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

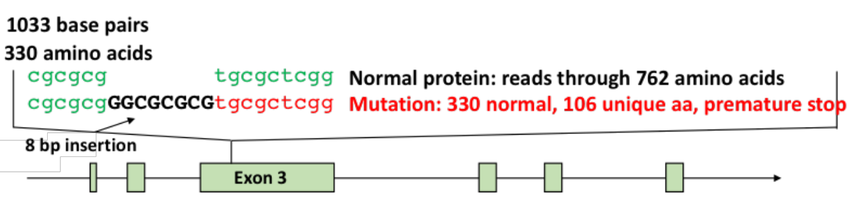
Chase Binion[1,2], Susan D'Costa[1], Matthew J. Rich[1,2], Garrett A. Sessions[1], Alex J. Roupas[1], Brian O. Diekman[1,2]

[1]Thurston Arthritis Research Center, University of North Carolina, [2]Joint Department of Biomedical Engineering, UNC & NC State

Introduction

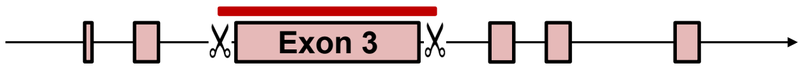
- Small leucine rich proteoglycans (SLRPs) regulate the formation and maintenance of cartilage matrix through altering collagen fibrillogenesis and modulating growth factor presentation.
- Chondroadherin-like (CHADL) is an SLRP that is expressed in chondrocytes during periods of high matrix synthesis. CHADL is relatively uncharacterized but may function to slow chondrogenic differentiation and promote matrix (Tillgren, 2015).
- A genome-wide association study (GWAS) has uncovered a rare mutation (rs523464664) in CHADL that is linked to a 7.7 fold increased risk for total hip replacement due to osteoarthritis when present on both copies of the gene (Styrkarsdottir, 2017).
- The rs523464664 mutation is an 8 bp insertion in the open reading frame of CHADL exon 3, causing both a frameshift and premature stop. While the transcript may be susceptible to nonsense mediated decay before translation, if made the truncated protein would contain 106 novel amino acids at the C-terminus (Styrkarsdottir, 2017).
- The efficient use of the CRISPR/Cas9 ribonucleoprotein (RNP) system has been established in primary human chondrocytes as a method to generate engineered cartilage with the complete knockout of target genes (D'Costa, 2019).

8 bp insertion mutation in CHADL (rs523464664)

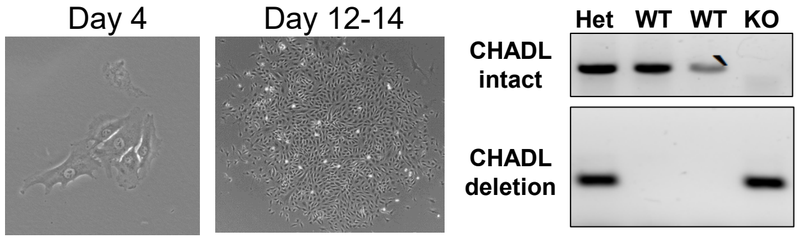


Homozygous CHADL knockout in primary human chondrocytes through genome editing

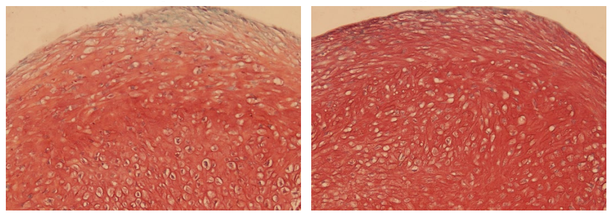
Guide RNAs target both sides of exon 3 for excision



Expansion and screening of single-cell derived colonies

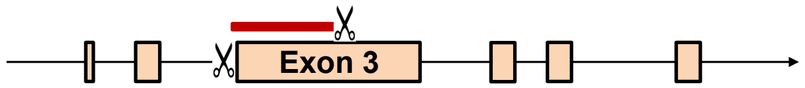


Safranin-O stain of engineered tissue after 28 days of pellet culture with 10 ng/ml TGF-β1

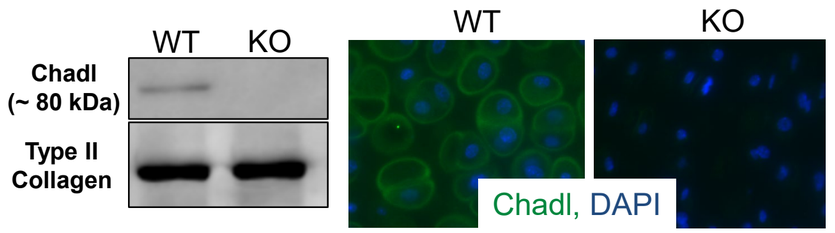


Characterization of Chadl knockout mice

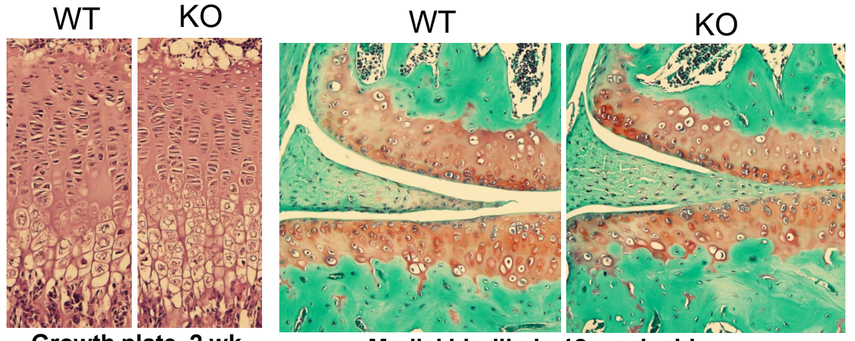
Chadl KO mice have a targeted 897 bp deletion in exon 3



