Integrated GWAS and Single-Cell RNA Sequencing Analysis Reveals the Role of Obesity in Ossification of the Posterior Longitudinal Ligament of the Spine

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AUTHOR DISCLOSURES
The authors report no conflicts of interest.

INTRODUCTION:
Ossification of the posterior longitudinal ligament (OPLL) is a highly heritable and debilitating spinal disorder characterized by heterotopic ossification (HO). Our previous studies demonstrated a causal contribution of obesity to OPLL susceptibility, yet the underlying genetic and cellular mechanisms are poorly understood. This study aims to elucidate these mechanisms by integrating human genome-wide association studies (GWAS) with single-cell RNA sequencing (scRNA-seq) of a rat HO model in obese and non-obese conditions to provide a comprehensive genetic-to-cellular mechanistic framework for obesity-driven OPLL.

METHODS:
Using GWAS summary statistics for OPLL and BMI, we employed Local Analysis of [co]Variant Association (LAVA) to assess local genetic correlations (r_g), Pleiotropic Analysis under a Composite Null Hypothesis (PLACO) to identify potential pleiotropic loci, and colocalization analysis (COLOC) to examine causal variants. Gene set enrichment analysis was conducted by Enrichr. To investigate the cellular mechanisms underlying obesity-associated HO pathogenesis, we utilized a rat model of Achilles tendon injury that recapitulates early-stage HO features (Fig. 1a). Male Zucker fatty (fa/fa) rats and age-matched non-obese littermates (fa/−) underwent unilateral Achilles tendon transection with the contralateral tendon as control. Tendons were harvested for 4 weeks post-injury for scRNA-seq, defining four groups: Non-obese Control (NC), Non-obese Injury (NI), Obese Control (OC), and Obese Injury (OI). We then applied UCell and single-cell Disease Relevance Score (scDRS) to quantify genetic risk enrichment associated with OPLL at single-cell resolution.

RESULTS:
We identified 50 loci with nominally significant local r_g between OPLL and BMI using LAVA. To explore shared genetic signals, we selected both OPLL and BMI lead variants from each of these 50 loci and annotated the nearest gene. Gene set enrichment analysis of the 50 nearest genes to OPLL lead variants at these loci revealed significant enrichment in BMI-related traits in the GWAS Catalog 2025 database (Fig. 2b), confirming these regions are genetically linked to obesity. Importantly, we found only 13 overlapping genes between OPLL and BMI. Gene enrichment analysis of the 13 shared genes revealed a significant enrichment in the MAPK signaling pathway in both KEGG and BioPlanet databases, suggesting that this pathway may constitute a shared mechanism linking BMI to OPLL. Although the variant rs7045837 near *NTRK2* was not reported in our previous OPLL GWAS, it exhibited a strong pleiotropic signal using PLACO with consistent directional effects across both traits (Fig. 2a), suggesting that rs7045837 may influence OPLL susceptibility via its effect on BMI. Colocalization analysis further supported rs7045837 as a candidate shared causal variant (PP.H4 = 0.806). Single cell analyses of tendons identified 9 major cell types based on unbiased clustering with UMAP analysis (Fig. 1b) and defined two distinct sets of transcriptional changes associated with obesity. First, we characterized the obesity-induced DEGs (obinDEGs), which reflect the chronic pathological state induced by obesity. These genes were significantly enriched for pro-fibrotic TGF-β and MAPK signaling pathways, and the most significant change was the downregulation of *Ntrk2* in pericytes. Second, we analyzed the obesity-aggravated DEGs (obagDEGs), which represent an exaggerated injury response. This gene set was dominated by the activation of the HIF-1α pathway, with *Hif-1α*, which is located near our previously identified OPLL GWAS risk locus, being a top DEG in progenitors. Integration of genetic signals with scRNA-seq using UCell identified chondrocytes as the cell type with the highest enrichment for both OPLL GWAS genes ($P = 3.6 \times 10^{-4}$) and an independent endochondral ossification gene set, with moderate correlation between the two scores across cell types (Pearson r = 0.73, P = 0.026; Fig. 1c). Progenitors demonstrated the strongest association with OPLL risk genes via scDRS (P = 0.026), which was independently validated in publicly available human posterior longitudinal ligament scRNA-seq data (PRJNA1067846; P = 0.012).

DISCUSSION:
Our study integrates human genetic risk and single-cell transcriptomic data to uncover the mechanisms by which obesity promotes heterotopic ossification in OPLL. Although the NTRK2 locus was not identified as genome-wide significant in prior OPLL GWAS, our pleiotropy analyses revealed it as a novel gene shared with BMI. Interestingly, our findings support a "priming and triggering" model for obesity-driven OPLL. Obesity first "primes" the tissue by establishing a chronic pro-fibrotic state, characterized by the downregulation of the novel pleiotropic gene *Ntrk2* in pericytes and activation of the MAPK pathway, implicating it in establishing a dysfunctional vascular niche that precedes ossification. This 'priming' phase sensitizes tissues to injury-induced hypoxia, which then 'triggers' exaggerated *Hif-1α*-driven chondrogenic programs in progenitors, which is directly linked to a known OPLL risk locus. Furthermore, by integrating GWAS data with our scRNA-seq dataset, we pinpoint progenitors as the genetic risk–harboring population, with chondrocytes serving as the pathologic effectors. Cross-species analysis confirms the conservation of this risk-enriched progenitor state in human OPLL samples.

SIGNIFICANCE/CLINICAL RELEVANCE:
This study uncovers obesity-linked genetic loci and cellular mechanisms driving heterotopic ossification, offering new insights for targeted strategies to prevent OPLL progression.

Fig.1

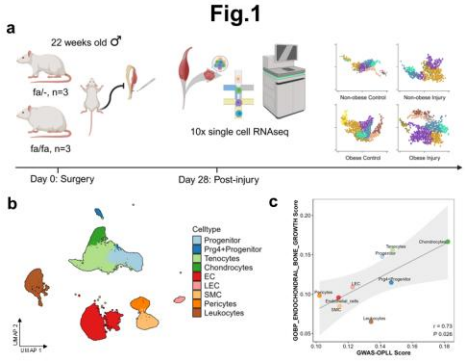

Fig.2