RIPK2 activity alters local inflammatory pathways in the joint to promote age-associated OA

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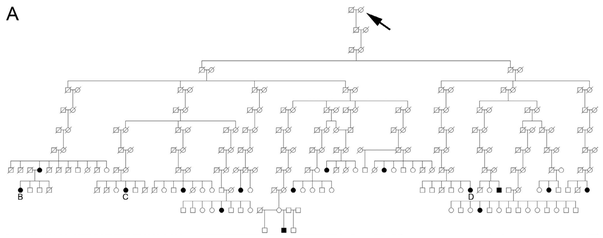
Background

The main obstacle to the development of disease-modifying therapies for OA is poor understanding of the disease process and lack of appropriate genetic models. We do not know the cell types or molecular pathways that normally function to limit susceptibility to OA and we have few genetic models that recapitulate human age-associated OA. Our goal is to discover molecular pathways that are vulnerability points for the development of OA and generate mouse models using human disease alleles. We discover these pathways by identifying rare mutations in coding sequences that have a strong effect on susceptibility in families with OA.

From our family analyses we identified 7 novel variants affecting components of the NOD/RIPK2 inflammatory signaling pathway that are associated with familial OA affecting the hand, shoulder, or foot. We introduced the OA-associated *RIPK2*^{104Asp} variant into the mouse and demonstrated that the *Ripk2*^{104Asp} allele acts dominantly to alter basal physiology and response to trauma in the mouse knee by increasing inflammation locally in the joint. In addition, uninjured 2-year-old *Ripk2*^{Asp104} mice have severe OA, yet we do not understand the primary molecular and cellular changes that underly OA development.

We hypothesize that RIPK2 drives local inflammatory pathways in the joint to promote age-associated OA.

Identification of families with NOD/RIPK2 mutations



NOD/RIPK2 pathway

Table 1. *NOD/RIPK2* Pathway Variants Identified in Independent Osteoarthritis Families

Gene	OA Phenotype (Family)	Variant	Minor Allele Frequency	Protein Domain Affected by Variant
NOD1	Finger Interphalangeal Joint OA (FIJ744)	c.G2114A:p.R705Q	0.0008	Leucine Rich Repeat Domain
NOD2	1st MTP Joint OA (UUHR2)	c.C2546T:p.A849V	0.00007	Leucine Rich Repeat Domain
NOD2	Finger Interphalangeal Joint OA (FIJ7)	c.G247A:p.A83T	0.00008	Caspase Activation and Recruitment Domain
IKBKB	Glenohumeral OA (SA735)	c.G1663A:p.G555R	0.00008	Scaffold Dimerization Domain
CARD9	Finger Interphalangeal Joint OA (FIJ9)	c.G722A:p.R241Q	0.00005	Structural Maintenance of Chromosomes
CHUK	1st MTP Joint OA (MTP25)	c.A376T:p.S126C	0.0008	Kinase Domain
*RIPK2**	1st MTP Joint OA (UUHR1)	c.A310G:pN104D	0.0004	Kinase Domain

* - Previously described in Jurynec, 2018.

Figure 1. Representative OA pedigree and mutations in the NOD/RIPK2 pathway.

The NOD/RIPK2 pathway regulates the cellular response to PAMPs and DAMPS

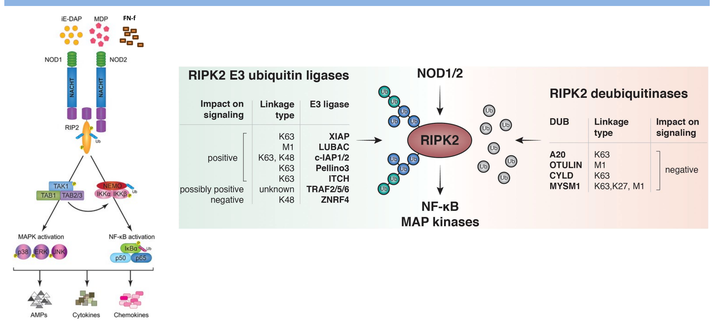


Figure 2. The NOD/RIPK2 pathway is activated by pathogen-associated molecular pattern (PAMPs) and damage-associated molecular pattern molecules (DAMPs), including the Fibronectin fragment (FnF). Its activity is modulated by post-translational modification.

