. When the nervous system is primed by a prior painful event, the same stressor induces visceral hypersensitivity that persists for months, a transition from acute to chronic pain. This animal model of overlapping pain (mimicking TMD and IBS) can be useful to investigate neural, immune and hormonal mechanisms that contribute to the generation and maintenance of chronic overlapping pain conditions in afflicted patients.

Acknowledgements

Disclosures
None

Comparing experimental pain sensitivity and endogenous pain modulatory processes in men and women

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Background
Previous research indicates that women experience a variety of clinical pain syndromes at a rate and severity higher than their male counterparts, including orofacial pain (among others). One explanation may be that increased pain sensitivity and disruption of endogenous pain modulatory processes contribute to these sex differences. However, previous studies addressing this hypothesis have resulted in mixed findings. Additionally, possible sex differences in pain sensitivity across the adult lifespan have not been fully assessed. Thus, two studies are presented in an effort to evaluate sex differences in pain sensitivity and endogenous pain modulation using quantitative sensory testing (QST).

Materials and methods
In both studies, healthy men and women each completed a QST battery. In Study 1, 48 young, healthy participants (ages 19-45) completed an ischemic pain task (IPT) that used a submaximal effort tourniquet procedure as well as a conditioned pain modulation (CPM) procedure for the assessment of endogenous pain inhibition [1]. Study 2 involved a separate sample of 241 healthy participants from across the adult lifespan (ages 19-76). Participants in the second study underwent QST procedures that assessed pain sensitivity including endogenous pain facilitation via temporal summation (TS) of heat and mechanical pain stimuli.

Results
In both studies, analyses revealed significant sex differences such that women demonstrated lower thresholds and tolerances to multiple modalities of pain stimuli, including ischemia (Study 1), heat (Study 2), and pressure (Study 2) when compared to men. Additionally, women demonstrated significantly decreased CPM (Study 1) and increased TS (Study 2) in comparison to their male counterparts (all p's<0.05).

Conclusions
This study provides evidence suggesting that women may be more pain sensitive and demonstrate less efficient pain inhibition as well as greater pain facilitation than their male counterparts. This is important, as women are often at increased risk for the development of chronic pain. It has been suggested that responses to QST may be better related to the clinical pain experiences of women compared with men. As such, it may be that less efficient endogenous pain modulation plays a contributory role to increased prevalence of clinical pain conditions – including orofacial pain – in women. Further research is necessary to confirm or refute such a hypothesis. Additionally, further investigations into interventions that may decrease pain sensitivity and enhance pain inhibition in women are warranted, particularly when considering possible impacts on clinical pain outcomes.

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Disclosures

The authors declare no conflicts of interest.

Epigenetic regulation of neuropathic pain

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Neuropathic pain, which is related to peripheral or central nervous system injury, is difficult to treat since it does not respond well to typical analgesics. Previous studies have shown that peripheral nerve injury can reduce the expression of pain related genes, such as potassium channels and opioid receptors, in primary sensory neurons [1,2]. Epigenetic mechanisms, including DNA methylation and histone modification, have been linked to the alteration of gene expression [3,4]. Here, we studied histone methylation in neuropathic pain genesis. Using quantitative real-time reverse transcription polymerase chain reaction (qPCR) analysis, Western blot analysis, or immunohistochemistry, we found increased mRNA and protein expression of G9a, a histone methyltransferase, in injured mouse dorsal root ganglion (DRG) after L4 spinal nerve ligation (SNL). A G9a inhibitor or genetic knockdown of G9a relieved SNL induced mechanical allodynia, thermal hyperalgesia and cold allodynia. We further found that G9a is required for nerve injury-induced epigenetic silencing of potassium channels (Kv1.2 and Kv1.4) and opioid receptors (mu, kappa and delta), which are key players in pain transmission. Functionally, DRG G9a overexpression increased mu opioid receptor-gated primary afferent neurotransmitter release, reduced voltage-gated potassium channel current, increased excitability in the DRG, and led to pain hypersensitivities. Conversely, DRG G9a inhibition/knockdown restored the decrease of morphine analgesia, prevented the development of morphine-induced tolerance, and mitigated neuropathic pain. Finally, we concluded that G9a may be a potential target for future neuropathic pain treatment.

