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SEVENTH SCIENTIFIC MEETING
OF THE TMJ ASSOCIATION

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

Meeting Abstracts

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Epidemiology of temporomandibular joint disorders and related painful conditions

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Epidemiology uncovers patterns of disease distribution in human populations and seeks determinants of those patterns. With recent emphasis on chronic pain as “a disease in itself”, it is informative to compare how the distribution of temporomandibular joint disorders (TMJD) compares with that of related pain conditions. Over two decades of National Health Interview Surveys (1989 to 2009), the prevalence of self-reported TMJD symptoms remained stable, affecting 5% of U.S. adults. In 2009, prevalence was greater in females than males, and increased with age to midlife before decreasing in older age. While racial- and ethnic-group differences were small, there was a pronounced income gradient, with greater prevalence at lower household income. Similar distributions according to gender, age and income occurred for headache and neck pain, although not for low back pain. There was also marked overlap of TMJD with those related pain conditions, irrespective of whether they occurred above or below the shoulders. Moreover, there was significant overlap of TMJD with non-painful medical conditions. In order to understand reasons for this overlap, prospective studies of TMJD incidence are needed to discover *determinants* of the disease. In the community-based OPPERA prospective cohort study, TMJD incidence was measured in 2,737 adults aged 18-44 years who had no significant history of TMJD when enrolled. During three years of follow-up, 19% of people per annum developed TMD symptoms and for a quarter of symptomatic episodes, pain intensity was severe. Examiner-verified, first-onset TMJD developed at an annual rate of 3.5% per annum, although the rate was approximately doubled in study participants who, at enrollment, reported related pain conditions. Likewise, TMJD incidence was strongly associated with a checklist of 20 non-specific health conditions reported at enrollment, ranging from depression to sleep apnea. Yet, by virtue of the study design, study participants had no TMJD at enrollment, meaning that the related pain conditions and other health conditions did not “overlap” concurrently with TMJD. Instead, they represent risk factors for development of TMJD. In fact, in multivariable analysis, related pain and other health conditions were among the strongest predictors of first-onset TMD. Furthermore, their effects on risk of developing TMJD were independent of conventional risk factors for TMJD.

Conclusion

Impaired general health, whether painful or not, is an important risk factor for development of painful TMJD.

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Disclosures

Gary Slade is a consultant and shareholder in Algyonics Inc., a company that does pain research.

Characterization of individuals with chronic pain: phenotyping approaches used in MAPP

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Pain is a complex perception affecting one's state of consciousness, functional status, and quality of life. Clinically, pain intensity is often the only facet of pain that is assessed; but two large research networks (i.e., the Orofacial Pain: Prospective Evaluation and Risk Assessment (OPPERA) [1] and the Multi-Disciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) [2]) have characterized pain in accordance with broader pain concepts. MAPP was designed to characterize individuals with urologic chronic pelvic pain syndromes (UCPPS) which consisted of diagnoses such as interstitial cystitis, bladder pain syndrome, chronic prostatitis, and chronic pelvic pain syndrome. Characterization of the sample of n=1039 individuals occurred at multiple sites and at multiple levels of analysis including: biomarkers, self-report questionnaires, quantitative sensory testing (QST), functional neurobiological studies, and structured and resting state neuroimaging studies. A comprehensive assessment occurred at baseline followed longitudinally by biweekly or bimonthly internet questionnaires and more extensive in-clinic visits at 24 and 48 weeks following baseline [3]. The self-report methods covered both urologic-specific and non-urological domains relevant to chronic pain which are consistent with the bio-psycho-social model of pain. Urological domains included urological diagnostics, symptoms and impact, sexual functioning, self-esteem, and social relationships. Non-urological-specific self-assessment included clinical pain, functional status, mood, co-morbid conditions, personality, attitudes/ beliefs, and early life trauma. Biological specimens linked to clinical data included cheek swabs and plasma, as well as urine for exploration of infectious etiology. Also linked to the clinical data was quantitative sensory testing (QST) which helped to characterize individuals with respect to pain threshold, and neuroimaging studies providing structural, functional and network connectivity data corresponding to central pain processing and modulation. Ultimately, the goal of such extensive phenotyping will be to identify subgroups of individuals with UCPPS who have distinct underlying pathophysiology to which more optimal treatment approaches can be aligned thus offering the potential for improved disease management and improved patient care.

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References

1. **Maixner, W. Diatchenko, L., Dubner, R. et al.**: Orofacial pain prospective evaluation and risk assessment study – the OPPERA study. **J Pain** 2011, 12:T4-11.
2. **Clemens, J. et al.**: The MAPP research network: A novel study of urologic chronic pelvic pain syndromes. **BMC Urology**. In Press.
3. **Landis, J.R. et al.**: The MAPP research network: Design, patient characterization, and operations. **BMC Urology**. In Press.

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Translational research in the genomic era: OPPERA study

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Background

Temporomandibular Disorder (TMD) tends to coexist with other chronic pain conditions in affected individuals and is characterized by a report of pain greater than expected based on the results of a standard physical evaluation. The pathophysiology of this condition is largely unknown, the scientific field lacks biological markers for accurate diagnosis, and conventional therapeutics have limited effectiveness. Growing evidence suggests that chronic pain conditions are associated with both physical and psychological triggers, which initiate pain amplification and psychological distress; thus, susceptibility is dictated by complex interactions between genetic and environmental factors [1].

Materials and methods

The large human study named OPPERA, Orofacial Pain Prospective Evaluation and Risk Assessment Study, measures both phenotypic and genotypic markers in the TMD patients. The phenotypic markers of greatest interest include measures of pain amplification and psychological measures such as emotional distress, somatic awareness, psychosocial stress and catastrophizing. Genetic markers are also measured in a study by genotyping 2,924 single-nucleotide polymorphisms representing 358 genes known to be involved in systems relevant to pain perception [1].

Results

The OPPERA findings provided evidence for few single single-nucleotide polymorphisms to be associated with risk of TMD [2]. Furthermore, several single-nucleotide polymorphisms exceeded Bonferroni correction for multiple comparison or false discovery rate thresholds for association with intermediate phenotypes shown to be predictive of TMD onset [3] One of the genes on which we focused our initial research efforts on was the epidermal growth factor receptor (EGFR). EGFR is activated by numerous endogenous ligands that traditionally promote cellular growth, proliferation and tissue regeneration. We first identified that SNPs in the gene loci encoding for EGFR and EREG are associated with the risk of chronic TMD in OPPERA and two other independent human cohorts. Subsequent experiments in animal models reveal the functional involvement of these proteins in the pain pathway, show pharmacological and genetic modulation of pain behavior in rodents and *Drosophila*, and define the relevant signaling pathway. EGFR–ErbB-4 heterodimer activation by EREG produces pain by regulating the PI3K/AKT/mTOR translational machinery and matrix metalloproteinase-9. As a result of these studies, EREG and EGFR–ErbB-4 can be viewed as novel targets for analgesic development.

Conclusions

Elucidation of the biological mechanisms by which these markers contribute to the perception of pain in these patients will enable the development of novel effective drugs and methodologies that permit better diagnoses and approaches to personalized medicine.

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This abstract is being presented on behalf of an OPPERA study group

References

1. Maixner W, Diatchenko L, Dubner R, Fillingim RB, Greenspan JD, Knott C, Ohrbach R, Weir B, Slade GD. Orofacial pain prospective evaluation and risk assessment study--the OPPERA study *J Pain*. 2011;12(11 Suppl):T4-11.e1-2
2. Smith SB, Maixner DW, Greenspan JD, Dubner R, Fillingim RB, Ohrbach R, Knott C, Slade GD, Bair E, Gibson DG, Zaykin DV, Weir BS, Maixner W, Diatchenko L. Potential genetic risk factors for chronic TMD: genetic associations from the OPPERA case control study. *J Pain*. 2011;12(11 Suppl):T92-101.
4. Smith SB, Mir E, Bair E, Slade GD, Dubner R, Fillingim RB, Greenspan JD, Ohrbach R, Knott C, Weir B, Maixner W, Diatchenko L. Genetic variants associated with development of TMD and its intermediate phenotypes: the genetic architecture of TMD in the OPPERA prospective cohort study. *J Pain*. 2013;14(12 Suppl):T91-101

