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, Phospho-Pro 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 ANALGESIA IN KNEE OSTEOARTHRITIS
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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 57.82±6.6 years; mean duration of OA was 12.1±10.0 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 reduction (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 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
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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 (30 regions per hand) were recorded. 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) which they performed 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 FMRI software (www.fmrib.ox.ac.uk/fs/).

Results: All hand OA participants reported pain despite 92% taking oral analgesic drugs. The mean VAS in hand OA participants was 39.31 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<0.0001). There was a global reduction in pain thresholds in the hand OA group despite the main radiological 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 were part of the brain 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...
associated with activation of brain regions mediating emotion and fear as evidenced by upregulation of the pre-frontal limbic regions in the hand OA group. Development of future therapies could be targeted at modulating activation of central pain processing centres in OA to improve pain management in patients.

81 RACIAL DIFFERENCES IN PAIN REPORTING IN SUBJECTS WITH OR AT RISK FOR SYMPTOMATIC RADIOGRAPHIC KNEE OSTEOARTHRITIS: DATA FROM THE OSTEOARTHRITIS INITIATIVE

P. Luneburg, L. Yerges-Armstrong, B. Mitchell, M.C. Hochberg. Univ. of Maryland, Baltimore, MD, USA

Purpose: African Americans have a higher prevalence of both radiographic and symptomatic radiographic knee OA (KOA) than Caucasians. While the reasons for this disparity are poorly understood, differences in risk factor patterns, coping mechanisms, and/or pathoanatomic characteristics have been suggested. Less is known, however, about differences in clinical examination features of the knee between the two groups and whether such differences may explain the greater frequency of pain and higher pain reporting in African Americans with knee OA.

Methods: Racial differences in pain reporting and severity as well as findings on physical examination of the knees were assessed using baseline data from subjects enrolled in the Osteoarthritis Initiative (OAI). The OAI is a multicenter, longitudinal observational study initiated in 2004 to examine biomarkers and risk factors for clinically significant knee OA. This analysis included 372 African Americans and 974 Caucasians with symptomatic radiographic KOA in at least one knee (Progression subcohort) and 495 African Americans and 2703 Caucasians at risk for developing symptomatic radiographic KOA (Incidence subcohort). In the current analysis, 3 distinct measures of pain severity (KOOS [0-100], WOMAC [0-20] and NRS [0-10]) and 7 physical exam subcohorts: P < 0.0001 for all comparisons) (Table 1). Furthermore, there were significant differences in physical examination findings between African Americans and Caucasians for the majority of physical examination features in both knees in both subcohorts (Table 2). After adjustment for these differences in physical examination findings as well as multiple confounding variables (see above), African-Americans continued to report greater pain severity than Caucasians both in the absence of symptomatic radiographic KOA (Incidence subcohort: P < 0.0001 for all pain outcomes) and in the presence of symptomatic radiographic KOA (Progression subcohort: P < 0.05 for all pain outcomes except left knee pain in the last 7 days measured by NRS).

Conclusions: These results demonstrate that African Americans report more pain than Caucasians, even after adjusting for sociodemographic factors, depressive symptoms and obesity, and that, regardless of the cause, this difference cannot be explained by findings on physical examination. Further analyses should examine the potential role of coping mechanisms, findings on magnetic resonance imaging of the knees and access to both nonpharmacologic and pharmacologic interventions.

82 P-188, ANTI-TNF-α AND OP-1: A NOVEL TRIAD IN THE PREVENTION OF POST-TRAUMATIC OSTEOARTHRITIS


Purpose: This continuing study investigated the utility of individual or combined pro-anabolic and anti-catabolic biologic interventions targeting chondroprotection and cartilage repair in an ex vivo acute injury model of post-traumatic osteoarthritis (PTOA) in human ankle cartilage.

