accounted for by differences in DNA methylation of the +37 CpG site that could influence the penetrance of rs143383 in susceptibility to OA and in other common musculoskeletal diseases.

76 ALLELIC EXPRESSION ANALYSIS IN PATIENT TISSUES OF THE OSTEOARTHRITIS AND OF THE LUMBAR DISC HERNIATION SUSCEPTIBILITY LOCI THAT MAP TO COL11A1

A.W. Dood, E.V. Raine, L.N. Reynard, J. Loughlin. Newcastle Univ., Newcastle upon Tyne, United Kingdom

Purpose: The arcOGEN genome-wide association scan (GWAS) has generated evidence of association to OA of single nucleotide polymorphism (SNP) rs2615977 from within intron 31 of COL11A1. This gene codes for the α1 polypeptide chain of type XI collagen and has long been considered a strong candidate for OA susceptibility, based on the important structural role of type XI collagen in the cartilage extracellular matrix and on the observation that penetrant mutations of COL11A1 can cause Sticker’s syndrome, which is a rare disease characterized by severe early-onset secondary OA. Furthermore, rs2615977, as mediating an effect on the expression of the COL11A1 gene transcription or transcript stability, resulting in allelic expression imbalance. The LDH SNP rs1676486 is an example of such a polymorphism, with the LDH-associated T allele of the SNP mediating decreased stability of the COL11A1 transcript relative to the C allele. In this study we therefore set out to assess: 1) whether the OA association to rs2615977 was marking allelic expression imbalance of COL11A1 and 2) whether the detrimental effect that rs1676486 has on COL11A1 transcript expression in LDH is also observed in the cartilage of OA patients.

Methods: Using RNA extracted from the cartilage of OA patients who had undergone elective joint replacement surgery, we assessed whether either SNP correlated with COL11A1 allelic expression by: 1) measuring COL11A1 expression by quantitative PCR and then stratifying the data by genotype at each SNP in turn and 2) accurately discriminating and quantifying the mRNA synthesized from both alleles of each SNP, using allelic-quantitative PCR performed on heterozygous patients. We studied a total of 73 male and female patients, who had undergone either a hip or a knee replacement. Linear regression was used to assess whether COL11A1 expression relative to genotype differed significantly from the null, whilst a 2-tailed Mann-Whitney exact test was used to assess the significance of any allelic differences.

Results: We found no evidence for a correlation between COL11A1 expression levels and genotype at either SNP; nor were there any correlations when the patients were stratified by the joint replaced (all P-values were greater than 0.05). This stratification analysis is vulnerable to the natural variability in gene expression between individuals, which can decrease its sensitivity and accuracy. For example, in individuals of the same genotype we observed up to a 250-fold difference in COL11A1 expression. The direct measurement of allelic output is not vulnerable to such effects. In our allelic expression analysis we found no evidence that genotype at the OA associated SNP rs2615977 correlated with allelic expression imbalance. However, we detected a complete correlation between allelic expression imbalance and genotype at the LDH associated SNP rs1676486: in all of the patients that we studied who were heterozygous for this SNP the LDH associated T allele produced significantly less transcript than the C allele, with an average allelic ratio of 0.36 and P-value less than 0.0001. When we tested this SNP for association in the arcOGEN GWAS of 7,410 cases and 11,009 controls, we did not however detect any compelling evidence of association to OA (all P-values were greater than 0.01).

Conclusions: Our findings support the polygenic nature of the genetic contribution to knee OA and the likelihood that contributing loci are likely to have very small effects. While it is potentially challenging to harmonize OA case and control definitions between different studies, ongoing meta-analyses may best be able to accommodate the complex genetic architecture of OA.