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Mechanisms of chronic pain

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Chronic pain is classified as nociceptive or neuropathic, depending on whether the integrity of the somatosensory nervous system is compromised by the underlying disease. Nociceptive pain results from the activation of receptors (nociceptors) sensitive to noxious stimuli. Prolonged or intense exposure to these stimuli, for example, chemical mediators released during inflammation, enhances the responsiveness of nociceptive nerve fibers [1]. This process, termed peripheral sensitization, involves a shift in the activation threshold of nociceptors and upregulation of voltage-gated sodium channels. Peripheral sensitization leads to increased action potential firing and transmitter release in the dorsal horn of the spinal cord, where somatosensory information is processed. Dorsal horn neurons react to the rising input with heightened excitability, a process termed central sensitization. Enhanced depolarization leads to the recruitment of N-methyl-D-aspartate (NMDA)-type glutamate receptors. NMDA and neuropeptide receptor activation produces a sharp increase in intracellular calcium, triggering signaling pathways and gene expression changes that promote a long-term shift in the activity of nociceptive circuits [2]. In some aspects, central sensitization even resembles long-term potentiation of excitatory transmission in the hippocampus. Central sensitization generates an exaggerated response to painful stimuli (hyperalgesia) and contributes to pain elicited by normally nonpainful stimuli (allodynia). Clinical findings suggest that pain hypersensitivity produces structural changes in the brain over time. These changes are, however, reversible upon pain relief. The pathophysiology of neuropathic pain is fundamentally different. Peripheral nerve lesion evokes stimulus-independent (ectopic) activity in nerve fibers. Innate immune cells react at the lesion site, in the dorsal root ganglion, where the cell bodies of peripheral somatosensory neurons reside, and in the dorsal horn of the spinal cord [3]. Active microglia of the dorsal horn releases chemical mediators that modulate the activity of neurons in the vicinity. One of these mediators, brain-derived neurotrophic factor, reduces the inhibitory effect of γ -aminobutyric acid (GABA) and glycine. Disinhibition opens polysynaptic connections in the dorsal horn, further enhancing the abnormal input from the lesioned nerve. Similarly to nociceptive pain, central sensitization occurs. Worsened by a relative deficit in transmitter uptake, increased glutamatergic transmission causes excitotoxic cell death, reducing the number of inhibitory interneurons. Their loss and a shift in descending modulatory pathways from the brainstem produce a profound imbalance between inhibition and excitation. The complexity of chronic pain mechanisms poses a major therapeutic challenge. Without biomarkers, it will remain difficult to develop targeted strategies for chronic pain reduction or prevention in the individual patient.

References

1. **Petho G, Reeh PW**: Sensory and signaling mechanisms of bradykinin, eicosanoids, platelet-activating factor, and nitric oxide in peripheral nociceptors. **Physiol Rev** 2012, 92:1699-1775.
2. **Kuner R**: Central mechanisms of pathological pain. **Nat Med** 2010, 16:1258-1266.
3. **Scholz J, Woolf CJ**: The neuropathic pain triad: neurons, immune cells and glia. **Nat Neurosci** 2007, 10:1361-1368.

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Study of chronic orofacial pain with preclinical models

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Background

Chronic orofacial pain conditions such as trigeminal neuropathic pain, reflex sympathetic dystrophy of the face, and temporomandibular pain disorders are debilitating pain conditions. They are known to respond poorly to treatment including opioids in human patients. There is a pressing need to better understand the mechanisms of these chronic orofacial pain conditions so that new and effective therapeutic targets can be identified. To achieve this goal, preclinical animal models that well represent human chronic orofacial pain conditions are needed. In the present study we used two preclinical models of chronic orofacial pain, the chronic constriction nerve injury of the infraorbital nerve (ION-CCI model) and the oxaliplatin-induced orofacial pain (oxaliplatin model). We applied newly developed orofacial operant behavioral assessment to study orofacial pain and to test effects on Kv7.2 channel activators in alleviating orofacial pain in these animals.

Materials and methods

Male Sprague–Dawley rats (300–450 g) were used in this study. In the ION-CCI model a chronic constriction nerve injury was created using unilateral ligation of the infraorbital nerve. For oxaliplatin model of orofacial pain, oxaliplatin was administered to rats (*i.p.*) at 2 mg/kg per day for five consecutive days. Orofacial pain behaviors in responses to mechanical and cold stimuli were assessed by the orofacial operant behavioral assessment method. To test the effects of Kv7.2 activators in alleviating orofacial pain in these two preclinical models, retigabine or CF341 was administered to these animals at the doses ranging from 0.19 to 15 mg.

Results

Rats of both ION-CCI model and oxaliplatin model showed significant orofacial mechanical allodynia and cold allodynia/hyperalgesia as examined by using the orofacial operant assessment method. Retigabine, a classical Kv7.2 channel activator, significantly alleviated orofacial cold allodynia in both ION-CCI model and oxaliplatin model. CF341, a newly synthesized Kv7.2 channel activator, also showed alleviation of the orofacial cold allodynia with efficacy similar to retigabine.

Conclusions

In both ION-CCI model and oxaliplatin model, orofacial operant behavioral assessment gives quantitative measurements of chronic orofacial pain and therapeutic effects of Kv7.2 activators. The preclinical models shown in this work are useful for both mechanistic study and drug development for chronic orofacial pain.

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Modeling TMJD pain in the laboratory mouse: role of TRP ion channels

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Trigeminal pain syndromes such as temporomandibular joint (TMJ) pain appear to have a particular potential to affect patients in a devastating manner. Prevalence of trigeminal pain disorders in the US is estimated at 20-30x10⁶, at >50-75x10⁶ including headaches/migraine. Neural circuit malfunction and maladaptive plasticity arise from altered primary sensory afferents. We have focused on a nerve cell that is the eminent gatekeeper of sensory afferent cues in the trigeminal system, trigeminal ganglion (TG) sensory neurons. These neurons, when damaged by physical, inflammatory or chemical injury, set up the ensuing maladaptive reprogramming and circuit malfunction, including pathological pain, in the CNS.

In recent years, the importance of TRP ion channels, expressed in nociceptor neurons, has been recognized in pain transduction in response to physical and chemical-irritant cues [1, 2]. TRP channels are non-selective cation channels with preference for Ca²⁺, so that nociceptor neurons can both be activated and reprogrammed[3, 4]. One such candidate TRP channel with robust expression in TG sensory neurons is TRPV4 [5-7].

We have recently developed a novel method of bite force measurement in the laboratory mouse as a clinically relevant metric of TMJ that significantly extends current practice for assessing TMJ pain[8]. Taking advantage of this novel technique, our study shows that TRPV4 expression in TG sensory neurons plays a critical role in TMJ pain. Also, the expression of several other pain-related TRP channels and activation of extracellular signal-regulated protein kinase (ERK) in the TG after TMJ inflammation are regulated by TRPV4.

In addition, we have adopted the formalin irritant-pain model to trigeminally innervated territories in laboratory mice and examined the involvement of TRPV4. We found TRPV4 to be critically involved in trigeminal nocifensive behavior evoked by whiskerpad injections of formalin, a finding supported by studies in Trpv4 null mice and with TRPV4-specific antagonists. Our results imply TRPV4 in MEK-ERK activation in TG sensory neurons, paralleling findings in chronic TMJ inflammation. Furthermore, cellular studies in primary TG neurons and in heterologous cellular systems with directed expression of TRPV4 suggest that TRPV4 can be activated directly by formalin to gate Ca⁺⁺.

Taken together, these results imply TRPV4 as a critical signaling molecule in irritation—and TMJ chronic inflammation evoked trigeminal pain. TRPV4-antagonistic therapies can therefore be envisioned as novel analgesics for specific targeting of trigeminal pain disorders, such as migraine, headaches, TMJD, facial and dental pain, and irritation of trigeminally-innervated surface epithelia.

References

1. **Levine, J.D. and N. Alessandri-Haber**, TRP channels: Targets for the relief of pain. *Biochim Biophys Acta*, 2007
2. **Liedtke, W.**, Molecular mechanisms of TRPV4-mediated neural signaling. *Ann N Y Acad Sci*, 2008. **1144**: p. 42-52
3. **Lindy, A.S., P.K. Parekh, R. Zhu, P. Kanju, S.v. Chintapalli, V. Tsviloskyy, R.L. Patterson, A. Anishkin, D.B. van Rossum, and W.B. Liedtke**, TRPV-channel-mediated calcium-transients in nociceptor neurons are dispensable for avoidance behavior. *Nat Commun*, 2014. **in press**
4. **Liedtke, W.**, TRPV channels' function in sensory transduction and cellular signaling cascades, in TRP ion channel function in sensory transduction and cellular signaling cascades, W. Liedtke and S. Heller, Editors. 2006, CRC Press / Taylor & Francis: Boca Raton, FL. p. 303-318.

(Continues on next page)

5. **Liedtke, W., Y. Choe, M.A. Marti-Renom, A.M. Bell, C.S. Denis, A. Sali, A.J. Hudspeth, J.M. Friedman, and S. Heller**, Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell*, 2000. **103**(3): p. 525-35
6. **Liedtke, W. and J.M. Friedman**, Abnormal osmotic regulation in *trpv4*^{-/-} mice. *Proc Natl Acad Sci U S A*, 2003. **100**(23): p. 13698-703
7. **Liedtke, W., D.M. Tobin, C.I. Bargmann, and J.M. Friedman**, Mammalian TRPV4 (VR-OAC) directs behavioral responses to osmotic and mechanical stimuli in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A*, 2003. **100 Suppl 2**: p. 14531-6
8. **Chen, Y., S.H. Williams, A.L. McNulty, J.H. Hong, S.H. Lee, N.E. Rothfus, P.K. Parekh, C. Moore, R.W. Gereau, A.B. Taylor, F. Wang, F. Guilak, and W. Liedtke**, Temporomandibular joint pain: a critical role for *Trpv4* in the trigeminal ganglion. *Pain*, 2013. **154**(8): p. 1295-304

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The neurobiology of oral cancer pain

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Oral cancer pain is more severe, on average, than pain from any other cancer. The public health problem of cancer pain is, ironically, exacerbated by improved chemo- and radio-therapies that prolong survival. The intensity of oral cancer pain escalates with disease progression; terminal patients generally experience debilitating pain during their final months of life. The etiology of oral cancer pain is not known and current treatment is ineffective. Cancer pain is hypothesized to result from a tumor-mass effect and/or activation of primary afferent nociceptors by mediators liberated by the cancer. Dr. Schmidt will discuss the molecular cross-talk between cancer and peripheral nervous system that might be responsible for pain. He will present data demonstrating a reciprocal proliferative effect between cancer and surrounding sensory nerves.