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Exploring the epigenetic mechanisms for individual pain vulnerability

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Background
Clinical data clearly show that there is considerable individual variance in response to pain stimuli and to pain drugs, and consequently in vulnerability to developing chronic pain. Many factors, including genetic background, prior pain experience and previous stressful life events, may contribute to the pain vulnerability. These psychological and environmental events could influence pain vulnerability by markedly changing an individual's epigenetic landscape that regulates gene expression in adaptive responses to those stressful events. However, individual variance in pain responses and pain vulnerability has been rarely addressed in current preclinical studies on animals. In this study, we examined individual variations in behavioral responses of both sensory and affective pain in rats, and explored a pain-induced epigenetic modification that may contribute to the pain variation and vulnerability for chronic pain development.

Materials and methods
Chronic condition of neuropathic pain was induced in rats by partial nerve ligation. Sensory pain responses were measured by the thermal paw withdrawal test and mechanical von Frey test. Affective emotion responses to pain were measured by the open filed test and elevated plus maze test. An AAV-CaMKII-ChR2-EGFP viral vector was injected into the parabrachial (PB) nucleus for optogenetic stimulation of the glutamatergic projections from PB neurons to central nucleus of the amygdala (CeA).

Results
We found that, while individual rats with chronic pain were not much variable in their behavioral response to sensory pain, the animals displayed considerable variance in their affective emotion behaviors, ranging from significant anxiety behavior to absence of the behavior at 30 days after the pain induction. In CeA, the protein level of DNA methyltransferases that catalyze de novo DNA methylation for gene repression was significantly altered by the pain condition. Photostimulation of the excitatory PB-CeA glutamatergic projections in CeA induced significant anxiety-like behavior in naïve rats.

Conclusions
The results indicate that individual variance in pain responses is mostly manifested in the affective component of pain in rats; and maladaptation in DNA methylation and its potential target of glutamate receptors in CeA may play an important role in the individual variance of affective pain.

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Disclosures
None

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

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Background

Pathological pain conditions in orofacial regions such as temporomandibular disorders (TMD) are often manifested by mechanical allodynia, a severe pain state that can be triggered by a gentle touch. Orofacial mechanical allodynia is a main clinical pain problem and is poorly managed by the currently available pain medicines. One main reason for the poor management of orofacial mechanical allodynia is the lack of scientific knowledge about how a gentle touch is sensed by our sensory system in orofacial regions.

Materials and methods

Rodent whisker hair follicles, important touch-sensing structures in orofacial regions, were used in our studies to explore cellular and molecular mechanisms underlying the sense of gentle touch in orofacial regions. Multiple approaches including electrophysiology, molecular biology, immunohistochemistry, pharmacology and mouse genetics were used in the studies.

Results

Our studies show that epidermal Merkel cells in whisker hair follicles are equipped with the recently cloned mammalian mechanical transducers Piezo2 ion channels and that Merkel cells are primary sites of sensory impulse initiation in response to a gentle touch to a whicker hair. We also demonstrate that tactile signals are synaptically transmitted from Merkel cells to their associated Aβ-afferent fibers to lead to the sense of touch.

Conclusions

Our studies provide novel insights into the transduction and transmission of gentle touch within whisker hair follicles and shall have implications in orofacial mechanical allodynia observed in temporomandibular disorders and other pathological pain conditions in orofacial regions.

Acknowledgements

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Disclosures

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Exploiting the immune response to illuminate host-microbiota interactions

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The composition of the gut microbiota is thought to have dramatic effects on the development and progression of a variety of diseases, including Inflammatory Bowel Disease (IBD), autoimmunity, and metabolic syndrome. However, identifying the specific bacteria that preferentially affect disease susceptibility and severity in humans remains a major challenge. In response to this problem, we developed a novel technology that uses the host's own immune response to the microbiota as a guide to identify specific members of the gut microbiota that preferentially modulate disease development. This approach specifically identified known disease-causing intestinal bacteria in a mouse model of microbiota-driven colitis. Furthermore, we were able to use this approach to identify specific bacterial strains from IBD patients that selectively conferred susceptibility to severe colitis when transplanted into germ-free mice. These studies thus: (i) establish a new strategy for the identification of disease- and immune-modulating members of the microbiota in humans; (ii) identify potentially disease-driving members of the intestinal microbiota in humans with IBD; and (iii) begin to establish causal, rather than correlative, connections between specific changes in the microbiota and human disease. Future studies using similar approaches will allow us to elucidate the full spectrum of reciprocal interactions between the microbiota and the host immune system. These studies will lead to a more complete understanding of the role of microbiota composition in human health and disease and will eventually enable the development of novel and specific microbiota-targeted therapeutics.

