

A novel genetic mouse model of age-associated OA

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INTRODUCTION: 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 gene mutations in coding sequences that have a strong effect on susceptibility to OA in families identified from a unique statewide population-based cohort, the Utah Population Database. 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. Whereas the knees of uninjured young *Ripk2*^{Asp104} mice appear normal histologically, the joints exhibit a set of marked gene expression changes reminiscent of overt OA. Although the *Ripk2*^{104Asp} mice lack evidence of chronically elevated systemic inflammation, they do exhibit significantly increased susceptibility to PTOA due to an increased inflammatory state within the joint. Here we test the hypothesis that the molecular changes in uninjured young *Ripk2*^{104Asp} mice presage overt OA and that the *Ripk2*^{104Asp} mice will be useful as a novel genetic mouse model of age-associated OA.

METHODS: We aged WT and *Ripk2*^{104Asp} mice to 24 weeks, 36 weeks, and 2 years of age. We performed molecular, histological, immunohistochemistry, and behavioral analyses to assess onset, progression, and functional outcomes associated with age-onset OA. Molecular analyses included measuring serum (systemic) and synovial fluid cytokines (local) and RNA-seq on whole joint tissue. Histological staining and OARSI scoring was performed on the right knee and the left knee was collected for RNA-seq analysis. As a proxy for pain, we quantify activity levels using open field locomotor boxes. These boxes allow us to measure multiple parameters including distance traveled, time spent at motion/rest, and rearing on hind limbs. WT and *Ripk2*^{104Asp} mice were acclimated to the boxes for 1 week prior to testing.

RESULTS: Histological analysis of knee joints of 24-week-old *Ripk2*^{104Asp} mice display no obvious histological signs of OA, yet expresses markers that are associated with overt OA (increased pNF-κB and Mmp13 and loss of Col2) (**Figure 1**). RNA-seq analysis of joints from 24 week-old mice indicate an upregulation of genes associated with an increased inflammatory response and those with an established link to OA pathogenesis. Given this result we hypothesized that *Ripk2*^{104Asp} mice would have an increased susceptibility to age-associated OA. We aged WT and *Ripk2*^{104Asp} mice to 2 years of age and examined the knee joints by histology. *Ripk2*^{104Asp} mice have severe OA as indicated by complete loss of articular cartilage, osteophyte formation, and synovitis (**Figure 2**) as compared to a mild OA phenotype (loss of proteoglycans) observed in WT mice. 36-week or 2 year old *Ripk2*^{104Asp} mice did not have elevated levels of serum cytokines, but 36-week old mice but had an increase in several cytokines in the synovial fluid (e.g., Eotaxin and Epo). This indicates that *Ripk2*^{104Asp} is acting locally to alter joint homeostasis. We next examined the functional consequence of accelerated age-associated OA in 36-week-old *Ripk2*^{104Asp} mice by quantifying changes in activity using open filed locomotor boxes. Compared to WT, 36-week-old *Ripk2*^{104Asp} mice had significantly reduced functional outcomes in every parameter measured, including a reduction in total distance traveled, increased rest time, and a decrease in vertical rises (**Figure 3**). Altogether these data indicate the *Ripk2*^{104Asp} allele alters the basal physiological state of the joint locally so that it expresses features normally associated with overt OA. Molecular changes are first detected in the knee joints of 24-week-old mice and a reduction in function is observed at 36 weeks of age.

DISCUSSION: Our studies demonstrate our innovative approach of combining genetic analysis of families with OA and functional analyses in the mouse to identify well-supported OA-associated alleles and pathways. 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 (changes in local gene, protein expression, and synovial fluid cytokines) portends overt OA and a reduction in multiple measures of activity. We are currently determining if *Ripk2*^{104Asp} alters the cellular composition of the joint (e.g., increased recruitment of immune cells) and if reduction of Ripk2 activity using novel inhibitors can reduce the severity of PTOA or age-associated OA. In sum, we have developed a novel genetic mouse model for the study of age-associated OA.

SIGNIFICANCE: 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.

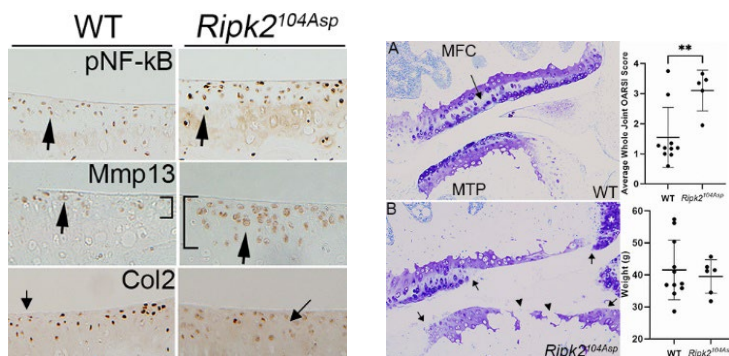


Figure 1. Uninjured *Ripk2*^{104Asp} knee joints express markers associated with OA at 24-weeks of age.

Figure 2. *Ripk2*^{104Asp} mice develop severe age-associated OA compared to WT. OARSI analysis of knee joints at 2 years of age.

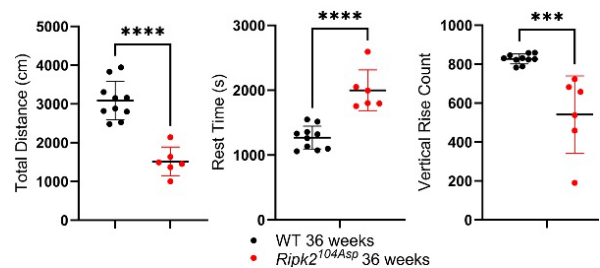


Figure 3. 36-week-old *Ripk2*^{104Asp} mice are less active than WT and have a decrease in vertical rises.