77 A GENETIC ANALYSIS OF OSTEOARTHRITIS OF THE KNEE IN NORTH AMERICAN CAUCASIANS: RESULTS FROM THE OSTEOARTHRITIS INITIATIVE AND JOHNSTON COUNTY OSTEOARTHRITIS PROJECT

L.M. Jerges-Armstrong1, C. Lu1, M.C. Hochberg2, B. Mitchell2, J.M. Jordan3, J.B. Rennert4, T. McSherry5, D.M. Taverna6, D. Duggan7, W.J. Mysiw8, R. Jackson9,1, Univ. of Maryland, Baltimore, MD, USA; 2Univ. of North Carolina, Chapel Hill, NC, USA; 3TGen, Phoeniz, AZ, USA; 4The Ohio State Univ., Columbus, OH, USA

Purpose: A strong genetic contribution to osteoarthritis (OA) is widely recognized although few loci have yet to be robustly associated with knee OA susceptibility. To identify genes associated with knee OA, we performed joint analyses across two large North American cohorts with well-characterized phenotypes of knee OA: the Genetic Components of Knee OA (GeCKO) Study, an ancillary study from the Osteoarthritis Initiative (OAI), and the Johnston County (JoCo) Osteoarthritis Project. Our goals were to 1) attempt replication of 14 single nucleotide polymorphisms (SNPs) previously reported in the literature to be associated with hip or knee OA, and 2) conduct an agnostic genome-wide association (GWA) analysis.

Methods: Cases had at least one knee with definite radiographic OA, defined as the presence of definite osteophytes (OARSI grade ≥1) regardless of the presence of joint space narrowing (equivalent to Kellgren-Lawrence [KL] grade ≥2). Controls were required to be free of radiographic evidence of OA in both knees (i.e., no evidence of both osteophytes and joint space narrowing, or KL grade = 0). Based on these definitions, there were 1,776 Caucasian cases in the GeCKO-OAI and JoCo cohorts, and 885 and 634 controls, respectively, included in the analyses. The 14 SNPs selected for replication were identified through a review of candidate gene and GWA studies in the literature. Genotyping was carried out on the Illumina 2.5M and 1M arrays in GeCKO-OAI and JoCo, respectively. Association analyses were carried out separately in each cohort with adjustments for age and sex.

Results: None of the 14 previously reported genetic variants for OA achieved even nominal significance (p<0.05) with knee OA in the GeCKO-OAI sample, despite a power to detect odds ratios of 1.17 or greater. A hypothesis-free GWA analysis was then carried out in the GeCKO-OAI sample on the 1.3M SNPs that passed genotype cleaning filters. No SNPs were associated with knee OA at GWA levels of statistical significance (P<5x10^-8). However, a total of 155 SNPs, representing 54 different chromosome regions, were suggestively associated with knee OA at a threshold of P<10^-4. We then tested these SNPs for association with knee OA in cases and controls from the JoCo Study. Of the 87 SNPs available for replication in JoCo, none were significantly associated with knee OA at a nominal P<0.01. At our tested levels of significance (i.e., P<10^-4 in GeCKO-OAI and P<0.01 in JoCo), we estimated that our sample would have 80% power to detect SNPs having odds ratios of 1.34 or higher among all SNPs tested in GeCKO-OAI and for the subset of SNPs tested for replication in JoCo for disease allele frequencies of 0.20 or greater.

Conclusions: Our findings support the polygenic nature of the genetic contribution to knee OA and the likelihood that contributing loci are likely to have very small effects. While it is potentially challenging to harmonize OA case and control definitions between different studies, ongoing meta-analyses may best be able to accommodate the complex genetic architecture of OA.
Methods: An oligonucleotide pull-down assay followed by isobaric mass tag labeling and tandem mass spectrometry was used to isolate and quantify the proteins binding to each rs143383 allele. Further trans-acting factors were identified using TransFac, ProPhit, Pheo 3.0, and TESS prediction software databases, followed by electrophoretic mobility shift assays (EMSA). Candidate proteins were investigated further using antibody supershifts, and their ability to modulate GDF5 expression assessed using RNA interference (RNAi). Luciferase reporter assays were used to assess the impact of over-expressing candidate proteins on the transcriptional activity of both rs143383 alleles. Chromatin immunoprecipitation (ChIP) was used to confirm the binding of these factors to GDF5 in-vivo.