Functional interactions between glutamate receptors and TRPV1 in trigeminal sensory neurons

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Background

Elevated glutamate levels within injured muscle exacerbate pain conditions and modulate functional properties of muscle nociceptors via peripheral glutamate receptors. Direct injections of capsaicin in muscle tissue significantly lower mechanical thresholds, and the blockade of TRPV1 attenuates mechanical hyperalgesia resulting from eccentric muscle contraction. This study explores how the two receptor-channel systems that have been independently implicated in muscle pain and hyperalgesia interact together, which should offer novel perspectives on how various receptors and channels in nociceptors operate as 'functional units'. We hypothesized that activation of peripheral glutamate receptors leads to TRPV1-dependent mechanical hyperalgesia via distinct intracellular signaling pathways.

Results

In the masseter muscle, direct application of NMDA induced a time dependent increase in mechanical sensitivity, which was significantly blocked when the muscle was pretreated with a specific TRPV1 antagonist, AMG9810. Calcium imaging analyses further corroborated that NMDA receptors and TRPV1 in trigeminal ganglia (TG) functionally interact. In dissociated TG culture, application of NMDA resulted in phosphorylation of serine, but not threonine or tyrosine, residues of TRPV1 in a time course similar to that of the development of NMDA-induced mechanical hyperalgesia. The NMDA-induced phosphorylation was significantly attenuated by CaMKII and PKC inhibitors, but not by a PKA inhibitor. Consistent with the biochemical data, the NMDA-induced mechanical hyperalgesia was also effectively blocked when the muscle was pretreated with a CaMKII or PKC inhibitor. We further demonstrated that the activation of NMDA receptors specifically increased the phosphorylation of S800 (p-S800) of TRPV1 at cell surface membrane and that A-Kinase anchoring protein 150 (AKAP150) was required for NMDA- and PKC-mediated p-S800 of TRPV1. Similarly, mechanical hyperalgesia induced by dihydroxyphenylglycine (DHPG), an agonist for Group I metabotropic glutamate receptors (mGlu1/5), in the masseter was attenuated by AMG9810. DHPG-induced mechanical hyperalgesia was suppressed by pretreatment with a decoy peptide that disrupted interactions between TRPV1 and AKAP150. DHPG also upregulated p-S800 of TRPV1 during which DHPG-induced mechanical hyperalgesia was prominent. Electrophysiological measurements in TG neurons demonstrated that TRPV1 sensitivity was enhanced by pretreatment with DHPG, and this was prevented by a PKC, but not by a PKA, inhibitor.

Conclusions

Our data suggest that activation of both NMDA receptors and mGlu1/5 in masseter afferents invokes phosphorylation of TRPV1 serine residues including S800, and that phosphorylation-induced sensitization of TRPV1 is involved in masseter mechanical hyperalgesia. These data support a role of TRPV1 as an integrator of glutamate receptor signaling in trigeminal muscle nociceptors.

Small-fiber polyneuropathy (SFPN), a common underlying diagnosis in syndromes involving unexplained chronic pain and multi-system symptoms

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Background

Syndromes involving unexplained chronic widespread pain (CWP) and multi-system symptoms are common, with 1-5% prevalence for fibromyalgia alone. They more often affect females and cause disability and high costs [1-3]. Other common syndromes include chronic fatigue, seronegative Lyme, and Gulf War Illness. Fragmentary syndromes include TMJD, POTS, CRPS, irritable bowel). These syndromes are particularly devastating in children and young adults, where they interfere with education and development and disrupt entire families [4-6]. SFPN is known to cause CWP and multi-system complaints in older adults. Unlike the syndromes above, SFPN can be objectively diagnosed by measuring innervation in lower-leg skin biopsies, and autonomic functions testing (AFT) of heart rate, blood pressure and sweating [7]. SFPN has several established causes including diabetes, infections, cancer, and toxins. Many causes are diagnosable, treatable, and sometimes curable [8]. Our work suggests that unrecognized SFPN contributes to several syndromes involving CWP and multi-organ symptoms.

Materials and methods

With IRB permission, we retrospective analyzed the medical records of 41 patients with onset of unexplained CWP and multisymptoms before age 21; most had objective testing for SFPN [9]. We also prospectively studied 27 adult patients with fibromyalgia and 30 healthy volunteers using history, examination, skin biopsies and AFT [10].

Results

Retrospective chart review identified definite (in 59%) and probable SFPN (in 17%) among the young patients with onset before age 21 [9]. We characterized the clinical features, diagnostic, and treatment options for this new early-onset SFPN. Studying children, who lacked the typical causes of late-onset SFPN, implicated autoimmune causality in most. Among patients treated with immunomodulatory therapies, pain and other symptoms improved in 2/3 [9]. Among adults with fibromyalgia, 41% of skin biopsies from subjects with fibromyalgia vs. 3% of biopsies from controls were diagnostic for SFPN, and symptom and examination scores were higher in fibromyalgia subjects than in controls (all $P \leq 0.001$) [10]. All fibromyalgia patients diagnosed with SFPN then had blood tests for all known causes [8]. None had diabetes but 62% had test-results consistent with dysimmunity, and some had genetic causes [10]. Other laboratories have now also linked fibromyalgia to SFPN [11-15].

Conclusions

Some patients with unexplained widespread pain and multi-system syndromes such as fibromyalgia have objectively diagnosable SFPN. SFPN can affect children and young adults, not just older adults. Multiple lines of evidence suggest that early-onset SFPN has novel causes that can be treated. The prevalence of SFPN among TMJD patients is unstudied.

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References

1. Lindell L, Bergman S, Petersson IF, Jacobsson LT, Herrstrom P: Prevalence of fibromyalgia and chronic widespread pain. *Scand J Prim Health Care* 2000, 18:149-153.
2. White KP, Speechley M, Harth M, Ostbye T: The London Fibromyalgia Epidemiology Study: the prevalence of fibromyalgia syndrome in London, Ontario. *J Rheumatol* 1999, 26:1570-1576.

(Continues on next page)

3. **White KP, Speechley M, Harth M, Ostbye T:** The London Fibromyalgia Epidemiology Study: direct health care costs of fibromyalgia syndrome in London, Canada. **J Rheumatol** **1999**, 26:885-889.
4. **Buskila D:** Pediatric fibromyalgia. **Rheumatic Disease Clinics of North America** **2009**, 35:253-261.
5. **van Geelen SM, Bakker RJ, Kuis W, van de Putte EM:** Adolescent chronic fatigue syndrome: A follow-up study. **Arch Pediatr Adolesc Med** **2010**, 164:810-814.
6. **Mikkelsen M, El-Metwally A, Kautiainen H, Auvinen A, Macfarlane GJ, Salminen JJ:** Onset, prognosis and risk factors for widespread pain in schoolchildren: A prospective 4-year follow-up study. **Pain** **2008**, 138:681-687.
7. **England JD, Gronseth GS, Franklin G, Carter GT, Kinsella LJ, Cohen JA et al.:** Practice Parameter: Evaluation of distal symmetric polyneuropathy: role of autonomic testing, nerve biopsy, and skin biopsy (an evidence-based review). Report of the American Academy of Neurology, American Association of Neuromuscular and Electrodiagnostic Medicine, and American Academy of Physical Medicine and Rehabilitation. **Neurology** **2009**, 72:177-184.
8. **England JD, Gronseth GS, Franklin G, Carter GT, Kinsella LJ, Cohen JA et al.:** Practice Parameter: Evaluation of distal symmetric polyneuropathy: role of laboratory and genetic testing (an evidence-based review). Report of the AAN, AANEM, and AAPMR. **Neurology** **2009**, 72:185-192.
9. **Oaklander AL, Klein MM:** Evidence of small-fiber polyneuropathy in unexplained, juvenile-onset, widespread pain syndromes. **Pediatrics** **2013**, 131:e1091-e1100.
10. **Oaklander AL, Herzog ZD, Downs HM, Klein MM:** Objective evidence that small-fiber polyneuropathy underlies some illnesses currently labeled as fibromyalgia. **Pain** **2013**.
11. **Üçeyler N, Zeller D, Kahn AK, Kewenig S, Kittel-Schneider S, Schmid A et al.:** Small fibre pathology in patients with fibromyalgia syndrome. **Brain** **2013**.
12. **Albrecht PJ, Hou Q, Argoff CE, Storey JR, Wymer JP, Rice FL:** Excessive peptidergic sensory innervation of cutaneous arteriole-venule shunts (AVS) in the palmar glabrous skin of fibromyalgia patients: Implications for widespread deep tissue pain and fatigue. **Pain Med** **2013**, 14:895-915.
13. **Serra J, Collado A, Sola R, Antonelli F, Torres X, Salgueiro M et al.:** Hyperexcitable C nociceptors in fibromyalgia. **Ann Neurol** **2013**.
14. **Giannoccaro MP, Donadio V, Incensi A, Avoni P, Liguori R:** Small nerve fiber involvement in patients referred for fibromyalgia. **Muscle Nerve** **2013**.
15. **Caro XJ, Winter EF:** Evidence of abnormal epidermal nerve fiber density in fibromyalgia: Clinical and immunologic implications. **Arthritis Rheumatol** **2014**.

