

RNA sequencing identifies increased immune system activation in the infrapatellar fat pad in women with hypertension and knee osteoarthritis

Folly M. Patterson¹, Priya Kulkarni¹, Glyn D. Palmer¹, Michael Strinden¹, Robert “Trey” Dalton¹, Janak Gaire¹, Hernan Prieto¹, Alejandro Almarza², Robert Caudle¹, Yenisel Cruz-Almeida¹, Kyle D. Allen¹
¹University of Florida, Gainesville, FL, ²University of Pittsburgh, Pittsburgh, PA
 f.patterson@ufl.edu

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INTRODUCTION: Hypertension is associated with increased osteoarthritis (OA) severity and pain [1], though it is unclear how this relationship is mediated. Further, there is evidence that this relationship is sex-dependent, with hypertension showing a greater association with radiographic knee OA [2] and pain [1] in women compared to men. As a highly vascularized endocrine and immune tissue, the infrapatellar fat pad (IFP) plays a significant role in OA progression [3] and may serve as a link between hypertension and knee OA development. As such, the objective of this study was to identify transcriptional changes in the IFP associated with hypertension in women, and differences between men and women with hypertension.

METHODS: All procedures were approved by the UF IRB. Informed consent was obtained from participants undergoing total knee arthroplasty for knee OA as part of the RE-JOIN Consortium study. Hypertensive status was identified through electronic medical records. Female participants with hypertension (n=5) were compared to females without hypertension (n=5) and to males with hypertension (n=4). IFP that would normally be discarded as surgical waste was collected, snap-frozen in liquid nitrogen, and stored at -80°C. Samples were thawed in RNAlater™-ICE and total RNA was extracted using the mirVANA miRNA isolation kit followed by ribodepletion with the Illumina Ribo-Zero plus rRNA Depletion Kit. Library construction was performed with the NEBNext Ultra II Directional RNA Library Prep Kit and evaluated with the 4200 TapeStation for average size and Qubit for concentration. Equimolar libraries were sequenced on the Illumina NovaSeq X Plus with samples run in quadruplet. Raw transcriptome reads were aligned to the GRCh38 reference genome using rsem and star packages with gene expression calculated using rsem. After normalization, filtering, and annotation of RNA reads, data dispersion was transformed using a log or variance stabilizing transformation depending on visualization of standard deviation. Gene expression fold-change ≥ 2 or ≤ -2 and p-value ≤ 0.05 were considered significant. Reactome was used to identify statistically overrepresented pathways in significantly up- and downregulated genes. All p-values were adjusted by false discovery rate (FDR).

RESULTS: There were 708 upregulated and 300 downregulated genes in hypertensive female IFP compared to normotensive female IFP (Fig. 1A). The top five upregulated Reactome pathways largely related to immune activation: FcγR activation, classical antibody-mediated complement activation, creation of C4 and C2 activators, scavenging of heme from plasma, and FCεRI-mediated MAPK activation (Fig. 1B). This suggests that hypertension in females with OA is associated with increased complement activation in the IFP. When compared to hypertensive male IFP, hypertensive female IFP displayed 418 upregulated and 317 downregulated genes (Fig. 1C). Here, upregulated genes mapped to only three pathways significant after FDR correction: chemokine receptors bind chemokines, Gα signaling events, and GPCR ligand binding (Fig. 1D). Together, these data indicate females with hypertension have greater upregulation of immune system and related signal transduction pathways compared to both normotensive females and hypertensive males.

DISCUSSION: Women experience greater knee OA and hypertension compared to men but the intersection of these two conditions in OA joint tissues remains understudied. This work found that immune activation in the IFP may be a key factor in the relationship between hypertension and knee OA in women. These data add to the body of evidence that the IFP is a significant contributor to OA pathogenesis [3]. Secreted factors from the IFP induce cartilage degradation, including leptin, a key hormone in obesity and hypertension [3, 4]. Limitations include a small sample size and exclusion of BMI and age as covariates. Future work will evaluate metabolomic shifts and sympathetic fiber sprouting in the IFP.

SIGNIFICANCE/CLINICAL RELEVANCE: This study is the first to consider the role of hypertension in transcriptional changes in the IFP in knee OA. These data will help identify molecular targets for personalized therapeutic development for OA.

REFERENCES: [1] Liu and Du, 2024, *BMC Musculoskelet Disord*; [2] Lo et al., 2022, *J Orthop Res*; [3] Collins et al., 2025, *Sci Rep*; [4] Hui et al., 2012, *Ann Rheum Dis*.

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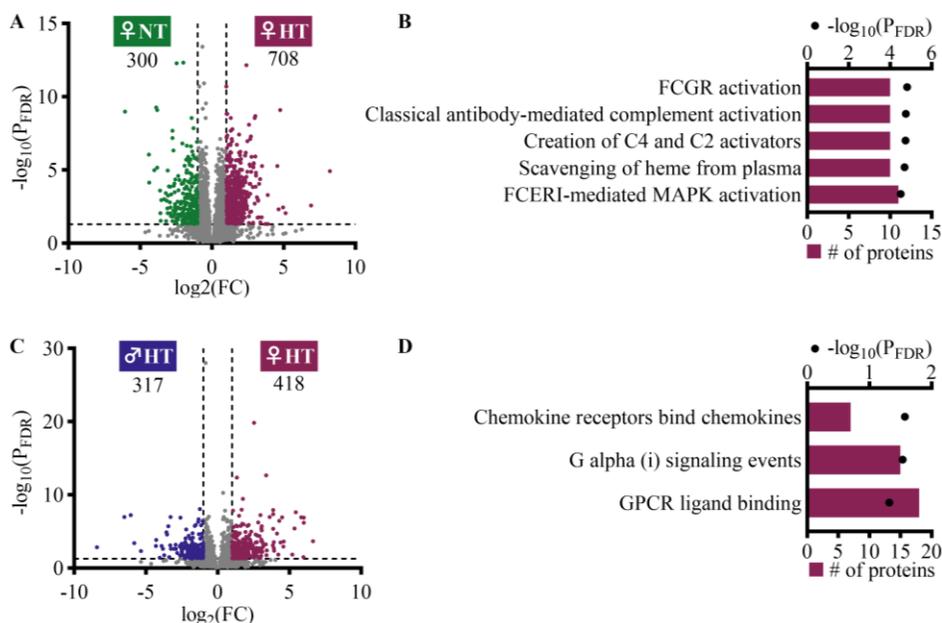


Fig. 1. A: Volcano plot of differentially expressed genes in hypertensive (HT) females (n=5) compared to normotensive (NT) females (n=5) and **B:** corresponding top five upregulated Reactome pathways. **C:** Volcano plot of differentially expressed genes in hypertensive females vs. hypertensive males (n=4) and **D:** corresponding upregulated Reactome pathways.