Disclosures
None

The impact of genetic testing for pain perception in the clinical management of chronic non-cancer pain

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Genetic markers of pain sensitivity, such as single nucleotide polymorphisms in the catechol-O-methyltransferase gene, have been shown to be associated with pain perception and have been used to provide objective information about a patient's pain.

A prospective, longitudinal study was conducted with 134 chronic non-cancer pain patients genotyped for pain perception-related catechol-O-methyltransferase haplotypes. Physicians were provided with patients' results and asked to document 1) their assessment of benefit of the genetic test; 2) treatment changes made based on the genetic test; and 3) patient clinical responses to changes implemented.

Based on genetic testing results, physicians adjusted treatment plans for 40% of patients. When medication changes were made based on genetic testing results, 72% of patients showed improvement in clinical status. When non-pharmacological actions were performed, 69% of physicians felt their patients' clinical status improved. Moreover, physicians believed the genetic test results were consistent with patient pain levels in 85% of cases.

These results demonstrate that providing personalized medicine with genetic information related to pain perception affected physician clinical decision-making for a substantial proportion of patients in this study, and that the availability and utilization of this information was a contributing factor in clinical improvement.

Our aim is to combine phenotypic and proven genetic factors that influence pain to produce a test that will: 1) identify individuals at risk of TMJ/TMD and 2) for those individuals at risk or suffering from TMJ/TMD, determine specific, actionable treatment recommendations based on their individual genetics.

Disclosures

Travel Awardee

Poster Abstracts

Poster A

A prediction error model of placebo analgesia and its extinction

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Background
Placebo analgesia can ameliorate the experience of pain[1,2]. There has yet to be a model created that can predict how an individual will respond to a placebo analgesic procedure and how long lasting these effects will be[3]. Therefore, the aims for this study were to use a prediction error model to: 1) predict placebo analgesia on an iterative trial-by-trial basis, 2) identify factors that lead to extinction or non-extinction of placebo analgesia, and 3) identify predictors of whether individuals experience placebo analgesia or not.

Materials and Methods
A prediction error model of placebo analgesia was developed and applied to data from two separate placebo analgesia experiments in humans using painful-like electrical stimulations and a placebo conditioning procedure. Both of these experiments measured expectancy and pain rating before and directly after the introduction of a placebo manipulation. We developed a model to provide predictions of placebo analgesic responses based on the specific treatment administered on each trial (placebo vs. control) and level of pain stimulation received by individuals during the conditioning. The model computed a perceived pain index as a simple weighted average of expectancy and actual pain stimulation.

Results
The implemented prediction error model provided a strong quantitative fit to the placebo analgesia data obtained from the two experiments. When fit separately, the ideal parameters across the two experiments were generally highly concordant, indicating that the model is generalizable across experiments that varied in non-essential aspects of procedure, indicating good external validity. Initial expectancy was one of the most important parameters in terms of the likelihood of an individual exhibiting placebo analgesia, whereas parameters such as learning rate did not appear to predict whether an individual experienced placebo analgesia or not. Initial expectancy also predicted the extent to which any placebo analgesia extinguished, with higher initial expectancies leading to slower extinction. However, learning rate was a significant predictor of extinction, with individuals with a slower learning rate demonstrating increased resistance to extinction, i.e., longer lasting placebo analgesia during test.

Conclusions
In this project, we tested the extent to which a prediction error model can account for placebo analgesia and its rate of extinction in two distinct experiments. The developed model was effective in predicting placebo analgesia and its extinction. These results point to a potential advantage in understanding the mechanisms of placebo analgesia. Researchers and clinicians may be able to develop clinical interventions that capitalize on the pain modulation to improve patient pain outcomes[4].

