Introduction: Osteoarthritis (OA) is one of the most common and painful musculoskeletal diseases known to affect tissues throughout the entire joint. Hallmarks of the disease include: progressive loss of the articular cartilage, meniscal degeneration, subchondral bone sclerosis, synovial proliferation, and osteophyte formation. A variety of OA related risk factors have been identified such as age, sex, traumatic injury, obesity, and genetics. A genetic predisposition to OA has been assessed in several twin and family risk studies and it has been suggested that the heritability of OA could be as high as 50-65%. In addition to important structural and extracellular matrix (ECM)-related factors, several genetic studies have identified molecules in the Wnt (Sfrp3,β-catenin), BMP (Bmp7,1a, Gdf5), and TGFβ (Lithp3) signaling pathways as important regulators of articular cartilage and joint maintenance in both mice and humans. Recently, it has been demonstrated that the Notch signaling pathway is a critical regulator of skeletal progenitor cell differentiation during both chondrogenesis and osteogenesis during development, and may also play a significant role in regulating chondrocyte and osteoblast function and terminal maturation.

Since the Notch pathway has been shown to be important during early development and maintenance in both mice and humans, it is being utilized to determine the dominant cell type responsible for maintaining the articular cartilage phenotype and also being utilized to determine the dominant cell type responsible for maintaining a normal joint homeostasis. Continuing studies are aimed at identifying the Notch signaling cells that are responsible for maintaining a normal articular cartilage phenotype. Furthermore, since multiple Notch signaling pathways are known to play a role in the development and maintenance of the articular cartilage, subchondral bone, synovium, and ligament changes during joint development and maintenance using the hindlimbs isolated from E14.5, E18.5, 2-week, 2-month, 4-month, and 8-month old mice. The University of Rochester Committee on Animal Resources approved all protocols.

Materials and Methods: To determine the role of canonical Notch signaling during joint development and maintenance in vivo, we generated Notch loss-of-function conditional mutant mice using Rbpjk floxed alleles in combination with the Prx1Cre transgene to specifically remove Notch activity in skeletal progenitors of the limbs (Prx1Cre; Rbpjk−/− mice). Lineage tracing analyses using Prx1Cre transgenic and Rosa26Reporter (R26R) mice were performed to identify the specific cells targeted. We performed whole skeletal staining, micro-CT 65%, in histology, in situ hybridization (ISH), immunohistochemistry (IHC), and polarized light microscopy to assess articular cartilage, subchondral bone, and meniscus and ligament changes during joint development and maintenance using the hindlimbs isolated from E14.5, E18.5, 2-week, 2-month, 4-month, and 8-month old mice. The University of Rochester Medical Center, Center for Musculoskeletal Research, Rochester, NY

Discussion: Our in vivo data demonstrate for the first time that Rbpjk-dependent Notch signaling is critical in maintaining postnatal articular cartilage and joint maintenance, but is dispensable for the formation of synovial joints during embryonic development. Since OA is viewed as a disease of the entire joint, we first took the approach of removing Rbpjk floxed alleles using the Prx1Cre transgene that targets a variety of mesenchymal cell types within the joint (articular cartilage, subchondral bone, synovium, ligaments). Loss of Rbpjk-dependent Notch signaling in these cells results in many of the same hallmark features observed in OA including: articular cartilage degeneration, subchondral bone sclerosis, osteophyte formation, and synovial expansion, while articular cartilage and meniscal fibrosis has not been prominently described in the literature or other genetic or injury induced models of OA. This may represent a unique feature to this genetic model and requires further research to identify the exact molecular underpinnings responsible for the phenotype. Furthermore, since multiple Notch signaling components are detectable in many of the tissues targeted by the Prx1Cre transgene, it remains to be determined precisely which Notch signaling cells are responsible for maintaining a normal morphological and functioning joint. Continuing studies are aimed at developing in vitro models of articular cartilage maintenance to study the effects of Notch inhibition on articular chondrocyte differentiation. A variety of tissue and cell specific inducible Cre transgenic mice are also being utilized to determine the dominant cell type responsible for maintaining the articular cartilage phenotype and normal joint homeostasis.

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