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A cellular mechanism of interactions between pain and depression

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Background

Depression is a common comorbid condition of chronic pain. The current mainstay of managing comorbid chronic pain uses combination drug therapy including opioid analgesics and antidepressants. However, the cellular mechanism underlying the comorbid interaction between chronic pain and depression remains unclear.

Materials and methods

Using rat models of genetically predisposed [Wistar-Kyoto (WKY) rats] or induced depressive behavior combined with persistent nociception, we examined whether 1) brain IDO1 (a rate-limiting enzyme in tryptophan metabolism) expression and activity would be increased in rats with genetically predisposed or induced depressive behavior, 2) rats with depressive behavior would exhibit exacerbated nociceptive behavior, and 3) brain IDO1 upregulation would be contributory to both depressive and nociceptive behaviors. Depressive behaviors were assessed by using forced swimming test, sucrose preference test, tail suspension test, and open field test. Nociceptive behaviors were assessed using von Frey filaments and hind-paw withdrawal to radiant heat stimulation. RT-PCR, Western blotting, HPLC, ELISA, immunohistochemistry and cell culture were used in the study.

Results

We demonstrate that brain IDO1 expression critically contributes to the comorbid interaction between pain and depression. (1) IDO1 expression was elevated in the hippocampus of rats with either genetically predisposed or anhedonia-induced depressive behavior. (2) Rats with elevated IDO1 expression exhibited a lower baseline mechanical and thermal nociceptive threshold, whereas depressive behavior was exacerbated in rats with persistent nociception. (3) IDO1 upregulation was mediated by the IL-6/JAK/STAT signaling, resulting in a shift of tryptophan metabolism toward the IDO pathway as well as a low serotonin content in the hippocampus. (4) Inhibition of IDO1 activity or IL-6/JAK/STAT-mediated IDO1 upregulation concurrently attenuated nociceptive and depressive behaviors in the same rats.

Conclusions

The results indicate that brain IDO1 expression critically contributes to the comorbid interaction between pain and depression and suggest that targeting brain IDO1 activity may offer a new therapeutic method for the treatment of comorbid chronic pain.

Acknowledgements

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Combined genetic polymorphisms and environmental factors in the etiology of a chronic TMJD murine model

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Background

TNFR1 and TNFR2 are receptors for the pro-inflammatory chemokine TNF α . Inflammation of the temporomandibular joint (TMJ) and hypersensitivity were induced in mice lacking both TNF α receptors (TNFR1/R2 -/-). After recovery from this initial priming TMJ insult lasting less than a week, a gastrointestinal (GI) chemical irritation is administered 3 weeks later. This “double-hit” initiates a recrudescence of inflammation and hypersensitivity persisting at least 18 weeks.

Materials and methods

TNFR1/2 -/- and wildtype (WT) mice were given a unilateral TMJ injection of complete Freund’s adjuvant (CFA, 5-10 mL) to induce hypersensitivity of the overlying facial area. Mechanical hypersensitivity threshold was determined with graded nylon von Frey monofilaments. Heat hypersensitivity was determined by latency to response with the hotplate test (50°C). Three weeks later, mustard oil (0.5% in peanut oil; 50 μ l) was infused into the colon. The prolonged inflammation and hypersensitivity that re-developed only in the TNFR1/2-/- mice allowed testing of several different compounds for their efficacy to reduce chronic inflammation-induced mechanical and heat hypersensitivity. Proteomic analysis of serum was performed to study cytokine level differences in weeks 2 and 10.

Results

While mechanical thresholds and heat response latencies in WT mice returned to baseline after both insults, the TNFR1/R2 -/- mice developed chronic mechanical and heat hypersensitivity which persisted at least 18 weeks. NMDA receptor antagonist MK801, P2X7 inhibitor A438079, reactive oxygen species scavenger phenyl-N-t-butyl nitron (PBN), and human TNF α neutralizing antibody Etanercept were tested to determine efficacy for reduction of the hypersensitivity. Both mechanical and heat hypersensitivity were attenuated by the test drugs with varying efficacies. A TRPV1 antagonist, capsaizepine, did not alter established heat hypersensitivity. At weeks 2 and 18, the TNFR1/R2 -/- and WT mice had very different cytokine profiles. At 2 weeks when the initial inflammation-induced hypersensitivity had resolved, serum levels of TNF α , RANTES, and MIG were twice as high in TNFR1/R2 -/- while CXCL11 was decreased compared to WT mice. After 18 weeks when hypersensitivity is chronic, G-CSF, IFN γ and TNF α serum levels in TNFR1/R2 -/- animals were significantly increased relative to the controls IL-16 and CXCL9 were decreased compared to WT samples.

Conclusions

Utilizing a “double hit” inflammatory model we demonstrated that TMJ inflammation can be “re-ignited” by a minor GI insult to become a chronic condition in mice with TNF receptor deficits. High levels of TNF α were accompanied by different inflammatory mediators after the acute inflammation compared to those present chronically with the TMJD recrudescence.

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Disclosures

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Roles of COMT, NPY and GCH1 in acute and chronic pain/stress response

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Pain is a pervasive stressor often experienced chronically, and strongly modulated by the brain's emotional circuitry. In recent years functional polymorphisms at several genes have been linked to pain response, encouraging the idea that both pain response and other aspects of emotionality can be better understood by genetic studies of acute and chronic pain, as illustrated by studies performed with several genes, two of which also alter anxiety and emotional responses. The functional COMT Val158Met locus, which had been tied to frontal cognitive function, trait anxiety and brain metabolic responses to emotional stimuli, was linked to the ability of acute pain to release endomorphin and displace ¹¹C carfentanil binding following a pain challenge (Zubieta et al, Science). The anxiety-associated Met158 allele predicts both lower pain threshold and stronger affective response to pain. This finding was replicated in a large sample of women prospectively followed for temporomandibular joint pain and measured for experimental pain (Diatchenko et al). These investigators later showed that the COMT haplotype linkage is better understood via the epistatic interaction of alleles to alter translatability of COMT mRNA. GTP cyclohydrolase (GCH1) represents a second gene influencing pain (Tegeder et al, Nat Neuroscience). The gene was identified as a candidate via array expression in the rat neurotomy model. In humans, a functional haplotype predicting GCH1 mRNA expression in lymphoblastoid cell lines. Consistent with the rat data, the high expression diplotype was linked to both clinical post lumbar surgery leg pain and to experimental pain in a large population of controls. Continuing the theme that genes that alter emotion can also alter pain responses, a functional polymorphism in the NPY (neuropeptide Y) promoter alters both amygdala and hippocampal emotional responses as well as predicting ¹¹C carfentanil displacement after a pain challenge (Zhou et al, Nature).

Molecular correlates of localized versus co-occurring chronic pain conditions

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Background

Complex chronic pain conditions, including temporomandibular disorder (TMD), vestibulodynia (VBD), irritable bowel syndrome (IBS), and widespread bodily pain (WBP), represent a significant healthcare problem. Current treatment regimens remain ineffective due to the conditions' unclear etiology and heterogeneous clinical manifestation. An emerging literature suggests that localized pain conditions occurring in isolation result from local increases in peripheral afferent activity, while those occurring in concert result from central dysregulations in pain processing. To understand the nature of these complex conditions and improve standards of care, the identification of unique biological signatures and pathways that map onto distinguishing clinical features is required. Thus, the objective of this session is to discuss the relationship between pain, related psychological characteristics, cytokine levels, and microRNA expression profiles in individuals with localized versus co-occurring chronic pain conditions.

Materials and methods

Two independent case-control studies were conducted. The first study included 344 participants: 103 with TMD, 66 with TMD+WBP, and 175 healthy controls. The second study included 78 participants: 33 with VBD, 23 with VBD+IBS, and 22 healthy controls. Both cohorts included assessments of experimental pressure and thermal heat pain (measured using an algometer and Peltier device, respectively), self-reported health and psychological phenotypes (measured using standardized questionnaires), and circulating cytokine protein levels (measured using a multiplex assay). The VBD cohort also included assessments of intracellular microRNAs (measured using OpenArray), which are small noncoding pieces of RNA that control gene expression.

Results

Compared to individuals with localized pain and healthy controls, those with TMD+WBP or VBD+IBS reported decreased general and physical health; increased somatization; increased interference of pain on daily activities; and increased remote bodily pain. All cases demonstrated increased circulating levels of the pro-inflammatory cytokine interleukin-8. However those with TMD or VBD demonstrated a compensatory increase in the anti-inflammatory cytokine interleukin-1 receptor antagonist, while those with TMD+WBP or VBD+IBS did not. Individuals with VBD displayed dysregulation of 10 microRNAs that cumulatively regulate pathways vital for pain processing and estrogen signaling. Those with VBD+IBS displayed dysregulation of 11 microRNAs important for pain processing, cellular physiology, and insulin signaling. Finally, cytokine and microRNA expression profiles were correlated with pain-relevant intermediate phenotypes.