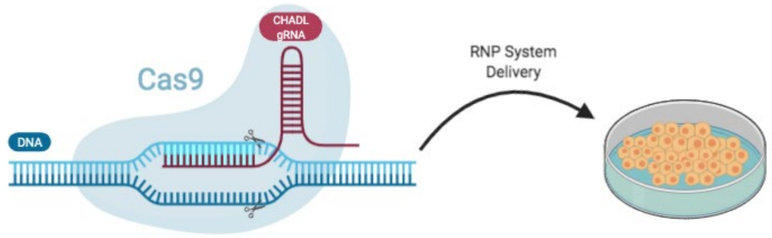
Complete loss of Chadl protein in hip cartilage from 2-week old mice



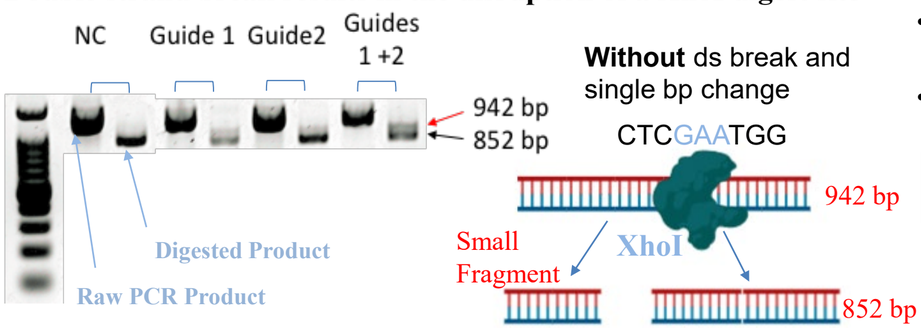
Normal skeletal development in Chadl KO mice



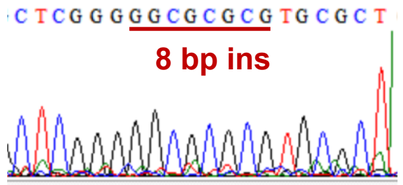
Preparation for generating mouse with 8 bp insertion: Modeling in Neuro2A cells



Double strand break results in the disruption of a XhoI digest site

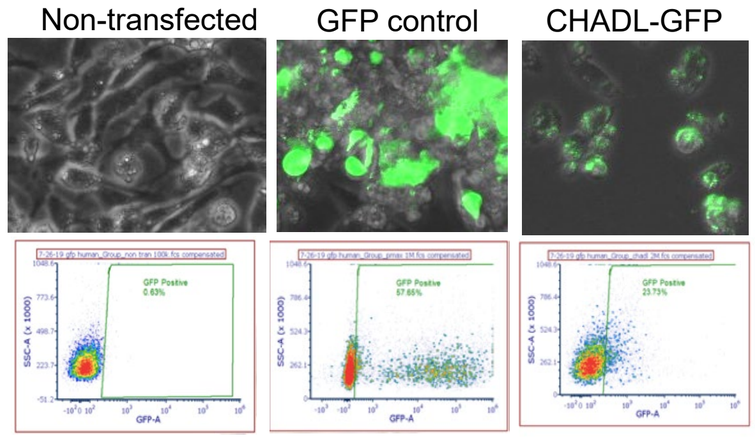


Sanger Sequencing on Colony E7

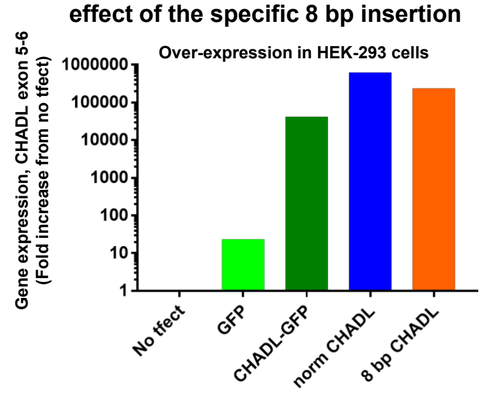


CHADL cDNA plasmid overexpression

Transfection efficiency in primary human chondrocytes



Multiple versions of CHADL plasmid to test effect of the specific 8 bp insertion



Discussion

- The high risk of OA with a genetic variant in CHADL suggests that this protein may regulate optimal cartilage function.
- The complete knockout of CHADL in primary human chondrocytes allows for investigation of protein function during cartilage matrix production.
- Chadl knockout mice show normal development, allowing for investigation of age-related OA without confounding factors.
- Inserting the 8 bp in murine neuro2A cells paves the way for making a novel genetically-engineered mouse.
- Overexpression of plasmid DNA with the 8 bp insertion allows for overexpression in HEK-293 and human chondrocytes.

References

Tillgren V, Ho JCS, Önnerfjord P, Kalamajski S. The novel small leucine-rich protein chondroadherin-like (CHADL) is expressed in cartilage and modulates chondrocyte differentiation. *The Journal of biological chemistry*. **2015**; 290(2):918-925. doi: 10.1074/jbc.M114.593541.

Styrkarsdottir U et al. Whole-genome sequencing identifies rare genotypes in COMP and CHADL associated with high risk of hip osteoarthritis. *Nature Genetics*. **2017**; 49(5):801-805. doi: 10.1038/ng.3816.

D'Costa S, Rich MJ, Diekman BO. Engineered cartilage from primary human chondrocytes with homozygous knockout of cell cycle inhibitor p21. In Press, *Tissue Engineering*.