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Poster B

Clinical characteristics of TMD at onset and predictors of persistence: preliminary results

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The prospective study of first-onset TMD that is part of OPPERA (Orofacial Pain: Prospective Evaluation and Risk Assessment) has assessed initially TMD-free individuals and identified clinical characteristics that are associated with higher risk of developing TMD (1). In the context of clinical care, such characteristics are more likely to be assessed when individuals are symptomatic and first present for care. However, it is not known whether any incidence risk factors assessed at initial consultation are useful for predicting TMD persistence. Thus, we evaluated clinical characteristics of the individual assessed at the time of TMD onset in order to develop a prediction model using variables that can be easily and reliably assessed by clinicians using the DC/TMD protocol(2) in order to identify first-onset TMD patients who are likely to have their symptoms persist versus those who are likely to recover within the subsequent six months. In addition, this study will also describe the clinical characteristics of TMD at onset and at the 6-month follow-up visit. The data presented here are from a nested case-control study of first-onset TMD that was part of the OPPERA project, which enrolled 260 incident cases of first-onset TMD and followed them over an approximately six-month period, when TMD case classification was re-assessed. TMD case classification was determined by a calibrated examiner using the RDC/TMD protocol. Additional variables that fulfill the DC/TMD protocol were also collected. At follow-up, 147 (56.6%) incident cases were re-examined, of which 72 (49%) presented "persistent" TMD and 75 (51%) no longer had examiner-verified TMD and were labeled "transient" TMD cases. Preliminary results show that a multivariable regression model including the number of masticatory muscles with pain on maximum unassisted opening, number of masticatory muscles tender to palpation, and count of comorbid conditions, performs best at distinguishing persistent from transient TMD. Specifically, the model provides an area under the ROC curve (AUC) of 0.77, as opposed to an AUC of 0.61 obtained with demographic variables only, suggesting that these clinical variables may be useful as predictive markers for the progression of the disorder. Clinically, this means that the time of onset represents a pragmatic point at which clinicians would be able to identify those at higher risk of persistence, allowing informed decision-making about who should receive treatment – and presumably which type of treatment – in order to avoid TMD persistence and eventual chronification of the disorder.

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Disclosures

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Poster C

The behavioral and neural effects of multipotent stromal cells in rodent models of persistent pain

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Multipotent stromal cells (MSCs) have shown potential to treat chronic pain. However, their efficacy and mechanisms of action under different pain conditions are still elusive. To extend our previous observation by utilizing bone marrow stromal cells (BMSCs), a major type of MSCs, we provide further evidence on the pain-attenuating effect of BMSCs in three rodent pain models. Women exhibit higher prevalence of orofacial pain than men, but it is unclear whether BMSCs produce pain relief in females as that in males. In a model of orofacial pain involving injury of a tendon of the masseter muscle, rat BMSCs (1.5 M cells, i.v.) reduced mechanical hyperalgesia and conditioned place avoidance behavior in female rats. The pain-attenuating effect of BMSCs in females lasted for 28-56 d, apparently shorter than that seen in males. To coincide preclinical findings with clinical conditions, we used human BMSCs in rats after L5 spinal nerve ligation (SNL) injury. Human BMSC (1.5 M cells, i.v.) also attenuated mechanical and thermal pain hypersensitivity and suppressed SNL-induced aversive behavior, and the effect persisted through the 8-week observation period. In a trigeminal slice preparation derived from mice with chronic constriction injury of the infraorbital nerve, BMSC treated-animals showed reduced amplitude and frequency of spontaneous miniature excitatory postsynaptic currents, compared to naïve and culture medium-treated mice. Electrical stimulation-evoked and NMDA receptor-dependent synaptic current was also reduced in BMSC-treated animals. These results suggest inhibition of trigeminal neuronal hyperexcitability and nociceptive primary afferent input. Further, GluN2A tyrosine phosphorylation and PKCγ immunoreactivity in the rostral ventromedial medulla (RVM), a key site for descending pain modulation, was suppressed at 8-week after BMSC in tendon-injured rats. As PKCγ activity related to NMDA receptor activation is critical in opioid tolerance, these results may explain long-term pain-relieving effect of BMSCs, which requires opioid receptors in RVM and apparently lacks the development of tolerance. These results provide convergent evidence that supports the use of BMSC in pain control.