Conclusions

Individuals with localized and co-occurring pain conditions differ with respect to clinical characteristics and molecular profiles, suggesting unique underlying pathologies that contribute to each subtype irrespective of the specific anatomic site(s) involved. Cytokines and microRNAs may represent valuable tools for differentiating between these subtypes.

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The epigenetic signature of chronic pain in the mouse brain

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Background

Peripheral nerve injury can be accompanied by long-term pain-related manifestations, such as affective and cognitive disturbances, suggesting the involvement of supraspinal mechanisms. One particular region of interest is the prefrontal cortex (PFC), an area implicated in depression, anxiety and cognitive impairment, all of which are frequently associated with chronic pain [1-4]. Clinically, pathological pain-related changes in the PFC in individuals with chronic low back pain can be reversed following effective pain management [5]. However, the mechanisms behind pain-induced brain plasticity remain poorly understood.

Epigenetics is a term used to describe modifications to genomic DNA that alter gene expression. DNA methylation is an epigenetic mechanism that is involved in gene regulation mainly by silencing promoter activity. We propose that long-term alterations in DNA methylation could provide a molecular substrate for chronic pain-related changes in the CNS, forming a “memory trace” for pain in the brain.

Materials and methods

Spared nerve injury or sham surgery was performed in male CD1 mice at three months of age. Six months after injury, mechanical hypersensitivity was confirmed, brains were collected and DNA and RNA were extracted. Global DNA methylation was measured by the luminometric methylation assay in various brain regions, including the PFC. Promoter methylation of individual genes was assessed by sodium bisulfite sequencing and functionally validated using an *in vitro* promoter assay. Finally, mRNA levels of the target genes were measured by RT-PCR.

Results

Six months following peripheral nerve injury, abnormal sensory thresholds and increased anxiety were accompanied by significant genomic DNA hypomethylation [6] and transcriptional reprogramming [7]. This was linked to the hypomethylation of individual genes, including (synaptotagmin 2) *syt2*, a known regulator of synaptic function. Furthermore, transcription of *syt2* was regulated by differential methylation of its promoter *in vitro* and *syt2* mRNA was upregulated in the PFC of injured animals. Thus chronic pain-induced changes in the PFC are detected long after the original injury, at a long distance from the site of injury.

Conclusions

We show that peripheral injury produces long-term changes in the PFC methylome and propose that DNA methylation mediates the changes in brain structure and cortical function that are associated with chronic pain.

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(Continues on next page)

References

1. **Schweinhart P, Bushnell MC:** Neuroimaging of pain: Insights into normal and pathological pain mechanisms. **Neuroscience letters** 2012, **520**:129-130.
2. **Apkarian AV, Bushnell MC, Treede RD, Zubieta JK:** Human brain mechanisms of pain perception and regulation in health and disease. **European Journal of Pain** 2005, **9**:463-484.
3. **Apkarian AV, Baliki MN, Geha PY:** Towards a theory of chronic pain. **Progress in neurobiology** 2009, **87**:81-97.
4. **Neugebauer V, Galhardo V, Maione S, Mackey SC:** Forebrain pain mechanisms. **Brain research reviews** 2009, **60**:226-242.
5. **Seminowicz DA, Wideman TH, Naso L, Hatami-Khoroushahi Z, Fallatah S, Ware MA, Jarzem P, Bushnell MC, Shir Y, Ouellet JA, Stone LS:** Effective treatment of chronic low back pain in humans reverses abnormal brain anatomy and function. **The Journal of neuroscience : the official journal of the Society for Neuroscience** 2011, **31**:7540-7550.
6. **Tajerian M, Alvarado S, Millecamps M, Vachon P, Crosby C, Bushnell MC, Szyf M, Stone LS:** Peripheral nerve injury is associated with chronic, reversible changes in global DNA methylation in the mouse prefrontal cortex. **PloS one** 2013, **8**:e55259.
7. **Alvarado S, Tajerian M, Millecamps M, Suderman M, Stone LS, Szyf M:** Peripheral nerve injury is accompanied by chronic transcriptome-wide changes in the mouse prefrontal cortex. **Molecular pain** 2013, **9**:21.

Disclosures

The authors declare no competing interests.

Epigenetic regulation of gene expression and cellular differentiation

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Epigenome is defined by a collection of various chromatin modifications, which maintains a chromatin environment that is either permissive or inhibitory for gene expression. While association of histone modifications with expressed or silent genes has been established, it remains unclear how changes in chromatin modifications relate to changes in gene expression. We used ChIP-Seq to analyze the genome-wide changes in chromatin modifications during short-term activation of naïve and memory CD4⁺ T cells by T cell receptor (TCR) signaling. In resting and activated T cells, expressed genes were strongly associated with “active” modifications (e.g. H3K4me1/2/3, H2A.Z) and RNA Polymerase II (Pol II), while silent genes were typically associated with repressive marks. However, we found that about 20-30% of silent genes were *poised*; they possessed positive modifications and sometimes even Pol II at their promoters. Interestingly, majority of genes induced during T cell activation were poised in resting T cells even before the TCR signaling was initiated. Our data suggest that T cell memory in which immunological memory state is determined epigenetically—by transcriptional memory and poising.

Integrating epigenetic data into molecular casual networks

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Genome-wide association studies (GWAS) have recently identified many risk loci for complex human diseases. However, genetics can explain only a fraction of disease variation. Epigenetics refers to cellular mechanisms that affect gene expression without modifying DNA sequence [1]. Epigenetic mechanisms reflect gene X environment interactions, which contribute to risk for many chronic diseases including obesity [2], hypertension [3], cancers [4], chronic inflammation [5], chronic pain [6], and chronic obstructive pulmonary disease (COPD) [7]. While these studies have provided an initial look into genetic or epigenetic factors affecting disease risk or disease severity, understanding the transcriptional regulation by genetic and epigenetic factors, such as DNA methylation and microRNA, may shed light on understanding the biological processes and molecular mechanisms associated complex human diseases.

By integrating genetic, epigenetic, and transcriptomic data we developed genetic causality tests [8, 9] and a novel methylation-based causality test. Then, we developed a method to construct a global Bayesian network [10-12] using the causal test results as priors. As a proof-of-concept, we applied these methods to genome-wide genetic, epigenetic, and transcriptomic data and phenotypic data generated from lung tissues of COPD patients and non-COPD controls, and identified multiple causal regulators for pathways associated with disease severity. We experimentally validated candidate genes in cell lines, mouse models, and in human tissues. Our results suggest that the integrative causal network can provide important insights into understanding the mechanisms underlying epigenetic regulations, altering transcriptional programs that lead to COPD pathogenesis and progression. These approaches can be applied to uncover molecular mechanisms underlying other diseases, such as chronic pain.

References

1. **Ubeda F, Wilkins JF:** Imprinted genes and human disease: an evolutionary perspective. **Advances in experimental medicine and biology** 2008, **626**:101-115.
2. **Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE, Lumey LH:** Persistent epigenetic differences associated with prenatal exposure to famine in humans. **Proc Natl Acad Sci U S A** 2008, **105**:17046-17049.
3. **de Jonge LL, Harris HR, Rich-Edwards JW, Willett WC, Forman MR, Jaddoe VW, Michels KB:** Parental smoking in pregnancy and the risks of adult-onset hypertension. **Hypertension** 2013, **61**:494-500.
4. **Kanwal R, Gupta S:** Epigenetic modifications in cancer. **Clin Genet** 2012, **81**:303-311.
5. **Bayarsaihan D:** Epigenetic mechanisms in inflammation. **Journal of dental research** 2011, **90**:9-17.
6. **Denk F, McMahon SB:** Chronic pain: emerging evidence for the involvement of epigenetics. **Neuron** 2012, **73**:435-444.
7. **Vucic EA, Chari R, Thu KL, Wilson IM, Cotton AM, Kennett JY, Zhang M, Lonergan KM, Steiling K, Brown CJ, et al:** DNA methylation is globally disrupted and associated with expression changes in chronic obstructive pulmonary disease small airways. **American journal of respiratory cell and molecular biology** 2014, **50**:912-922.
8. **Schadt EE, Lamb J, Yang X, Zhu J, Edwards S, Guhathakurta D, Sieberts SK, Monks S, Reitman M, Zhang C, et al:** An integrative genomics approach to infer causal associations between gene expression and disease. **Nat Genet** 2005, **37**:710-717.
9. **Millstein J, Zhang B, Zhu J, Schadt EE:** Disentangling molecular relationships with a causal inference test. **BMC Genet** 2009, **10**:23.
10. **Zhu J, Sova P, Xu Q, Dombek KM, Xu EY, Vu H, Tu Z, Brem RB, Bumgarner RE, Schadt EE:** Stitching together multiple data dimensions reveals interacting metabolomic and transcriptomic networks that modulate cell regulation. **PLoS Biol** 2012, **10**:e1001301.
11. **Zhu J, Wiener MC, Zhang C, Fridman A, Minch E, Lum PY, Sachs JR, Schadt EE:** Increasing the Power to Detect Causal Associations by Combining Genotypic and Expression Data in Segregating Populations. **PLoS Comput Biol** 2007, **3**:e69.
12. **Zhu J, Zhang B, Smith EN, Drees B, Brem RB, Kruglyak L, Bumgarner RE, Schadt EE:** Integrating large-scale functional genomic data to dissect the complexity of yeast regulatory networks. **Nat Genet** 2008, **40**:854-861.