Uninjured Ripk2^{104Asp} knees express markers associated with OA and develop severe age-associated OA

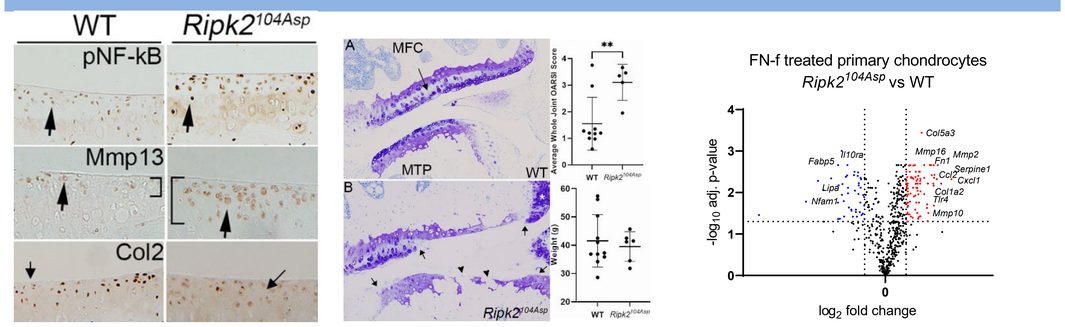


Figure 3. Uninjured *Ripk2*^{104Asp} knee joints express markers associated with OA at 24-weeks of age and develop severe age-associated OA compared with WT. OARSI analysis of knee joints at 2 years of age. *Ripk2*^{104Asp} primary mouse chondrocytes have an amplified catabolic response to the FN-f.

Ripk2^{104Asp} mice have reduced activity and altered bone properties at 36 weeks of age despite no histological evidence of OA

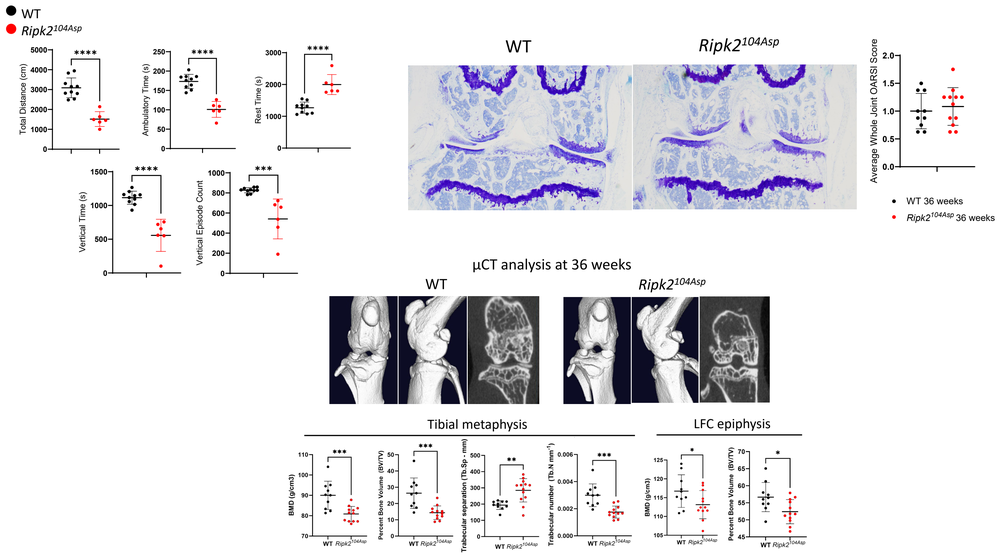
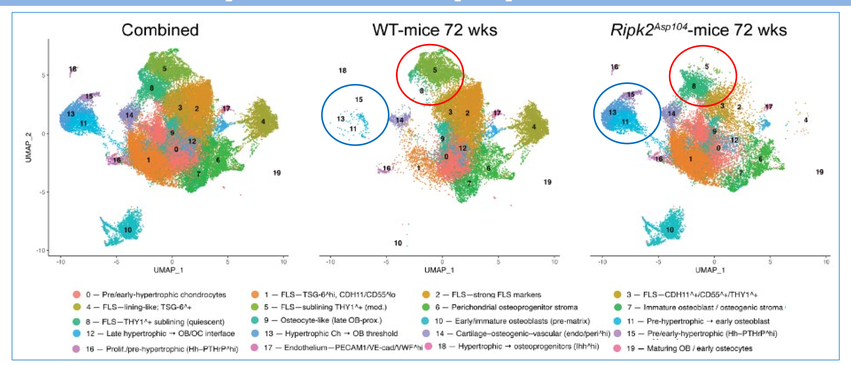


Figure 4. *Ripk2*^{104Asp} mice have reduced activity and bone properties at 36 weeks of age despite no histological evidence of OA. 36 weeks was the first time point at which we detected significant changes in behavioral activity.