Poster D

Structural and functional abnormalities in chronic orofacial pain disorders: a meta-analytic study

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Chronic orofacial pain (COFP) disorders are prevalent and debilitating pain conditions affecting the head, face and neck areas. COFPs comprise several etiologically different disorders, either arising from the periphery, such as musculoskeletal abnormalities in the orofacial region and neuropathic and neuralgic conditions, or from the central nervous system, such as functional pain disorders. A notable clinical challenge in the treatment of these disorders is the interindividual variability in the manifestation and clinical measures of the chronic pain. The diversity of chronic orofacial pains, and interindividual factors that contribute to the clinical presentation of these disorders make them particularly difficult to diagnose and to treat. COFPs are associated with complex patterns of central neural mechanisms reflected in the structure and function of the brain. Finding convergent structural and functional brain abnormalities across these phenotypically different COFPs could allow us to understand the CNS mechanisms common to all these conditions. Here, we review the COFP neuroimaging literature and perform three coordinate-based meta-analyses to examine (1) structural and (2) functional brain abnormalities of patients with COFP compared to healthy, pain-free subjects. The structural MRI meta-analysis identified 12 peer-reviewed articles met the study criteria, and revealed widespread gray matter abnormalities in COFP: gray matter increases in nociceptive processing regions, including the orofacial region of the primary somatosensory and motor cortices (S1, M1), thalamus, the basal ganglia, medial and dorsolateral prefrontal cortices (mPFC, dlPFC), the anterior cingulate cortex (ACC) and the insula (INS). COFP patients had lesser grey matter in the hand region of S1/M1, the hippocampus, the bilateral superior temporal gyrus, and the posterior cingulate cortex. The functional brain imaging meta-analysis identified 22 peer-reviewed studies comparing COFP patients and pain-free subjects that met study criteria, and showed that patients had greater activation in several regions, including the STG, DLPFC, S1, M1, the secondary somatosensory cortex (S2), the basal ganglia and the hippocampus. Patients, however, had less activation in bilateral thalamus and INS. These results suggest that the structural and functional abnormalities may be related, and that there is increased nociceptive drive in COFP, and decreased pain modulation. Overall, these findings of structural and functional abnormalities in the brain of COFP patients could be used to develop novel diagnostic and prognostic tools.

Poster E

Lack of evidence for ectopic sprouting of genetically labeled Aβ touch afferents in inflammatory and neuropathic trigeminal pain

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Background
Mechanical and in particular tactile allodynia is a hallmark of chronic pain in which innocuous touch becomes painful. Previous cholera toxin B (CTB)-based neural tracing experiments and electrophysiology studies had suggested that aberrant axon sprouting from touch sensory afferents into pain-processing laminae after injury is a possible anatomical substrate underlying mechanical allodynia. This hypothesis was later challenged by experiments using intra-axonal labeling of A-fiber neurons, as well as single-neuron labeling of electrophysiologically identified sensory neurons. However, no studies have used genetically labeled neurons to examine this issue, and most studies were performed on spinal but not trigeminal sensory neurons which are the relevant neurons for orofacial pain, where allodynia oftentimes plays a dominant clinical role.

Materials and methods
We recently discovered that parvalbumin::Cre (Pv::Cre) labels two types of Aβ touch neurons in trigeminal ganglion. Using a Pv::CreER driver and a Cre-dependent reporter mouse, we specifically labeled these Aβ trigeminal touch afferents by timed taxomifen injection prior to inflammation or infraorbital nerve injury (ION transection).

Results
We examined the peripheral and central projections of labeled axons into the brainstem caudalis nucleus after injuries vs controls. We found no evidence for ectopic sprouting of Pv::CreER labeled trigeminal Aβ axons into the superficial trigeminal noci-receptive laminae after inflammation or nerve injury. Furthermore, there was also no evidence for peripheral sprouting.

Conclusions
CreER-based labeling prior to injury precluded the issue of phenotypic changes of neurons after injury. Our results suggest that touch allodynia in chronic orofacial pain is unlikely caused by ectopic sprouting of Aβ trigeminal afferents.

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Disclosures
None of the authors have conflicts of interest with respect to this work.