Pain in the network of genetic and epigenetic control

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In a significant proportion of patients pain persists despite sophisticated analgesic therapy. In addition, current available treatments provide sufficient pain relief only in a fraction of chronic pain patients. This triggers intensive research and drug development activities. The study of the genetic regulation of pain and its inhibition is thereby considered as a key approach to the development of effective treatments. Inherent parts of this approach are, besides changes in the function of the gene products due to genetic variants, alterations in the expression of pain-relevant genes due to genetic variants at sites relevant for gene transcription, splicing or DNA stability. Moreover, mechanisms of gene expression control, which emerge from several independent lines of contemporary research, point at so far unappreciated complexity of gene expression control exceeding current paradigms. This involves micro RNA control, that according to novel analysis acts on all levels of gene expression including transcriptional fine-tuning, mRNA processing up to translation, which form together with classical epigenetic mechanisms including DNA methylation and histone modifications a regulatory apparatus of pain gene expression. Novel research results clearly indicate that genetics and epigenetics not only affect the pain phenotype and treatment, but the interactions with the genome are bidirectional. Thus, several lines of research evidence point at a high complexity of the genetic control of pain and it is increasingly recognized that this complexity of pain has to be addressed. These developments are accompanied by intensive methodological progresses in modern bioinformatics that increasingly enable the comprehension and utilization of the complexity of the genetic control of pain and its inhibition. Examples of these developments are the exploitations of knowledge base information enabling the identification of relevant biological processes addressed by pain relevant genetic or epigenetic mechanisms. Moreover, analyses of pain phenotypes distributions allow identifying the underlying molecular bases, or may be used to classify pain patients for patterns of symptoms, which facilitates the association of complex pain-relevant genotypes. These developments promise, perhaps for the first time optimistically, a major step toward the understanding of the complexity of pain and the development of effective individualized treatments.

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The authors have declared that no competing interests exist.

Targeted genome and epigenome editing using engineered TALE and CRISPR/Cas9 technologies

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The impact of reverse genetics, synthetic biology, and gene therapy has been restricted by the limitations of conventional genetic engineering technologies. To expand the capacity for genetic modification of mammalian cells, we are engineering artificial DNA-binding proteins, including zinc finger proteins, TAL effectors, and CRISPR/Cas9 to regulate and edit endogenous mammalian genes. For example, we have engineered both protein-based and RNA-guided transcriptional activators and repressors targeted to human genes relevant to medicine, science, and biotechnology. Delivery of combinations of transcription factors led to synergistic effects on gene activation and tunable expression levels. This approach recapitulates the previously intractable complexity of natural regulation of mammalian genes that is the product of cooperative actions of many transcription factors. We have also developed novel methods for controlling the activity of these proteins, such as optogenetic regulation of protein dimerization with blue light. Genome-wide analysis of the DNA-binding, gene regulation, and chromatin remodeling of these targeted epigenome modifiers has demonstrated their exceptional specificity. In other studies we have engineered synthetic nucleases to stimulate gene targeting to genomic safe harbor sites. This approach is particularly useful for generating isogenic cell lines. We showed that this method leads to a decrease in the variability of transgene expression within a clonal cell line and between multiple clones relative to conventional techniques. Finally, we have used similar methods to correct mutations causing genetic disease. We engineered synthetic nucleases targeted to the human dystrophin gene that is mutated in Duchenne muscular dystrophy patients. When we delivered these nucleases to cells from patients with this disease, the correct gene reading frame and expression of the functional dystrophin protein were restored *in vitro* and following cell transplantation *in vivo*. We further demonstrated that these nucleases were well-tolerated and did not lead to off-target alterations of the exome in several corrected clonal cell populations. Collectively, these studies demonstrate the potential of engineered DNA-binding proteins to enable new approaches in medicine, science, and technology.

Disclosures

Charles Gersbach is an inventor on patent applications and consultant in the area of genome editing.

Imaging orofacial pain in mice

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Nociceptors in the dorsal root ganglia (DRG) and trigeminal ganglion (TG) play an essential role in initiating pain by detecting painful stimuli through their peripheral axons and sending signals to the spinal cord via their central axons [1]. Pathological conditions such as inflammation and nerve injury can sensitize nociceptors, causing heightened pain sensitivity and often leading to chronic pain conditions like TMJ disorders. Despite its importance in understanding the mechanism of nociceptor sensitization, monitoring neuronal activity of nociceptors in tissue explants or in live animals is still technically challenging due to the interference of the surrounding tissues. Recently, we have developed a novel approach to directly monitor neuronal activity and hyperactivity after injury and revealed the contribution of central terminal sensitization of primary nociceptive neurons to molecular mechanisms underlying the maintenance of trigeminal neuropathic pain. We generated *Pirt-GCaMP3* mice in which GCaMP3, a genetic-encoded Ca^{2+} -sensitive indicator [2], is specifically expressed in >95% of all DRG and TG neurons under the *Pirt* promoter [3]. Because of the specific expression of the Ca^{2+} sensor (i.e., only in DRG and TG and not in skin cells or spinal cord neurons), we detected robust neuronal hyperexcitability in TG explants and TG's axons in the skin explants and trigeminal brain-stem slices of animals with nerve injury compared with naïve or sham-treated mice. In addition, we are developing techniques to image DRG neuronal activity in live mice in response to various sensory stimuli applied to sensory peripheral receptive fields. The advantages of the functional imaging using *Pirt-GCaMP3* mice include simple tissue preparation and imaging procedures, intact sensory somatotopic organization, and simultaneously monitoring a large population of neurons and nerves. Previous and ongoing studies using this technique have revealed new mechanisms underlying chronic pain conditions including orofacial pain.

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References

1. Basbaum, A.I., Bautista, D.M., Scherrer, G., and Julius, D. (2009). Cellular and molecular mechanisms of pain. *Cell* 139, 267-284.
2. Tian, L., Hires, S.A., Mao, T., Huber, D., Chiappe, M.E., Chalasani, S.H., Petreanu, L., Akerboom, J., McKinney, S.A., Schreiter, E.R., et al. (2009). Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. *Nat Methods* 6, 875-881.
3. Kim YS, Chu Y, Han L, Li M, Li Z, Lavinka PC, et al. Central terminal sensitization of TRPV1 by descending serotonergic facilitation modulates chronic pain. *Neuron*. 2014;81(4):873-87.

Disclosures

Dr. Caterina is an inventor on a patent on the use of products related to TRPV1, which is licensed through UCSF and Merck, and may be entitled to royalties related to these products. He is on the Scientific Advisory Board for Hydra Biosciences, which develops products related to TRP channels. These conflicts are being managed by the Johns Hopkins Office on Policy Coordination.

Carbonic anhydrase-8 gene therapy inhibits the ITPR1-cytosolic free calcium pathway producing analgesia and anti-hyperalgesia

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Background

Calcium dysregulation is linked with various forms of neuropathology including seizure disorders, multiple sclerosis, Huntington's disease, Alzheimer's, spinal cerebellar ataxia (SCA) and chronic pain. Carbonic anhydrase-8 (Car8) is an allosteric inhibitor of inositol trisphosphate receptor-1 (ITPR1), which regulates intracellular calcium release fundamental to critical cellular functions including neuronal excitability, neurite outgrowth, neurotransmitter release, mitochondrial energy production, cell fate and neuroplasticity through the regulation of transcription and protein synthesis. Herein, we test the hypothesis that Car8 regulation of ITPR1 and cytoplasmic free calcium release is critical to nociception and pain behaviors, and a potential therapeutic target for persistent pain.

Materials and methods

The homozygous 'waddles' (wdl) mouse is a Car8 null mutant (MT) due to a 19 Bp deletion in exon 8 that occurred spontaneously in the C57BLKS (WT) background producing a truncated unstable protein. MT and WT mice were purchased from Jackson Labs. Behavioral testing was conducted using von Frey filaments and Hargreaves methods as previously described.[1,2] AAV2 vectors were constructed from WT cDNA (ATCC) and Car8 was mutagenized to produce the 19 Bp deletion (negative control) using site directed mutagenesis, and WT and MT constructs were packaged in AAV8 viral particles. Car8 AAV-mediated gene transfer (<1E+14/1.5 microL) of WT (V5-mCar8WT) and MT (control V5-mCar8MT) via sciatic nerve injections was used to rescue nociceptor hypersensitivity in wdl mice. Immunostaining, westerns, RT-PCR, and calcium imaging were performed as described previously.[3,4]

Results

We show that the homozygous 'waddles' (wdl) MT mice exhibiting mechanical allodynia and thermal hyperalgesia compared to WT mice. Dorsal root ganglia (DRG) from MT mice also demonstrate increased steady-state ITPR1 phosphorylation (pITPR1) and cytoplasmic free calcium release compared to WT mice. Overexpression of V5-Car8WT protein in MT nociceptors complements Car8 deficiency, down regulates pITPR1, and abolishes thermal hyperalgesia and mechanical allodynia. We further demonstrate that inflammation down regulates Car8 nociceptor expression, producing a deficiency relative to ITPR1, and increased pITPR1 in WT mice as a potential mechanism of hypersensitivity and calcium dysregulation. Finally, nociceptor gene transfer of V5-Car8WT (but not V5-Car8MT gene transfer) produces analgesia and anti-hyperalgesia in subacute and chronic inflammatory pain models.

