Characterizing a genetic variant with high risk of osteoarthritis using knockout mice and engineered cartilage

Chase Binion[1,2], Susan D'Costa[1], Matthew J. Rich[1,2], Garrett A. Sessions[1], Alex J. Roupas[1], Brian O. Diekman[1,2]

[1]Thurston Arthritis Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC and North Carolina State University, Raleigh, NC

Corresponding author: bdiekman@email.unc.edu

Genetic predisposition is a key driver of the risk for osteoarthritis (OA) and genome-wide associated studies (GWAS) have identified genetic variants that increase the risk of OA. The presence of two copies of an 8 bp insertion frameshift mutation in the coding region of chondroadherin-like (CHADL) results in a 7.7-fold increased risk of total hip replacement (Styrkarsdottir et al, 2017). CHADL is a small leucine rich proteoglycan (SLRP) that is localized to the pericellular matrix of cartilage (Fig. A. left). The specific expression pattern and the lack of a phenotype in those harboring a single risk allele supports the interpretation that the complete loss of functional CHADL protein compromises the quality of cartilage tissue, but the mechanisms are unknown. To investigate the role of Chadl in vivo, we have developed a cohort of mice with and without the genetic loss of Chadl (Fig. A, right). Chadl knockout mice have normal size/weight, joint structure, and growth plate morphology, highlighting that Chadl is not required for skeletal development. To complement in vivo studies, we have used a CRISPR/Cas9 genome editing approach to delete CHADL from primary human chondrocytes. Single-cell derived colonies were screened using PCR (Fig. B), with 23 out of 88 colonies having the homozygous deletion of CHADL. A subset of these colonies were used to make chondrogenic pellet cultures that stained positively for sulfated glycosaminoglycans (GAGs) by Safranin-O after 28 days of stimulation with TGF-β1 (Fig. C), providing a system to investigate the way that CHADL loss affects cartilage synthesis.