Title

Metabolomic Approach In Osteoarthritic Patients After Itis Diet Intervention

Authors

Saich, JD Murillo
Mannochio-Russo, H
Coras, R
et al.

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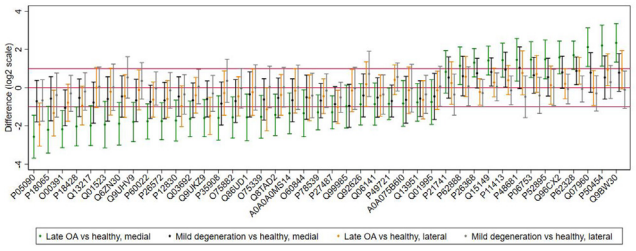
Figure 1. Proteins follow the dose-response expression pattern from healthy to late-stage OA.



in the mild degenerative group. The group where more modulated proteins were found with an absolute fold change (log2) of 1 or larger and a p-value <0,05 was end-stage OA^{medial} where 76 proteins were upregulated and 148 downregulated followed by end-stage OA^{lateral} with 25 proteins upregulated and 45 downregulated. In contrast, the group with less modulated proteins was mild degenerative-OA^{lateral} where only Glypican-6 (GPC6) and Complement C1q tumor necrosis factor-related protein 8 (C1QT8) were found upregulated, followed closely by mild degenerative-OA^{medial} with [T-complex protein 1 subunit zeta (TCPZ), eukaryotic translation initiation factor 4B (IF4B), and Spermidine synthase (SPEE)] upregulated and Sulfhydryl oxidase 1 (QSOX1) downregulated. The metrics for the modulated protein in the early group are shown in Table 1.

A further selection of the proteins was carried out to extract those that follow a specific dose-response pattern of expression considering that the late-stage OA^{medial} group represents the most advanced disease status, while mild degenerative-OA^{lateral} represents the least diseased as compared to healthy. Both mild degenerative-OA^{medial} late-stage OA^{lateral} groups were considered intermediate stages of the disease process. Furthermore, only proteins where the CIs included large differences (at least 1.5 on log 2 scale) for and the comparison of mild degeneration vs control were selected. According to these criteria, 13 proteins were found upregulated and 29 downregulated (Figure 1).

Furthermore, the Gene Ontology (GO) pathway enrichment analysis performed in STRING revealed that tissue development, immune response, and ECM organization were the biological processes more strongly represented in our data set.

Conclusions: Here, the proteomic profile of the lateral and medial meniscus of patients with early signs of OA degeneration was analysed for the first time by using state-of-the-art mass spectrometry data-independent acquisition. However, the profile expression of most proteins in the mild degeneration group was found similar to healthy donor menisci. The largest differences were found between medial end-stage OA versus healthy donors, whereas the mild degenerative stage was relatively similar to healthy donors. The study provides a landscape of those proteins that exhibit a dose-response pattern from healthy to end-stage-OA suggesting an early modulation in the OA process.

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METABOLOMIC APPROACH IN OSTEOARTHRITIC PATIENTS AFTER ITIS DIET INTERVENTION

J. D. Murillo Saich [1], H. Mannochio-Russo [2], R. Coras [1], M. Sala-Climent [1], M. Cedeno [1], A. Quan [3], M.K. Hose [3], E. Gentry [2], N.E. Lane [4], P. Dorrestein [2], M. Guma [1,5]. [1] *Univ. of California San Diego, La Jolla, CA;* [2] *Univ. of California San Diego, Collaborative Mass Spectrometry Innovation Ctr., La Jolla, CA;* [3] *VA San Diego Hlth.care System, San Diego, CA;* [4] *Univ. of California Davis, Davis, CA;* [5] *VA San Diego Hlth.care System, San Diego, CA, United States, San Diego, CA*

Purpose: Osteoarthritis (OA) is the most common form of arthritis. Currently, there is no effective medical disease-modifying therapy available for OA. Prior randomized controlled clinical trial (RCT) evidence supported the beneficial effects of anti-inflammatory micronutrients on pain and physical function in knee OA (KOA). Recent paradigm-shifting studies suggest that gut microbiome-derived pro- and anti-inflammatory metabolites contribute to pain and