(Continues on next page)

Conclusions

Our findings indicate Car8 controls an intracellular calcium-regulating pathway critical to nociception, inflammatory pain and possibly other neuropathological states. Car8 and ITPR1 represent important new targets for persistent pain conditions. Herein we provide a proof-of-concept for Car8 nociceptor directed gene therapy for inflammatory pain.

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References

1. **Chaplan, S.R., Bach, F.W., Pogrel, J.W., Chung, J.M. & Yaksh, T.L.** Quantitative assessment of tactile allodynia in the rat paw. **J Neurosci Methods** 53, 55-63 (1994).
2. **Hargreaves, K., Dubner, R., Brown, F., Flores, C. & Joris, J.** A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. **Pain** 32, 77-88 (1988).
3. **Zhuang, Z.Y., Xu, H., Clapham, D.E. & Ji, R.R.** Phosphatidylinositol 3-kinase activates ERK in primary sensory neurons and mediates inflammatory heat hyperalgesia through TRPV1 sensitization. **J Neurosci** 24, 8300-8309 (2004).
4. **Bianchi L, Gerstbrein B, Frøkjær-Jensen C, Royal DC, Mukherjee G, Royal MA, Xue J, Schafer WR, Driscoll M.** The neurotoxic MEC-4(d) DEG/ENaC sodium channel conducts calcium: implications for necrosis initiation. **Nat Neurosci.** 2004, Dec;7(12):1337-44.

Preclinical and translational studies of fenobam, an mGlu5 NAM, for the treatment of pain

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Background

Metabotropic glutamate receptor 5 has been suggested by many rodent studies to be a promising target for the development of novel analgesic drugs. The lack of approved compounds has prevented proof-of-concept studies in human subjects. Here we describe preclinical and translational studies of the mGlu5 negative allosteric modulator (NAM), fenobam.

Materials and methods

Fenobam was tested for analgesic efficacy and toxicity in mouse models. We also tested the plasma levels after oral dosing of fenobam in healthy volunteers, and collected any adverse events following oral dosing compared to placebo.

Results

The mGlu5 NAM Fenobam is effective in a wide variety of preclinical pain models in mice with no evidence of the development of analgesic tolerance on daily dosing. No obvious toxicities were observed in mice, or in several studies in healthy human volunteers.

Conclusions

Fenobam has robust analgesic activity and shows a good safety profile. Fenobam therefore represents a useful tool for proof-of-concept studies in human subjects.

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Travel Awardee

Poster Abstracts

Poster A

Association between *INADL* genetic variant and a subgroup with high risk for TMD in the OPPEA study

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Background

A major impediment to addressing the epidemic of persistent pain conditions is the development of classification procedures that capture the mosaic of signs and symptoms that accompany these disorders. According to the bio-psycho-social model, the measurable features of an idiopathic pain condition such as temporomandibular disorder (TMD) are associated with abnormalities in sensory, psychological, neuroimmune, and autonomic systems, which arise due to the interaction of genetic and environmental risk factors. Using a high-dimensional dataset derived from a large TMD case-control study, we have developed a new method to integrate clinically assessed intermediate phenotypes across bio-psycho-social domains, clustering subjects in a manner that provides clinically useful prognostic and diagnostic information. We performed a candidate gene association study to identify genes that influence cluster assignment in order to characterize the genetic determination of these clusters.

Materials and methods

Cluster analysis was performed to identify subgroups within the Orofacial Pain: Prospective Evaluation and Risk Assessment (OPPEA) study, which included 1,031 TMD cases and 3,247 controls. TMD status was confirmed using the Research Diagnostic Criteria for TMD (RDC/TMD); each participant was also assessed for psychological characteristics, medical history, and sensitivity to experimental pain. Supervised 3-means clustering was applied to the pain sensitivity and psychological data using the 15 features most strongly associated with TMD. Subjects were genotyped using a candidate gene panel of 2,924 single nucleotide polymorphisms (SNPs) corresponding to 358 pain-relevant genes. We assessed association between cluster identity and SNP genotypes using logistic regression.

Results

In the contrast between Cluster 1 (n=1180, characterized by low pain sensitivity and low psychological distress) and Cluster 3 (n=273, high pain, high distress), the strongest association was with the SNP rs2498982 (minor allele frequency = 0.39), in the *INADL* gene (standardized OR=1.57, p=1.1x10⁻⁵). This gene is a scaffolding protein regulating tight junctions in sensory neurons, including interactions between channels involved in nociception such as ASIC3. No statistically significant (after Bonferroni correction) associations were observed in the contrast of Cluster 2 (n=1571, moderate pain, low distress) with Cluster 3 although the strongest signal (p=4.4x10⁻⁴) was again observed in *INADL*, indicating this gene may distinguish individuals in Cluster 3, with the highest risk of TMD. We also identified other genes potentially contributing to molecular pathways affecting cluster assignment.

Conclusions

Using a novel clustering method to classify individuals into diagnostic categories, we have identified a genetic variant in *INADL* associated with the subgroup with the highest risk for TMD.

Acknowledgments

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Disclosures

Smith, Fillingim, Slade, Diatchenko, and Maixner declare financial relationships with Algynomics, Inc.

Poster B

Case-control analysis in resting and evoked inflammatory profiles

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Background

While case-control differences in inflammation have been reported in several chronic pain conditions, the question of whether Temporomandibular Disorder (TMD) is also characterized by a heightened pro-inflammatory profile has not been investigated thoroughly. Inflammatory profiles can be evaluated by a number of methods including measuring basal (resting) levels and reactivity following a painful stressor. Therefore, the objective of the study was to evaluate case-control differences in a) resting levels of inflammation and b) impact of experimentally induced pain on inflammatory responsiveness. The current study tested the hypothesis that individuals with TMD will show a greater pro-inflammatory profile at rest and following pain induction compared to controls. Exploratory analysis of the associations between inflammation and clinical outcomes were also conducted.

Materials and methods

Individuals with (n=9) and without (n=20) TMD were recruited from the University of Florida. Blood was collected in EDTA tubes before and up to 90 minutes following an experimental testing procedure including heat (forearm, cheek) and cold immersion (foot). Blood was placed on ice, processed, and stored at -80°C. Simultaneously measurement of pro- (TNF α , IL-6, IL-8) and anti- (IL-4, IL-5, IL-10) inflammatory levels was performed with multiplex kits (Millipore). A visual analog scale (VAS) was used to measure cold and heat pain. To assess reactivity, area under the curve with respect to increase (AUC_i), which controls for baseline values, was assessed for each cytokine across time. Group differences in variables derived from resting and reactivity measures were evaluated as dependent variables in separate analyses of covariance controlling for age, menstrual cycle, and time of collection. Associations between resting inflammatory levels, clinical measures of pain and disability were assessed with partial correlations.

Results

Markers of resting inflammation were higher in the TMD group (all p 's < .05) and positively associated with clinical measures (all p 's < .05). While no case-control differences were observed in pain sensitivity, an increase in inflammation was observed in both groups following pain induction. Compared to the control group, pro-inflammatory markers were significantly higher (all p 's < .001) while a trend was observed for lower levels of anti-inflammatory markers (all p 's < .10) in individuals with TMD.

Conclusions

The current study suggests that individuals with TMD may exhibit a pro-inflammatory imbalance (i.e., enhanced release of pro-inflammatory markers; blunted release of anti-inflammatory markers), which may contribute to the clinical phenotype. Additional research is needed to determine the significance of these findings.

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Disclosures

None

Poster C

Confocal microscopy reveals nerve fiber similarities in fibromyalgia and patients with dry eyes with a normal ophthalmic exam

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Introduction

Small fiber neuropathy has been shown in a subset of fibromyalgia patients, but this is a non-specific finding that has been noted in several chronic pain states. Small nerve fiber morphology can be measured non-invasively in the cornea, an area densely innervated with small fibers, using in-vivo confocal microscopy (IVCM). We used this technique to determine if 1) neuropathy is present in the corneas of fibromyalgia patients and 2) if those who suffer from idiopathic, chronic dry eyes (i.e., dry eye symptoms without dry eyes on exam) might also have compromised small fibers.

Methods

Fibromyalgia patients (n=19), chronic dry eye sufferers (n=12), and healthy controls (n=24) underwent IVCM to measure the morphology of corneal nerve fibers. In addition, participants also received an ophthalmic evaluation to measure tear function.

Results

ANOVA revealed significant differences across the three groups in corneal nerve length. Post-hoc tests showed that nerves were significantly shorter in fibromyalgia patients (mean = 1.85 mm), compared with both dry eye subjects (2.66 mm) and healthy controls (2.68 mm). When the dry eye group was broken down into subgroups of those displaying normal vs. decreased tear function, it was revealed that corneal nerves were significantly shorter in the dry eye subjects with a normal tear function (2.03 mm), compared to those with altered tear function (3.12 mm). Nerve length was not significantly different between fibromyalgia patients and dry eye subjects with normal tear function.