Poster F

Mechanical and inflammatory mediated degeneration of mandibular cartilage is associated with altered NG2-type VI collagen colocalization and cytosolic NG2 residues in articular chondrocytes

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Background
TMJ degenerative joint disease (DJD) is of an unknown etiology and has few available interventional options. One key aspect of DJD is pervasive chondrocyte cell death or chondroptosis that limits homeostatic repair of the condylar cartilage following mechanical or inflammatory damage. This is an unappreciated target for both clinical interventional and translational research. Cell-matrix derived signaling is an established regulator of both cell viability and tissue homeostasis. The major component of the pericelllular matrix surrounding chondrocytes of the mandibular condylar cartilage (MCC) is type VI collagen. A known receptor for type VI collagen is the transmembrane proteoglycan, Nerve/glial antigen 2 (NG2). Colocalized NG2-type VI collagen has been illustrated in various cell types and is known to be a key regulator of cell proliferation, migration, differentiation, and viability. NG2 has a hypothesized role as a transcriptional regulator through the cleavage of its ectodomain and subsequent nuclear translocation of the intracellular domain. Despite its regulatory roles in various cell types, the significance of the NG2-ype VI collagen relationship has yet to be elucidated in the proliferative chondrocytes of the MCC.

Materials and methods
Using a surgical instability mouse model, DJD was induced in the temporomandibular joint of male c57 mice. Tissue was collected at 0, 4, 8, 12 and 16 weeks post-operatively. NG2-type VI collagen colocalization was calculated from immunofluorescence confocal microscopy using WCIF_ImageJ plugin (Toronto Western Research Institute, Canada), and with a proximity ligation assay confirming the spatial correlation between residues of NG2 and its ligand, type VI collagen.

Results
In non-surgical controls, NG2-type VI collagen colocalization coefficients were high in articular, prechondroblastic, and chondroblastic chondrocytes but not hypertrophic chondrocytes. On the medial aspect of 4, 8 and 12 week post-operative joints, colocalization coefficients slightly decreased. At 8 weeks post-operative, cytosolic localization of the NG2 intracellular domain was observed in articular cells. These changes were also associated with remarkable spatiotemporal changes in NG2 expression relative to structural matrix proteins such as type VI collagen. Type VI collagen was found to encompass proliferative chondrocytes in a tight band at 0 and 4 weeks post-operative, become progressively more diffuse and spatially disorganized as degeneration progressed (n=3). This degenerative change was concentrated on the medial aspect of the joint.

Conclusions
Together, these data support the hypothesis that the transmembrane proteoglycan NG2 colocalizes with type VI collagen in the pericellular matrix, that NG2-type VI collagen interactions mediate cell-matrix derived signaling, and that disruption of this signaling could promote cartilage degeneration.

Acknowledgments

Poster G

Lateral thalamic control of nociceptive response after whisker pad injection of varicella zoster virus

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Background

Herpes Zoster (HZ) leads to post-herpetic neuralgia (PHN) in twenty percent of patients causing chronic pain that can be debilitating. Currently using a novel orofacial model for herpes zoster associated pain we are able to investigate mechanisms controlling the pain responses that are not yet understood. Herpes zoster infection which results from reactivation of a latent varicella zoster virus (VZV) will produce orofacial pain in about a quarter of HZ patients. Studies suggest central pathways involving the thalamus could control pain related to HZ, and preliminary studies in our lab suggest vesicular GABA transporter (VGAT) in the lateral thalamus influences orofacial pain.

Methods

VZV was injected into the whisker pad of Sprague Dawley male and female rats. Affective and motivational aspects of pain were measured using the Place Escape/Avoidance Paradigm. Thalamic neuronal activity was modulated after injecting an adeno-associated virus (AAV) expressing an engineered acetylcholine Gi-protein coupled receptor. This receptor inhibits neuronal burst firing when bound by clozapine-n-oxide (CNO). VGAT expression was attenuated in the thalamus by injecting an AAV construct that expressed a VGAT silencing shRNA.

Results

VZV induced nociception was significantly decreased after administering CNO in both male and female rats. Nociception significantly increased concomitant with increased thalamic c-fos expression after attenuating thalamic VGAT expression.

Conclusions

This data establishes that the lateral thalamus (posterior, ventral posteromedial, ventral posterolateral and reticular thalamic nucleus) controls VZV induced nociception in the orofacial region, and that GABA in this region appears to reduce the response to VZV induced nociception possibly by gating facial pain input.