Methods: Fifteen normal tali obtained from organ donors through the Gift of Hope Organ and Tissue Donor Network were impacted using a 4mm cylindrical indenter with 6000N following a model previously described. 8mm cartilage explants comprising the 4mm impacted core and the immediately adjacent 4mm non-impacted ring were cultured for 14 days in the presence of 5% serum and osteogenic protein-1 (OP-1), two doses of interleukin (IL)-1 receptor antagonist (IL-1Ra); tumor necrosis factor-α (TNF-α) antagonist, N-Acetyl-L-Cysteine (NAC), two pan caspase inhibitors, P-188 surfactant, or the combination of P-188, OP-1, and anti-TNF-α (Triad). Controls included untreated impacted and non-impacted explants. Tissue and media samples were collected on days 0, 1, 2, 7, and 14 to assess cell survival and metabolic activity. Samples were analyzed for cell viability (live/dead assay), apoptosis (Tunel assay), matrix integrity (Safranin O staining, Mankin score), and proteoglycan synthesis and content.

Results: A single impact to human articular cartilage resulted in cell death/apoptosis within the impacted core, which if untreated, expanded to the adjacent non-impacted ring. Sustainable significant increase in cell survival was observed under the treatment with OP-1 and more so in Triad (24% and 43%, p < 0.001 respectively). These same treatments were the most significant in stimulating PG synthesis (5-fold and 6-fold, p < 0.001 respectively). Two of the triad components (TNF-α antagonist and P-188), when given alone did not show any significant effect on PGs. P-188, OP-1, and TNF-α antagonist in combination showed a better preservation of matrix integrity as evidenced by normal pattern of Safranin O staining throughout the entire section. Mankin score was within range of non-impacted control and was about 2-fold lower than the impacted subcohorts: P < 0.0001 for all comparisons) (Table 1). Furthermore, there were significant differences in physical examination findings between African Americans and Caucasians for the majority of physical examination features in both knees in both subcohorts (Table 2). After adjustment for these differences in physical examination findings as well as multiple confounding variables (see above), African-Americans continued to report greater pain severity than Caucasians both in the absence of symptomatic radiographic KOA (Incidence subcohort: P < 0.0001 for all pain outcomes) and in the presence of symptomatic radiographic KOA (Progression subcohort: P < 0.05 for all pain outcomes except left knee pain in the last 7 days measured by NRS).

Conclusions: These results demonstrate that African Americans report more pain than Caucasians, even after adjusting for sociodemographic factors, depressive symptoms and obesity, and that, regardless of the cause, this difference cannot be explained by findings on physical examination. Further analyses should examine the potential role of coping mechanisms, findings on magnetic resonance imaging of the knees and access to both nonpharmacologic and pharmacologic interventions.

Table 1

<table>
<thead>
<tr>
<th>Race/ethnicity</th>
<th>Incidence</th>
<th>Progression</th>
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<tbody>
<tr>
<td>African American</td>
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<tr>
<td>Caucasian</td>
<td>3.41 (3.17)</td>
<td>6.01 (3.47)</td>
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<tr>
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<td>7.61 (17.7)</td>
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<tr>
<td>Caucasian</td>
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<td>3.69 (3.36)</td>
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Table 2

<table>
<thead>
<tr>
<th>Race/ethnicity</th>
<th>Incidence</th>
<th>Progression</th>
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<tbody>
<tr>
<td>African American</td>
<td>106 (21.5%)</td>
<td>283 (10.6%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>283 (10.8%)</td>
<td>141 (39.2%)</td>
</tr>
<tr>
<td>African American</td>
<td>104 (28.8%)</td>
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</tr>
<tr>
<td>Caucasian</td>
<td>141 (39.2%)</td>
<td>293 (30.4%)</td>
</tr>
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References:

1. IL-RA; tumor necrosis factor-α (TNF-α) antagonist, N-Acetyl-L-Cysteine (NAC), two pan caspase inhibitors, P-188 surfactant, or the combination of P-188, OP-1, and anti-TNF-α (Triad). Controls included untreated impacted and non-impacted explants. Tissue and media samples were collected on days 0, 1, 2, 7, and 14 to assess cell survival and metabolic activity. Samples were analyzed for cell viability (live/dead assay), apoptosis (Tunel assay), matrix integrity (Safranin O staining, Mankin score), and proteoglycan synthesis and content.

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