The NOD/RIPK2 signaling pathway is a susceptibility factor for osteoarthritis

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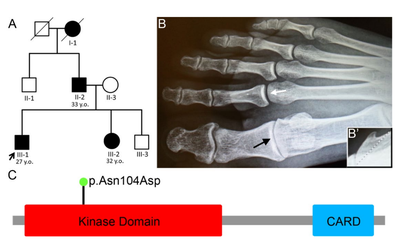
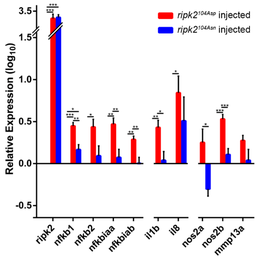
Background

The molecular pathways that are rate-limiting in the onset and progression of osteoarthritis (OA) are unknown, consistent with the complete lack of disease-modifying drugs available at this time. Knowledge of these pathways is required for identifying individuals at risk for disease, for understanding mechanisms that trigger or amplify disease processes, and for development of effective therapies. One proven approach toward identifying pathways and biological processes whose normal functions limit disease has been to identify gene variants responsible for highly penetrant familial forms of the disease. Increasing evidence demonstrates there are no/few differences between the genes contributing to "monogenic" disease and those contributing to complex disease. Pathways that can be mutated to have determinate effects promoting OA will also be vulnerable to the modest genetic or environmental perturbations that underlie common spontaneous forms of OA. Despite its promise, to date there have been relatively few studies of familial OA. We have used a unique medical genetics resource, the Utah Population Database, to identify a large number of multigenerational families with dominantly inherited OA. Here we employ genomic analyses of these families and functional analyses in mice to test the hypothesis that perturbation of the NOD/RIPK2 proinflammatory pathway is sufficient to significantly elevate susceptibility to OA.

A Proinflammatory RIPK2 Mutation Associates with Early-Onset OA

Our previous work (Jurynec et al, 2018) identified an allele of RIPK2 that associates with early-onset OA. Functional analyses of the disease allele in zebrafish indicated that it is hyperactive and promotes a heightened proinflammatory response.

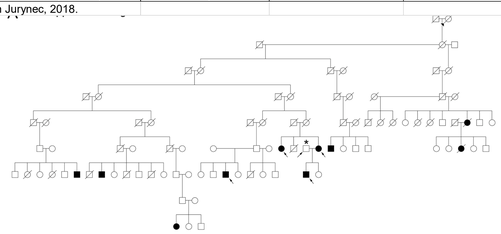




Rare alleles of NOD-RIPK2 pathway genes are associated with multiple types of familial OA

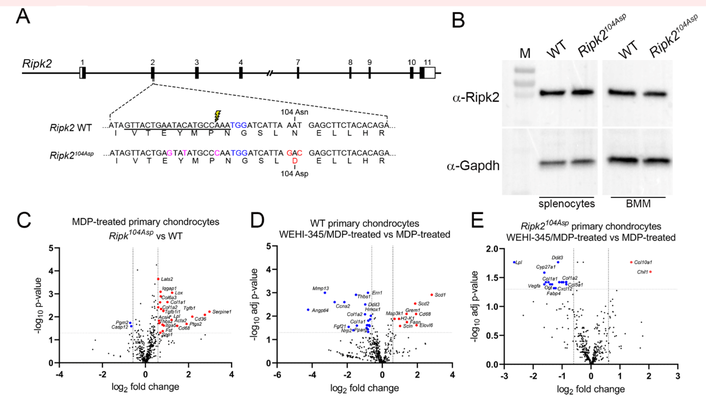
Gene	OA Phenotype (Family)	Variant	Minor Allele Frequency	Protein Domain Affected by Variant
NOD1	Finger Interphalangeal Joint OA (FIJ744)	c.G2114A:p.R705Q	0.0008	Leucine Rich Repeat Domain
NOD2	1st MTP Joint OA (UUHR2)	c.C2465T:p.A822V	0.00007	Leucine Rich Repeat Domain
NOD2	Finger Interphalangeal Joint 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.

