THE CONTRIBUTION OF COMPLEMENT TO THE HYPERALGESIA PHENOTYPE IN POST-TRAUMATIC OSTEOARTHRITIS: POTENTIAL ROLE OF FACTOR D/ADIPSIN IN SENSORY NEURON SENSITIZATION

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INTRODUCTION: Osteoarthritis (OA) is the leading cause of musculoskeletal pain and a primary driver of care-seeking behavior. However, there is lack of understanding of the mechanisms controlling the relationship between pain and structural damage in OA. Understanding pain signals independent of structural damage has been difficult using the existing pre-clinical models. Moreover, obese patients with OA can experience more severe pain for a given amount of structural damage compared to non-obese OA patients, suggesting an interconnected axis between adipose, nociception, and pain in OA that may be distinct from structural damage. Mice that lack fat-derived adipsin (also known as complement factor D, FD KO) are protected from cartilage damage induced by the destabilization of the medial meniscus (DMM) but demonstrate increased sensitivity to pressure-pain hyperalgesia, providing a unique opportunity to study OA pain independently of structural changes. The goal of this study was to determine the mechanism by which FD modulates pain in OA by conducting bulk RNA sequencing of the dorsal root ganglia (DRG) sensory L3-L5 nerves in FD KO mice with DMM and assessing nerve terminal sprouting in these knees.

METHODS: FD KO mice and WT controls (7-12/group) were fed chow (10% kcal fat) or high-fat diet (HFD, 60% kcal fat). FD KO mice were transplanted with mouse embryonic fibroblast (MEF)-derived fat implants to restore circulating FD, which was confirmed by western blot. At 16-weeks, mice underwent destabilization of the medial meniscus (DMM) unilaterally to induce OA. At 26-weeks, forelimb grip strength, knee hyperalgesia (via small animal algometer) and tactile allodynia (Electronic von Frey) were assessed. Knee joints were evaluated using a Modified Mankin Score and immunohistochemistry (IHC) for C3, C5-9, tyrosine hydroxylase (sympathetic sprouting), and calcitonin gene-related peptide (CGRP, sensory sprouting). Bulk RNA sequencing was performed on L3-L5 dorsal root ganglia (DRG). Data were analyzed by repeated measures of ANOVA (genotype, limb, diet) and *post hoc* testing.

RESULTS: Chow-fed FD KO mice were protected from DMM-induced structural OA (Fig. 1A-B) but not synovitis or osteophyte formation (data not shown). Additionally, these mice demonstrated reduced pressure-pain thresholds and increased tactile allodynia (Fig. 1C). The structural OA change was reversed with MEF implant (Fig.1A-B). FD KO mice on HFD showed no protection against DMM-induced structural OA (Fig. 1A). There was no evidence of an alternate complement pathway bypass mechanism to explain the lack of protection suggesting that complement is dispensable for structural damage under HFD conditions (data not shown). However, HFD FD KO mice demonstrated lower pressure-pain threshold, or increased hyperalgesia at the knee (Fig. C), despite having a similar Modified Mankin Score to HFD WT DMM limbs (Fig. 1A-B). Hyperalgesia was not explained by difference in synovitis (Fig. 1D), suggesting that other mechanisms contribute to the hyperalgesia phenotype. Restoration of alternative complement pathway activity using MEF transplantation in both diet groups induced a reversal of pain thresholds in FD KO to levels observed in WT DMM limb levels, suggesting a role for FD in OA pain (Fig. 1C). Gene ontology (GO) analysis of DRG from WT and FD KO mice with DMM-induced OA revealed differentially expressed genes associated with neutrophil infiltration and histone modifications. A total of 524 differentially regulated genes were detected, and pathway analysis revealed enriched terms for histone modification, suggesting that FD modulates chromatin accessibility in DRGs (Fig. 2). Gene ontology assessments revealed top hits for neutrophil activation in immune responses, neutrophil degranulation, neutrophil mediated immunity, and Th-17-driven inflammation. Lastly, FD KO mice with DMM-induced OA demonstrate increased CGRP-expressing sensory neuron terminals in the lateral aspect of the joint, where pressure-pain hyperalgesia measurements are collected (data not shown).