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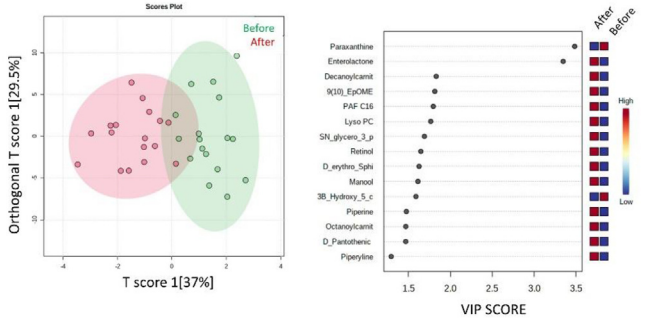

	Fold Change	log2(FC)	After diet
Enterolactone	5.985	2.5813	Higher
Cholic acid	2.6705	1.4171	Higher
Paraxanthine	0.43919	-1.1871	Lower
Stercobilin	0.45528	-1.1352	Lower

inflammation in KOA. The aim of this study is to determine if an anti-inflammatory (ITIS) diet intervention will diminish pain symptoms in KOA by modulating circulating metabolites.

Methods: Patients diagnosed with KOA with a visual analog pain knee score of between 20-80 during the last 7 days, and without changes in therapy during the previous 3 months were offered a 4-week anti-inflammatory diet and recruited in our study. This diet comprises ingredients that increase the omega-3/6 ratio and anti-inflammatory compounds (such as turmeric, anti-oxidants, and prebiotics), in addition to probiotics. It also eliminates pro-inflammatory ingredients such as lactose, gluten, and red meat. We established their clinical and biological baseline on their first visit (day -14). On their second visit (day 0), we collected clinical parameters. Patients were instructed on how to follow the diet and were asked to follow a daily diet log. On their third visit (day 28), we evaluated study feasibility outcomes, diet adherence, and clinical parameters. We also collected blood on day -15 and +28. The trend in clinical changes was examined between days -14 and +28. Bile acids and other bioactive lipids profiling were detected in plasma by untargeted mass spectrometry (MS), and the annotations were obtained using the GNPS platform. The annotated features were compared before (-15) and after diet (+28). Data processing and statistical analysis were performed in R and Metaboanalyst. Data were normalized by sum and the features are presented as peak areas.

Results: In an ongoing clinical trial, 17 KOA (41.2% women, age average: 64 years, standard deviation (SD): 8.72) were recruited to go through

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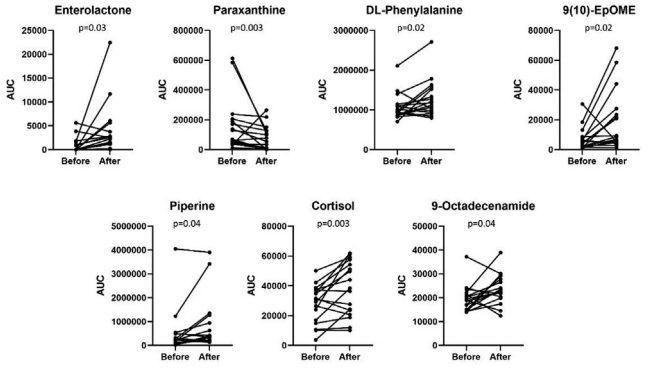

the complete trial. Clinical outcomes improved after the 4-week anti-inflammatory diet (WOMAC osteoarthritis index before and after the ITIS intervention were 53.34±14.20 vs. 42.13±16.22, p=0.032; and WOMAC Pain scores were 12.43±3.70 vs. 9.33±3.06, p= 0.01). The plasma analyses from OA patients detected 2458 compounds; among them, 170 metabolites were annotated based on spectral library searches within the GNPS platform. Figure 1 shows significant discrimination of the metabolic profile between before and after diet by Orthogonal partial least square analysis (OPLS-DA). The variance importance in projections shows enterolactone (formed by the action of intestinal bacteria on plant lignan precursors -increased after diet), and paraxanthine (a down-stream metabolite of the caffeine -decreased after diet) as the metabolites responsible for this separation. Figure 2 shows some of the significant metabolites after the ITIS diet intervention. In addition, DL-phenylalanine, 9(10)-EpOME, piperine, cortisol, and 9-octadecenamide also increased after diet intervention.