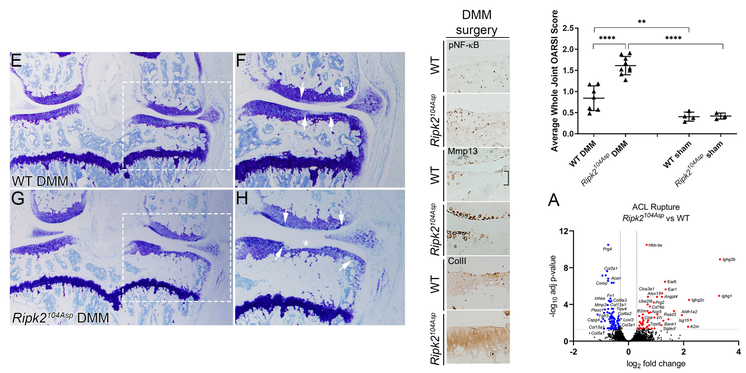


Generation of the Ripk2^{104Asp} mouse

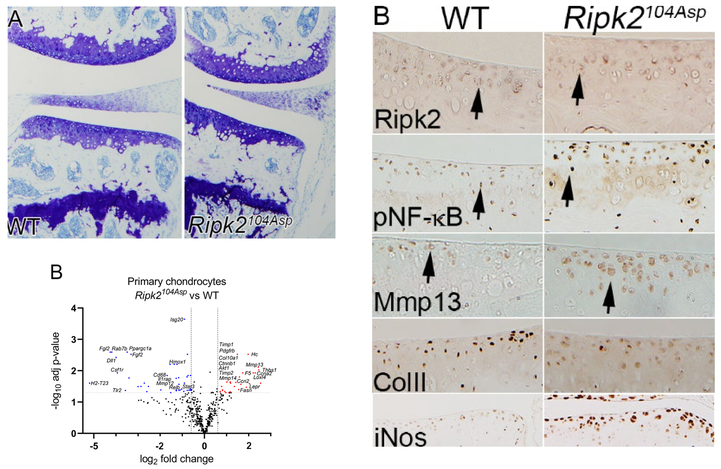
A single copy of the Ripk2104Asp allele is sufficient to alter gene expression in primary articular chondrocytes



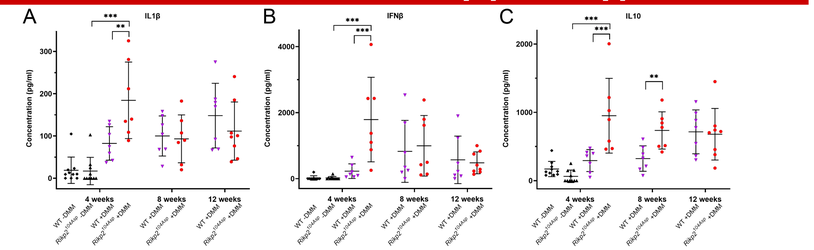
The Ripk2^{104Asp} allele acts dominantly and is sufficient to confer increased susceptibility to OA



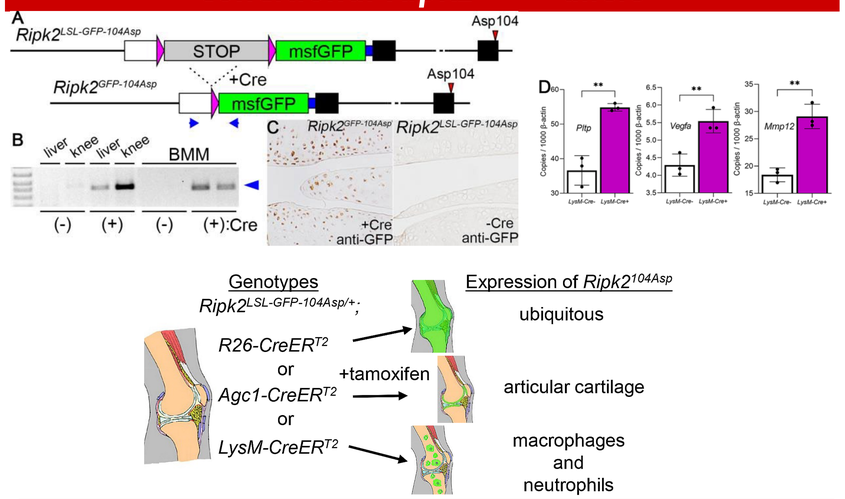
Uninjured Ripk2^{104Asp} knee joints appear primed to develop OA



The Ripk2^{104Asp} mice do not exhibit a chronically elevated inflammatory phenotype



Generation and preliminary characterization of a conditional Ripk2^{104Asp} allele



Conclusions

In sum, 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, as evidenced by gene expression in chondrocytes isolated from young mice and altered expression of pNF-κB, iNos, Mmp13, and ColII in mature animals. Nevertheless, the elevated activity of the NOD/RIPK2/NF-κB pathway caused by the variant allele has a very modest effect on tissue remodeling under normal laboratory conditions.

We propose modulation of the NOD/RIPK2 signaling pathway is a general vulnerability factor for OA. Our data indicate that modification of the NOD/RIPK2 pathway can render multiple joints (both weight and non-weight bearing) susceptible to OA. While the initiating factor may be different between joints and individuals, our work has shown that altered NOD/RIPK2 signaling is a predictive indicator of susceptibility to OA. Further pursuit of this signaling pathway and the spatiotemporal requirement for its activity may lead to assays for detection of early stages of disease and have therapeutic potential.

Acknowledgements

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