DISCUSSION: FD KO mice exhibited a paradoxically heightened pain phenotype post DMM, suggesting that this model can be used to dissect the clinical discordance between pain and joint structural damage. The bulk sequencing data from DRGs supports the notion that neutrophils migrate to DRGs and contribute to chronic pain initiation and maintenance in peripheral nerves. FD triggers DRG-dependent neuroimmune changes, potentially by regulating neutrophil infiltration of DRGs, altering chromatin accessibility, the pattern of gene expression, and ultimately pain phenotype. Increased CGRP+ sensory neurons in the FD KO post DMM mice suggests that complement modulates expression of a key neurotransmitter critical to the transmission of pain messages. Ongoing work is aimed at ablating CGRP expression to understand whether the observed increased CGRP+ sensory neurons in FD KO explains the heightened pain phenotype. Insights gained may inform whether therapeutic targeting of complement activity will be beneficial in OA pain treatment.

SIGNIFICANCE: Leveraging the FD KO model of increased hyperalgesia, and state-of-the-art spatial and multiomic approaches will help uncover new mechanistic pathways and targets that are "druggable" for the development of complement-based treatment of OA and musculoskeletal-specific pain.

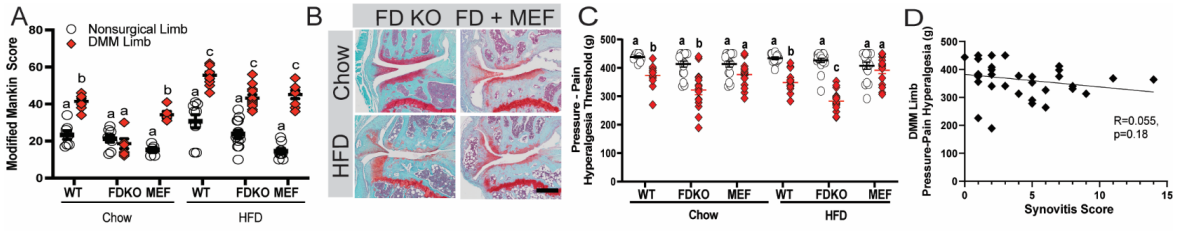

Figure 1. Modified Mankin Score (A), red diamonds indicate limbs challenged with destabilization of the medial meniscus (DMM), circles contralateral limbs, Mouse Embryonic Fibroblast (MEF) indicates FD KO + MEF; (B) Medial tibial plateau Safranin-O/Fast Green histology sections of DMM limbs (black bar indicates 100μm); (C) pressure-pain hyperalgesia measured by SMALGO on DMM (red diamonds) and nonsurgical (circles) limbs; (D) relationship between synovitis score and DMM-limb pressure-pain hyperalgesia is not significant (p=0.18), indicating that the hyperalgesia phenotype is not explained by synovitis. Different letters indicate p<0.05 by ANOVA, data are shown as mean ± standard error.

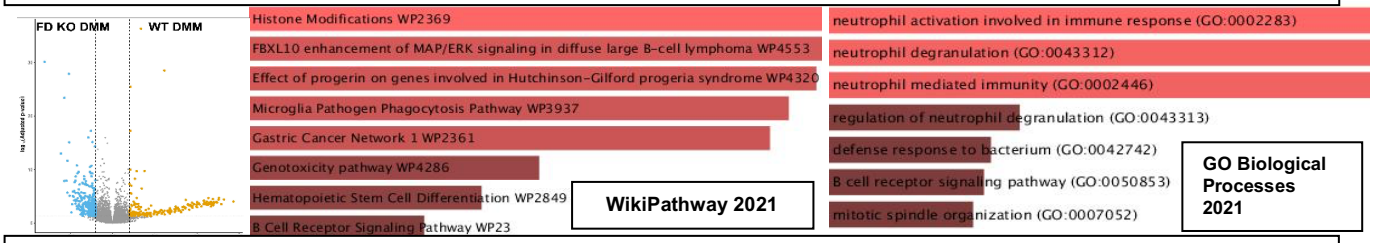

Figure 2.Volcano plots from bulk PolyA sequencing of L3-L5 DRGs from FD KO (blue) and WT DMM limbs, n=3/group. FDKO DMM DRGs exhibited 218 upregulated genes vs. WT, and WT demonstrated 306 upregulated genes vs. FD KO, for a total of 524 differentially regulated genes. Pathway analysis revealed several genes related to histone modifications were differentialyl upregulated in FD KO DRGs with DMM, and genes associated with neutrophil activation involved in immune response using GO terms.