Results: Tandem mass spectrometry identified the transcriptional co-activator p15 as binding more avidly to the T-allele of rs143383. Upon p15 knockdown, overall GDF5 expression increased while the allelic expression imbalance was attenuated. Furthermore, GDF5 was enriched following ChIP with an anti-p15 antibody. By EMSA supershift, we identified the more avid binding of transcription factors Sp1 and Sp3 to the T-allele of rs143383. RNA-mediated silencing of Sp1 and Sp3 significantly increased the expression of GDF5, and modulated the AEl observed. Furthermore, results from ChIP revealed that these two factors bind GDF5 in-vivo.

Conclusions: p15 and Sp3 bind to GDF5, this binding is modulated by genotype at the rs143383 SNP, and these factors differentially regulate the expression of GDF5. Now that these trans-acting factors have been identified, they can serve as targets for modulating the OA genetic deficit mediated by rs143383.

79 BRAIN FUNCTIONAL PROPERTIES PREDICT PLACEBO ALTERNATE IN KNEE OSTEOARTHRITIS

A. Mansour, M. Baliki, T.J. Schnitzer, A.V. Apkarian. Northwestern Univ., Chicago, IL, USA

Purpose: Chronic pain is the primary complaint associated with OA, serving as a predictor of physical dysfunction and muscular weakness. As blinded, randomized placebo-controlled trials of therapeutic agents in OA have repeatedly demonstrated a marked reduction in pain response to placebo, studies of the brain response to placebo may inform on brain properties of OA itself. Although mechanisms of placebo response have been repeatedly studied in humans, we are the first to investigate its underlying brain properties in a clinical drug study design. Here we investigate the possibility of identifying brain properties that predispose different OA patients to placebo analgesia.

Methods: 20 patients meeting ACR criteria for knee OA were enrolled into this single-blinded 4 week study. At baseline, patients were instructed that they might be receiving either active drug or placebo; however they all were given placebo for 2 weeks after which the treatment was stopped. At baseline and at the end of the 2 weeks the patients underwent an fMRI brain scan as well as a structural high resolution T1 brain scan; efficacy reports were collected on both occasions. Two weeks post cessation of medication patients were contacted again and same efficacy reports were recollected. The primary efficacy measure was Visual Analogue Scale 24 hour average pain rating. Secondary efficacy measures included WOMAC Index; Pain detect, Beck depression Index (BDI), and Pain catastrophizing scale (PCS). In addition, 17 healthy age- and gender-matched participants without knee pain were scanned at baseline to serve as controls. For the analysis, the patient groups were stratified as responders vs. non-responders to the placebo treatment based on a decrease in visual analogue scale (VAS) pain rating equal to or greater than 20% from baseline. Analysis was then conducted to determine differences in brain fMRI data between OA patients and controls as well as between OA-placebo-responders vs. OA-placebo-non-responders.

Results: Of the 20 patients enrolled, 9 were male and 11 female; mean age was 57.826.6 years; mean duration of OA was 12.1510.0 years. 3 patients discontinued prior to their week 2 visit; of the remainder, 8 responded to placebo (mean VAS dropped from 72.5+/-14.1 to 32.5+/-20.5, i.e., 56% decrease in VAS) whilst 9 did not show any significant pain relief (VAS changed from 66.7+/-9.2 to 70+/-4.7, i.e., a mean increase of 7%). OA vs controls: By comparing the general number of connections across all brain regions between OA patients and controls we conclude that the region constituted by the medial prefrontal cortex (mpfc) and nucleus accumbens as well as the caudate is more connected (p < 0.05, corrected for multiplicity) in patients with OA than in controls. OA-responders vs. OA-non responders: The secondary somatosensory cortex (S2) was generally more linked in the OA non-responders (p<0.05). In this group, the S2 region displayed significantly more connectivity with the ventro-lateral prefrontal cortex (vlpfc). Moreover, the vlpfc showed more connectivity (p<0.05) with the part of the brain network identified for OA, namely mpfc. Both S2 counts and vlpfc-mpfc connectivity predicted with very high accuracy (AUC=.97 and .96, respectively) responders to placebo.