Conclusions: Modulating diet has the possibility to complement medication and improve the quality of life for KOA patients by modulating circulating pro- and anti-inflammatory metabolites. Further studies are needed to provide insight into specific long-term dietary interventions or supplements as a complementary treatment in KOA, and to establish the scientific basis for using diet to adjust the circulating metabolome to improve KOA management.

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COMMONALITY BETWEEN THE PROTEOME SIGNATURES OF CLINICAL RESPONSE TO SURGICALLY SUSTAINED AND ACUTE TRAUMATIC CARTILAGE INJURIES IN TWO HUMAN KNEE COHORTS

C.H. Hulme [1,2], A. Williams [3,4,5], T.L. Vincent [5], F.E. Watt [4,5,*], K.T. Wright [6,*]. [1] *Keele Univ., Oswestry, United Kingdom;* [2] *Robert Jones and Agnes Hunt Orthopaedic Hosp., Oswestry, United Kingdom;* [3] *Fortius Clinic, London, United Kingdom;* [4] *Imperial Coll., London, United Kingdom;* [5] *Univ. of Oxford, Oxford, United Kingdom;* [6] *Keele Univ., Keele, United Kingdom*

Purpose: Autologous Chondrocyte Implantation (ACI) is a two-stage surgical procedure aimed at repairing chondral/osteochondral defects. The first arthroscopic surgery requires a harvest of articular cartilage from a low weight-bearing region of the knee, after which chondrocytes are extracted and culture expanded, before implantation into a symptomatic defect 3-4 weeks later. The harvest procedure can be seen as a controlled surgical acute 'injury' to the cartilage, which we have shown initiates a marked inflammatory response measurable in synovial fluid (SF), particularly in patients who do not demonstrate clinical improvement. Separately, we have shown that acute traumatic knee injuries are also associated with a rapid inflammatory response measurable in SF which is associated with longitudinal outcomes. This study compares the SF responses to ACI cartilage harvest injury and acute traumatic knee injury, to i) determine the proteome signatures associated with each type of injury and ii) compare the proteomes of these two groups in terms of their longitudinal clinical response.

Methods: Isobaric tag for relative and absolute quantitation (iTRAQ)-LC-MS/MS was used to assess the proteome of pooled SF samples from the knees of two defined groups, 'improvers' and 'non-improvers', in two respective cohorts: i) those collected immediately prior to Stages I and II of ACI and ii) from those within 8 weeks of acute clinical injury in the Knee Injury Cohort at the Kennedy (KICK) (n=10 for each of the 6 groups). ACI improvement was determined by change in Lysholm score at 12 months (the Lysholm is a scale of 0-100; 100 represents a 'perfect' functioning knee); non-improvers were defined as not demonstrating at least a 10 Lysholm point improvement at 12 months. For the ACI cohort, mean improvement was 33 points (range 17-54) and mean worsening (i.e. non-improvers) was 11 points (range 4-46). KICK non-improvers were defined as having new symptomatic (positive NHANES (National Health and Nutritional Examination Study) symptoms or radiographic OA (Kellgren-Lawrence grade 2+) or KOOS (Knee Injury and Osteoarthritis Score) in the lowest quintile at 2 years post-injury. Non-improvers were categorised as individuals who did not fit the 'improver' definition. Proteins that were differentially abundant between improvers or non-improvers in both cohorts and functional analysis of the differential proteins was carried out using Ingenuity Pathway Analysis software (Qiagen, UK).

