PIEZO1 variants that reduce open channel probability are associated with familial osteoarthritis

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INTRODUCTION: The synovial joint is a mechanosensitive organ that responds to various physical forces including compressive, shear, and hydrostatic stresses. These joint cells sense (mechanosensing) and respond (mechanotransduction) to daily physical forces to maintain homeostasis of the joint. Disruption of mechanosensing or mechanotransduction can lead to molecular and cellular changes in the joint that are associated with the development of osteoarthritis (OA). Many physiological and environmental risk factors are associated with increased risk of developing OA, including genetics, traumatic joint injury, aging, obesity, and altered biomechanics. Mechanosensing and mechanotransduction can be influenced by many of the same factors, which can be exacerbated by mechanical overloading, injury or unloading. Despite the importance of mechanobiology in joint homeostasis and disease, we do not fully understand how alterations of the proteins that sense and respond to physical forces contribute to the development of OA.

PIEZO1 is a major mechanosensitive cation channel in the joint directly regulated by mechanical stimulus. Genetic studies in mice have indicated that loss of PIEZO1 in cartilage is protective against injury induced OA. A recent *in vitro* study has demonstrated that activation of PIEZO1 with a chemical agonist promotes expression of pro-chondrogenic genes and increased deposition of sulfated glycosaminoglycans. These data suggest that PIEZO1 may have context dependent roles in maintaining homeostasis of the joint.

Despite the significant genetic contribution to OA, there is only one reported genetic association of *PIEZO1* variants with the OA phenotype. The genetic analysis of families with dominant forms of OA allows us to identify highly penetrant coding alleles that have determinate effect promoting OA, independent of prior biases on how protein activity may affect the OA phenotype or the tissues specific requirement of the mutant alleles. Non-null alleles further allow for functional studies to determine how the coding mutations affect protein activity and function in a physiologically relevant context. Here we report the identification of four families with age-associated OA with independent OA-susceptibility alleles in PIEZO1. We perform functional analyses *in vitro* and *in vivo* to determine how the mutations alter PIEZO1 channel activity and how this altered activity contributes to OA susceptibility. Our data indicate that reduced PIEZO1 channel activity increases susceptibility to age-associated OA and increased PIEZO1 channel activity may be protective in the absence of acute injury. We hypothesize that PIEZO1 may have context dependent or tissue specific roles in injury vs aging.

METHODS: We used the Utah Population Database to identify 151 independent families with dominant inheritance patterns of OA. Whole exome sequence analysis was performed on informative family members and we identified four independent families with *PIEZO1* mutations. To determine the functional impact of the *PIEZO1* mutations and the previously identified GWAS allele we performed single channel electrophysiological analysis, biophysical analysis of permeation and gating properties, chemical modulation by Yoda1, and gene expression analysis in primary human chondrocytes. Furthermore we generated a mouse harboring one of the familial mutations, *Piezo1*^{R1398W}. We analyzed the response to injury induced OA and aging in WT and *Piezo1*^{R1398W} mice.

RESULTS: Our electrophysiological and biophysical analyses demonstrated that the familial mutations are hypomorphic, reducing the open probability of the channel. In contrast, the GWAS allele is hypermorphic leading to an increased open probability (Figure 1). These data indicate that the GWAS allele, which has been suggested to be protective against joint replacement, and the familial mutations have opposite effects on PIEZO1 channel activity. Expression of *Piezo1*^{R1398W} in primary human chondrocytes protects against IL1B-induced OA associated gene expression. These results led us to the hypothesis that reduced PIEZO1 activity promotes age associated OA while potentially protecting against injury induced OA. To test this we examined the molecular phenotype in uninjured and injured WT and *Piezo1*^{R1398W} mice. Joints isolated from young uninjured *Piezo1*^{R1398W} mice have elevated RNA expression of IL1B and elevated IL1B in the synovial fluid compared with WT. In contrast to the uninjured mice, *Piezo1*^{1398W} mice have a reduced local and systemic inflammatory response to injury compared with WT)(Figure 2). PIEZO1 likely has tissue dependent roles as well. *Piezo1*^{1398W} paw synovial fibroblasts have an augmented response to IL1B treatment hypothesis, indicated by the increased expression of *Il6*, *Il1b* and *Ccl2*. Finally, aged *Piezo1*^{1398W} mice have reduced locomotor activity compared with WT, a phenotype that is sexually dimorphic as this reduction in activity was not observed in male *Piezo1*^{1398W}.

DISCUSSION: In summary we have identified four independent *PIEZO1* mutations that are associated with age dependent familial OA. Our electrophysiological and biophysical results indicated that the familial mutations reduce PIEZO1 channel activity, whereas the GWAS allele results in increased PIEZO1 channel activity. Analysis of a mouse harboring a human OA mutation, *Piezo1*^{R1398W}, supports the hypothesis that reduced PIEZO1 activity contributes to age associated OA and protects against injury induced OA.

SIGNIFICANCE/CLINICAL RELEVANCE: Our data provides support that PIEZO1 has a context and possibly cellular/tissue dependent roles in maintaining joint homeostasis. Modulation of PIEZO1 activity during aging vs injury may be a new avenue for OA therapeutics.

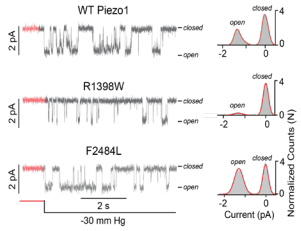

Figure 1. Single-channel currents of WT and OA-associated PIEZO1 variants. Single-channel current recordings acquired at -60mV, before (red) and after -30 mmHg (grey) pressure pulse. Respective all-point current-amplitude histograms showing the closed (0 pA) and open (-1.5 pA) state of the channel.

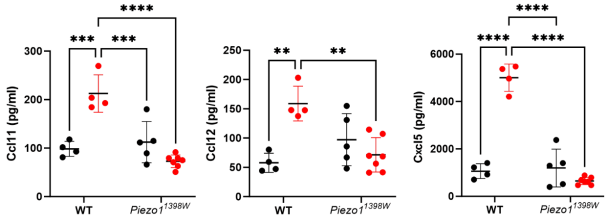

Figure 2. The *Piezo1*^{1398W} allele alters the immune response to ACL rupture. Quantification of serum cytokines in uninjured and ACL ruptured (5 weeks post injury) WT and *Piezo1*^{1398W} mice. WT mice upregulate the systemic expression of Ccl11, Ccl12, and Cxcl5 in response to knee injury, but this response is absent in mice harboring the *Piezo1*^{1398W} allele. Black dots indicate uninjured mice and red dots indicate ACL ruptured mice. Significance determined by two-way ANOVA.