Conclusions

Nerve length is significantly reduced in the cornea of fibromyalgia patients, indicating that small fiber abnormalities in fibromyalgia are not limited to the skin. More research is needed to determine whether these abnormalities are a cause or a symptom of the syndrome. Individuals who experience idiopathic dry eyes but whose tear function is normal also show evidence of corneal neuropathy, similar to fibromyalgia patients. These data suggest that idiopathic dry eyes may be an ophthalmic manifestation of centralized conditions such as fibromyalgia and other associated chronic overlapping conditions.

Poster D

TRPV4-mediated trigeminal pain: behavior assessments and mechanisms

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Trigeminal pain represents one of the worst pains that humans can suffer. One of the obstacles towards development of rationally targeted therapies is rooted in shortcomings of available animal models for trigeminal pain. Another roadblock is lack of clear understanding of molecular and cellular mechanisms that underlie this type of pain.

TRPV4 is a polymodally activated Ca²⁺-permeable nonselective cation channel that is activated by a variety of factors, including chemical, osmotic, mechanical, moderate heat and low pH stimuli. Previous studies detected that it is highly expressed in trigeminal ganglion (TG) sensory neurons with small diameter, indicating it might function in trigeminally mediated pain.

We first demonstrated that the TRPV4 channel is critical for TMJ-inflammation evoked pain behavior in mice, and that TG pro-nociceptive changes are Trpv4-dependent. As a quantitative metric, bite force was recorded as evidence of masticatory sensitization, in keeping with human translational studies. In Trpv4^{-/-} mice with TMJ-inflammation, attenuation of bite force was significantly reduced compared to WTs. TMJ-inflammation and mandibular skeletal changes were apparent after CFA injections, but remarkably independent of Trpv4 genotype. Intriguingly, as a result of TMJ-inflammation, WT mice exhibited significant up-regulation of TRPV4 and phospho-ERK in TMJ-innervating TG neurons, absent in Trpv4^{-/-} mice. Mice with genetically impaired MEK/ERK phosphorylation in neurons showed a similar resistance to reduction of bite-force as Trpv4^{-/-} mice. Thus, TRPV4 is necessary for masticatory sensitization in TMJ-inflammation, and likely functions up-stream of MEK/ERK phosphorylation in TG neurons in-vivo.

Next, we tested whether TRPV4 ion channels might be critical for irritant-evoked trigeminal pain behavior. Our results demonstrate TRPV4 to be critically involved in trigeminal nocifensive behavior evoked by whisker-pad injections of formalin. We have used Trpv4^{-/-} mice and TRPV4-specific antagonists in mice to support this conclusion. Furthermore, our results imply TRPV4 to activate MEK-ERK in TG neurons. Importantly, cellular studies suggest that TRPV4 can be activated directly by formalin to gate Ca²⁺ ions.

Last, we developed a novel behavioral assay of water licking for assessing trigeminal irritant pain. We found that formalin-induced irritation in the V2 territory decreased the water licking times and increased the latency of first water licking in WT, which were significantly attenuated in Trpv4^{-/-} mice.

Taken together, our results imply that TRPV4 represents a novel pro-nociceptive target in trigeminal pain including TMJ, and thus a potential target for novel pain alleviating strategies for TMJ and other trigeminal pain disorders.

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Disclosures

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

Poster E

Associations between *ACTN3* and *OPPERA* pain-related genes in malocclusion

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Background

We have investigated an orthognathic surgery population to determine how variation in masticatory muscle gene expression and genotype plays a key role in development of both jaw-deformation malocclusion and temporomandibular joint disorders (TMD). A gene of particular interest is *ACTN3* since the common R577X polymorphism results in α -actinin-3 protein loss, reduced myofiber Z-disc structural integrity in skeletal muscle and decreased osteoblast/osteoclast activity in bone formation. Secondly, since the prevalence of TMD in this population is quite high (30%) we sought to determine if genes related to pain processes—previously identified in the Orofacial Pain: Prospective Evaluation and Risk Assessment Study (OPPERA) were differentially expressed.

Methods

After obtaining masseter muscle and saliva-DNA samples from subjects during orthognathic surgery, we identified associations between genotype, muscle gene expression, fiber type properties, malocclusion classification, facial asymmetry, gender and TMD. Genotype and gene message quantities were determined using TaqMan chemistry. Morphometry of muscle fiber types was conducted on tissue cross sections stained with myosin heavy chain-specific antibodies using NIH Image software. Jaw Pain and Function questionnaire and clinical examinations were used to diagnose TMD. Malocclusion diagnosis was determined by the type of treatment plans executed during surgery.

In a separate pilot analysis muscle samples were analyzed for gene expression differences on Affymetrix HT2.0 microarray expression chips containing 70, 534 transcripts. Principal Components Analysis and False Discovery Rate corrections were applied to comparisons with Partek Genomics Suite software.

Results

We identified associations between *ACTN3* genotypes and skeletal class II ($p=0.003$) and deep bite ($p=0.03$) malocclusions, masseter muscle fiber type properties ($p=0.02$) and an almost significant association for presence of TMD, which was often limited to masticatory muscle pain ($p=0.08$). Global gene expression analysis identified significant differences for approximately 200 *OPPERA* pain process genes in subjects with asymmetry and TMD, compared to subjects with malocclusion only. Differential expression in masseter muscle for one of these genes, *CACNA2D1* (voltage-dependent calcium channel subunit alpha-2/delta-1, active in neuropathic pain), was confirmed with additional quantitative RT-PCR experiments by gender ($p=0.0008$) and between women with and without myalgia ($p=0.05$).

Conclusions

These results indicate that *ACTN3* genotypes make significant contributions in the development of malocclusion as a musculoskeletal condition. Dentofacial deformity subjects, especially females, have a high prevalence for TMD, diagnosed clinically as masticatory muscle myalgia. Differences in α -actinin-3 protein levels may predispose muscle to contraction-induced damage, or altered calcineurin-mediated nociception.

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Poster F

Repetitive transcranial magnetic stimulation (rTMS) of the primary motor cortex for treating facial neuropathic pain – preliminary results of a randomized, sham-controlled, cross-over study

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Background

Repetitive transcranial magnetic stimulation (rTMS) noninvasively triggers action potentials in underlying brain cortex intended for therapeutic benefit. Currently marketed for refractory depression, rTMS is under investigation for treating several neurological conditions including pain. Facial neuropathic pain (NP) appears to respond well to rTMS [1]. The primary motor cortex (M1) is established as the best site to stimulate to relieve pain, but the best target within M1 remains unknown [1,2]. MRI guidance of rTMS permits repeated precise application to specific targets. The aim of this study was to compare pain relief obtained by applying MRI-guided rTMS to the M1 representation of subjects' painful face vs. to adjacent M1 cortex innervating their non-painful hand.

Materials and methods

In this ongoing randomized, single-blinded, placebo-controlled, cross-over study, eight adults with facial NP provided informed consent for head MRI and rTMS. Subjects undertook the 3 study phases in random order. In one phase, rTMS was applied to the contralateral M1 representation of their painful face. In the others, rTMS was applied to the adjacent hand area, or sham-rTMS was administered to the hand area using a spacer below the coil. In each phase, 1500 stimuli were administered using a Nexstim NBS 3.2 with a double 70mm coil. Five-second trains of 10 Hz pulses were applied at 80% of resting motor threshold with 55 second inter-train intervals. Each study-phase involved recording baseline data for 3 days, 5 consecutive daily sessions of rTMS, followed by 3 washout weeks. The primary outcome was change in the 0-10 numeric pain rating score. Secondary outcomes measured physical and mental functioning using the SF-36, McGill pain questionnaire and Beck depression Inventory.

Results

Among the 8 subjects studied so far, 4 had V1 postherpetic neuralgia, 2 had ocular NP, and one each had trigeminal neuralgia and idiopathic facial pain. Reductions in mean pain intensity were >20% on treatment days 3-5 during both active treatments, but not during sham treatment. Pain reverted to baseline within two weeks after ending treatment. 3/8 subjects had pain reductions >30% when the cortex innervating their hand was stimulated and 2/8 had reductions >30% when cortex innervating their face was stimulated. Secondary outcomes did not change and there were no significant adverse events.

Conclusions

These preliminary results corroborate the efficacy of rTMS for facial NP. Recruitment is ongoing to test the hypothesis that stimulating adjacent healthy cortex may relieve pain better than stimulating cortex representing the painful face [3].

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References

1. O'Connell NE, Wand BM, Marston L, Spencer S, DeSouza LH: Non-invasive brain stimulation techniques for chronic pain. *Cochrane.Database.Syst.Rev.* 2014;4:CD008208
2. Leung, A., Donohue, M., Xu, R., Lee, R., Lefaucheur, J. P., Khedr, E. M. et al.: rTMS for suppressing neuropathic pain: a meta-analysis. *J.Pain* 2009, 10, 1205-1216.
3. Lefaucheur, J. P., Drouot, X., Menard-Lefaucheur, I., Zerah, F., Bendib, B., Cesaro, P. et al. : Neurogenic pain relief by repetitive transcranial magnetic cortical stimulation depends on the origin and the site of pain. *Journal of Neurology, Neurosurgery & Psychiatry* 2004, 75, 612-616.

Disclosures

None of the authors have any conflicts of interest to disclose.