## ADRB2, pain and opioids in mice and man

O. Kambur[1,2,*], A. Samoshkin[3,4], M. Kaunisto[5], J.S. Wieskopf[3,6], J.S. Mogil[3,6], L. Diatchenko[3,4,7], E. Kalso[1,8]

[1] *Pharmacology, University of Helsinki, Faculty of Medicine, Helsinki, Finland*
[2] *Department of Pain Research and Management, Oslo University Hospital, Oslo, Norway*
[3] *Alan Edwards Centre for Research on Pain, McGill University, Montreal, QC, Canada*
[4] *Department of Anesthesia, McGill University, Montreal, QC, Canada*
[5] *Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland*
[6] *Department of Psychology, McGill University, Montreal, QC, Canada*
[7] *Faculty of Dentistry, McGill University, McGill University, Montreal, QC, H3A 1G1, Canada*
[8] *Helsinki University Central Hospital (HUCH), Pain Clinic, Helsinki, Finland*
*E-mail address:* oleg.kambur@gmail.com (O. Kambur).

**Aims.** We aim to characterize the effects of variation within *ADRB2*-gene on pain and opioid requirements in human patients. We will assess ADRB2-OPRM1-6TM heterodimer as a molecular mechanism, potentially explaining pronociceptive and antianalgetic effects, using preclinical *in vitro* and *in vivo* models. We will further assess clinical significance *via* its genetic proxy, rs563649 in humans.

**Methods.** In humans, experimental and postoperative pain and opioid responses were assessed in 1000 breast cancer surgery patients. Association of *ADRB2* (n = 40) and *OPRM1* (n = 1) polymorphisms was assessed using linear regression and analysis of variances (ANOVA). *In vitro* methods involved immunofluorescence microscopy (IF), cellular localization and translocation of 6TM/β2AR-heterodimers and $Ca^{2+}$-measurements. Behavioral *in vivo* characterization was performed in mice using formalin, von Frey, hot plate and cold plate tests after administration of morphine, specific OPRM1-6TM agonist IBNtxA and ADRB2-antagonist ICI118,551.

**Results.** In humans, several *ADRB2* SNPs were associated with pain and opioid phenotypes. The strongest associations were seen between cold pain phenotypes and rs17108817 & rs11957757 ($p < 0.0001$). *In vitro*, coexpression with $\beta_2$-Ars increased translocation of 6TM-MOR to plasma membrane and $Ca^{2+}$responses after treatment with selective 6TM-agonist, IBNtxA, compared with the cells expressing OPRM1-6TM alone. *In vivo*, co-administration of $\beta_2$AR selective antagonist ICI 118,551 increased analgesic efficacy of opioids in a synergistic manner and reduced opioid-induced hyperalgesia.

**Conclusions.** Our findings suggest that ADRB2 and genetic variation in *ADRB2*-gene are involved in the modulation of human pain and opioid responses. 6TM-MOR/$\beta_2$-AR heterodimerization represents a molecular mechanism causing excitatory cellular effects and sufficient explanatory potential to explain pronociceptive and antianalgesic effects. Our animal findings further confirmed the concept of $\beta_2$-AR and 6TM-MOR interaction *in vivo*. We suggest that co-administration of β-blockers with opioids might increase efficacy and safety of OPRM1 agonists.

http://dx.doi.org/10.1016/j.sjpain.2016.05.017

## Retrospective analysis of pediatric patients with CRPS

L. Kiehelä*, J. Pouttu, K. Hamunen

*Helsinki University Hospital (HUH), Department of anesthesiology and intensive care, Pain Clinic, Finland*
*E-mail address:* lauri.kiehela@hus.fi (L. Kiehelä).

**Aims:** The aim of the study was to describe the clinical features and clinical pathway of pediatric CRPS patients in HUH Children's Hospital.

**Methods:** This retrospective study included patients admitted to the pediatric pain clinic for CRPS during six years 2008–2013. Data on time from the first symptoms to diagnosis, first appointment at the pain clinic, and symptom resolution, as well as demographic and clinical symptoms were extracted from patient records.

**Results:** Forty patients were clinically diagnosed with CRPS during the time period without using the Budapest Criteria. 75% of the patients were female, median age was 12, and in 90% of the cases the CRPS was localized in the lower extremity. The median time from first symptoms to the CRPS diagnosis was 8.5 weeks (range 0–47), to first appointment with the pain physician 10 weeks (range 2–47), and to symptom resolution 35 weeks (range 10–131). Eleven out of forty patients (27.5%) were not symptom-free at the end of the treatment period. Most common clinical finding was allodynia or hyperalgesia of the afflicted area (70%).

**Conclusions:** Compared to an earlier study performed in our hospital (retrospective study of seven years, $n = 18$), the number of patients has more than doubled, maybe due to better awareness of the syndrome. Our findings about demographics and localization of CRPS are in agreement with previous literature. Time to reaching diagnosis, the small number of consultations and radiographs show that CRPS is well known among pediatric orthopedic surgeons. Many Budapest criteria that are now considered important diagnostic findings were not highly prevalent in our patients (e.g. prior trauma, color changes of skin), although patient records were not always clear about the findings leading to diagnosis. The Budapest criteria should be used to standardize the diagnosis also in our pediatric patients.

http://dx.doi.org/10.1016/j.sjpain.2016.05.017

## Activation of epidermal growth factor receptors (EGFRs) following disc herniation induces hyperexcitability in the pain pathways

M. Kongstorp, A. Moen, D.P. Jacobsen, F. Haugen, A. Mahmood, J. Gjerstad*

*The National Institute of Occupational Health, Oslo, Norway*
*E-mail address:* Johannes.Gjerstad@stami.no (J. Gjerstad).

**Aims:** Low back pain and sciatica after disc herniation may be caused by mechanical compression of the nerve roots, but also by the release of pro-inflammatory agents including growth factors from the nucleus pulposus (NP).

**Methods:** Here, in an animal model mimicking the clinical situation following disc herniation, CLIA protein analyses, extracellular single-cell recordings in the spinal dorsal horn and qPCR were performed to examine the nociceptive signaling due to disc herniation.

**Results:** The present data demonstrated that EREG may be released from NP – and that administration of EREG onto the spinal dorsal nerve roots increased spontaneous activity in nociceptive neurons. An up-regulation of EGFR and HER4 in the dorsal horn as