Aged Ripk2^{104Asp} mice have changes in osteocyte and synovial cell populations

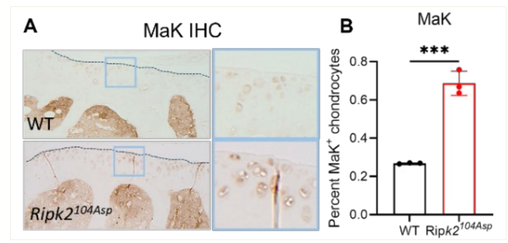


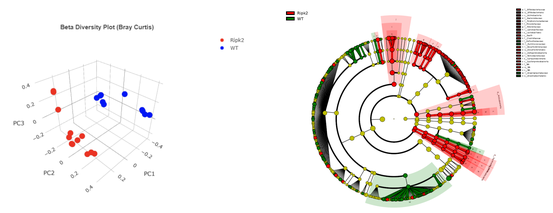
5 – FLS sublining (Thy1+/mod). Gained in *Ripk2*^{Asp104} mice. Increased T-cell and Hippo signaling.
8 – FLS lining cells (Thy1+,quiescent). Lost in *Ripk2*^{Asp104} mice. Increased antigen processing and TNF signaling.

11- Pre-hypertrophic/early osteoblast – Gained in *Ripk2*^{Asp104} mice. Activation of endochondral ossification pathways and cartilage-to-bone conversion.
13- Hypertrophic chondrocytes – osteoblast threshold – Gained in *Ripk2*^{Asp104} mice. Increased hormonal and GPCR responsiveness, glycolytic and autophagy signatures.
15 – pre/early hypertrophic chondrocytes (Hh-PThrP high) - – Gained in *Ripk2*^{Asp104} mice.

Figure 4. Whole joint single cell analysis on 72-week-old WT and *Ripk2*^{Asp104} mice.

Aged Ripk2^{104Asp} mice have an altered metabolic state and microbiome





Increase in Lactobacillus, Saccharibacteria, and Actinobacteria
Reduction in Bacteroidales and Ruminococcaceae

Figure 5. *Ripk2*^{104Asp} mice have an altered metabolic state (increased MaK staining at 2 years of age) and microbiome (2 years of age) compared with WT mice.

RIPK2 is a new therapeutic target

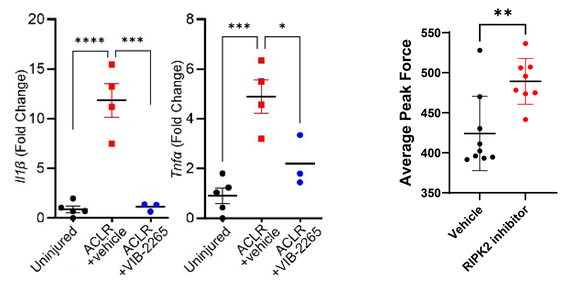


Figure 6. RIPK2 inhibitors reduce expression of OA-associated proinflammatory gene expression 5-days-post ACL rupture and increase the pain threshold in the knee 8 weeks post DMM.

Conclusions

- Animals carrying the single amino acid change encoded by the *Ripk2*^{104Asp} variant have a magnified response to joint injury that leads to a predisposition to develop OA.
- The allele creates a chronically hyperactive inflammatory state in the joint with early signs of defective joint maintenance, which leads to severe age-associated OA.
- Early signs of defective joint maintenance portends overt OA and a reduction in multiple measures of activity.
- Cellular analyses indicated that changes in osteocytes and synovium are key drivers of joint remodeling during aging.
- **In sum, developing new age-associated OA animal models with human susceptibility alleles is useful for understanding mechanism of disease, identifying and developing biomarkers for early detection of OA, and therapeutic development.**

Acknowledgements

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