Results: In the ACI cohort, 113 proteins were differentially abundant between improvers and non-improvers following cartilage harvest (p<0.05; fold change (FC)±2.0), including insulin-like growth factor-binding proteins 3 & 6 (both were 99 fold higher in non-improvers) and chondroadherin (11 fold lower in non-improvers). Further, 61 proteins were differentially abundant between KICK improvers and non-improvers (p<0.05; FC±2.0): the most differentially abundant proteins included protein- glutamine gamma-glutamyltransferase E and Complement factor H-related protein 2 (both increased 99 fold in KICK non-improvers). Thirty-seven common differentially abundant proteins (FC±2.0) were identified across the two sets of injury cohort outcome comparisons, with 13 proteins following the same direction of change according to post-injury improvement/non-improvement. These included matrix metalloproteinase 2 (increased 8.5 and 3.5 fold in both non-improvers in ACI and KICK, respectively); apolipoprotein-A1 (increased 2.8 fold in both ACI and KICK non-improvers) and caspase-14 (increased 12.8 and 9.7 fold in non-improvers following ACI and KICK, respectively). Several pathways and functions were identified as differentially regulated in non-improvers compared with improvers in both cohorts. These included LXR/RXR activation (ACI: p=$1.42x10^{-26}$, z-score= 0.91; KICK: p=$6.04x10^{-14}$, z-score=2.69) and release of lipids (ACI: p=$1.94x10^{-6}$, z-score=2.67; KICK: p=$1.1x10^{-3}$, z-score=1.14). Suggested common up-stream regulators to both injury mechanisms were also identified, including TP63 (ACI: p=$1.72x10^{-5}$, z-score =1.88; KICK: p=$4.26x10^{-3}$, z-score=1.67) and leptin (ACI: p=$5.36x10^{-7}$, z-score=1.22; KICK: p=$3.21x10^{-4}$, z-score=2.22).

Conclusions: Differential SF proteome signatures can be identified between clinical improvers and non-improvers following both ACI cartilage harvest and clinical acute knee injury. Interestingly there are a number of consistently differentially abundant proteins in non-improvers across both types of injury. Furthermore, commonly regulated/dysregulated biological pathways, functions and upstream regulators can be potentially identified. In addition, these data suggest that the ACI cartilage harvest procedure provides a clinically representative and controllable 'model' of acute cartilage injury in humans; furthermore, that understanding this connective tissue injury response in disparate models such as this is clinically relevant to understanding, predicting and potentially modifying patient outcomes in these different settings.

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INTEGRATION OF MULTI ANCESTRY OSTEOARTHRITIS GENETIC ASSOCIATION FINDINGS, IN SILICO TRANSCRIPTOMICS AND DRUG REPURPOSING HIGHLIGHTS MECHANISM FOR EFFICACY OF ANTI-EPILEPTICS FOR OSTEOARTHRITIS PAIN

M.-L.N. McDonald [1,2], P. Lakshman Kumar [1,2], V. Srinivasasainagendra [1,2], A. Nair [1,2], A. Rocco [1], A.C. Wilson [1], J. Chiles [1], J. Richman [1,2], S.A. Pinson [1], R. Dennis [3], V. Jagadale [3], C. Brown [4,2], S. Pyarajan [5], H.K. Tiwari [1], M. Bamman [6,2], J. Singh [1,2]. [1] *Univ. of Alabama at Birmingham, Birmingham, AL;* [2] *Birmingham VA Hlth.care System, Birmingham, AL;* [3] *Central Arkansas Veterans Hlth.care System, Little Rock, AR;* [4] *Louisiana State Univ. Hlth.Sci. Ctr., New Orleans, LA;* [5] *VA Boston Hlth.Care, Boston, MA;* [6] *Florida Inst. for Human & Machine Cognition, Pensacola, FL*

Purpose: To perform a multi-ancestry genetic association study of osteoarthritis in 484,374 participants in the Million Veteran Program (MVP) and UK Biobank (UKB) harnessing *in silico* transcriptomics and drug repurposing for mechanistic insight.

Methods: Osteoarthritis cases and controls were identified using International Classification of Disease, 9th revision, common modification (ICD-9-CM) and ICD-10-CM codes. Additional exclusion criteria were applied to controls comprising codes for frequent concomitant findings. Genotypes variants were typed using Affymetrix Axiom Biobank Arrays and imputed using the 1000 Genomes reference panel. Ancestry stratified and multi-ancestry analyses were performed using PLINK2 and BOLT-LMM in each study separately adjusting for age, sex, BMI and principal components capturing population structure in participants between 40-80 years of age. MetaXcan was to perform a transcriptomics -wide imputation to identify OA expression quantitative loci (eQTL) in adipose subcutaneous, brain amygdala, brain frontal cortex, and skeletal muscle tissue. Fine-mapping was performed using PAINTOR. Enrichment of drug repurposing targets was examined among significant OA eQTLs (P<0.05) using Genome for REPositioning drugs (GREP) software.

* Joint Author