Conclusions: In this study, we have shown that brain information sharing (functional connectivity) is different in OA in contrast to healthy control subjects. Furthermore, analysis of activity of components of this network predicts the response to placebo. Thus, placebo analgesia is predictable from brain connectivity (information shared between brain regions), prior to start of the placebo treatment trial. These results suggest that distinct sub-groups of OA have distinct propensities for placebo based on their brain states. The specific characteristics that distinguish between these groups remains to be identified. The results of the present study have important implications regarding OA, clinical trials in OA, and for future designs of treatments for OA.

80 PAIN PERCEPTION IN HAND OSTEOARTHRITIS: RELATION BETWEEN CLINICAL NODES, RADIOLOGICAL SEVERITY, PAIN THRESHOLD AND BRAIN PAIN PROCESSING

N. Sofat, J. Wajed, C. Smeed, M. Hermanssohn, F. Howe, T.R. Barrick. St George’s, Univ. of London, London, United Kingdom

Purpose: Hand osteoarthritis (OA) is a prevalent condition for which treatments are based on analgesia and physical therapies. However, the majority of patients continue to have symptoms of pain and reduced function despite optimisation of current available treatments. We hypothesised that inflammation in the hand joints due to osteoarthritis enhances sensitivity and firing of peripheral nociceptors, thereby causing chronic pain. Our primary objective was to evaluate pain perception in a cohort of participants with hand OA by assessing the characteristics of nodal involvement, pain threshold in each hand joint and radiological severity. Our secondary objective was to assess if a standardised painful hand task showed evidence of brain pain processing pathways using functional brain neuroimaging.

Methods: Participants with proximal and distal interphalangeal (PIP and DIP) joint hand OA and non-OA controls were recruited. Clinical parameters of local joint disease including clinical nodes, VAS (visual analog score) for pain (0-100 mm scale), HAQ (health assessment questionnaire), Kellgren-Lawrence scores for radiological severity in each hand joint (30 regions per hand) and standard hand radiographs and pain thresholds performed with algometers (Wagner Instruments, USA) over each joint were assessed. Central brain pain processing in all participants in the cohort was then evaluated using a standardised finger flexion-extension task. Subjects underwent functional brain neuroimaging in a 1.5 Tesla MRI scanner (GE-Healthcare) and we determined the finger flexion-extension task to measure central components of pain processing. Activation of central pain processing pathways was then evaluated using group analyses with FMRIb software (www.fmribo.x.ac.uk/fsl).

Results: All hand OA participants reported pain despite 92% taking oral analgesic drugs. The mean VAS in hand OA participants was 59.31 mm +/-8.19 compared with 4.00 mm +/-1.89 in the control group. Hand OA participants also had HAQ scores 8-fold higher than controls indicating high levels of functional impairment (p<0.05). Objective measures of pain using algometers on 780 joints in total demonstrated lower pain thresholds across not only DIP and PIP, but also CMC, MCP and wrist joints in the OA group versus controls (p<0.001). There was a global reduction in pain thresholds despite the hand OA group reporting pain intensity changes and nodal disease being found in the PIP and DIP joints respectively. Pain threshold in the OA group did not vary significantly with increasing radiological severity. Functional brain MRI during the painful finger flexion-extension task demonstrated increased activation of the thalamus, cingulate gyrus, frontal and somatosensory cortex in the hand OA group that was not observed in the control group (p<0.05). The activated brain regions we observed following a standard placebo pain stimulus were a key predictor of chronic pain perception. In the OA group, pain perception correlated with imaging measures of pain processing regions.

Conclusions: Our data demonstrate that hand OA subjects are sensitised to pain due to increased firing of peripheral nociceptors. Hand OA subjects demonstrate lower pain thresholds globally in their hands compared with controls and have enhanced central sensitisation as demonstrated by increased activation of central brain pain processing pathways. Our findings also suggest that pain processing in OA is