Functional Analysis of OA-Associated *PIEZO1* Human Variants on OA Susceptibility

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Disclosures: None

INTRODUCTION: Osteoarthritis (OA) is a debilitating disease affecting millions worldwide. Remodeling and degeneration of the synovial joint provokes increasingly painful symptoms and decreased function in those it affects. Despite its prevalence, there are currently no effective disease-modifying therapies. The main obstacle in developing such therapies is a poor understanding of the mechanisms driving the development of OA. Our goal is to discover genes and molecular pathways in humans that are vulnerability points in the development of OA and generate mouse models with human disease alleles. PIEZO1 is a mechanosensitive Ca^{2+} ion channel in synovial joints. In vitro, PIEZO1 is activated by hyperphysiological forces, such as those sustained during an injury. Activation of the channel leads to Ca^{2+} influx, changes in gene expression, and increased cell death. Recent data has indicated contradictory roles for *PIEZO1* in OA susceptibility. Using the Utah Population Database (UPDB), we have identified four independent families with dominant *PIEZO1* mutations. By studying these non-null human disease alleles *in vitro* and in mouse models, we aim to understand the mechanisms and role of *PIEZO1* in OA. Doing so will resolve the role of *PIEZO1* in OA susceptibility *in vivo*, aid in the effort to discover markers for OA susceptibility, elucidate molecular pathways involved in OA development, and aid in the formulation of novel OA disease-modifying therapies.

METHODS: We take an approach used infrequently in the OA field. We study many unrelated families with clear-cut inherited forms of OA to identify susceptibility alleles that have strong determinate effects. To discover pathways that when modified lead to strong and unambiguous susceptibility to OA, we analyzed the exomes of >170 unrelated families from the UPDB that had a significant enrichment of dominantly inherited OA. We then use electrophysiology to determine the effect of the OA-associated variants on *PIEZO1* channel activity. We then use CRISPR/Cas9 technology to generate an animal that contains the human OA-associated *PIEZO1* disease allele (changing a single, highly conserved amino acid). Polymerase chain reaction (PCR) and restriction enzyme digest confirm successful human disease variant genotype in targeted animals. To study the effect of *PIEZO1* deletion in cartilage after anterior cruciate rupture (ACLR), we used *Aggrecan*-Cre^{ERT2} to delete *PIEZO1*. Gene expression is analyzed via qPCR analysis of RNA collected from whole mouse knee joints.

RESULTS: Analysis of families from the UPDB yielded four independent families with unique OA-associated *PIEZO1* variant alleles. All variants are in highly conserved amino acids. We next tested how these variants affect channel activity. Electrophysiological studies showed that all four OA-associated *PIEZO1* alleles decreased the open channel probability indicating that these alleles are hypomorphic, thereby reducing PIEZO1 channel activity. Mice with *Piezo1* deleted from articular chondrocytes show no difference after ACLR compared to WT controls. We next examined mice containing one human OA-associated *PIEZO1* allele. Uninjured mice carrying the OA-associated *PIEZO1* allele showed significantly increased levels of *Il1β* compared to WT. These data indicate that mice carrying a hypomorphic allele of *PIEZO1* have altered gene expression that may make them more susceptible to injury induced or age-associated OA.

DISCUSSION: Our results provide novel insight into the role *PIEZO1* plays in the development of OA *in vivo*. Published *in vitro* studies show increased *PIEZO1* calcium channel conductance in chondrocytes leads to weakened cytoskeletal support and increased susceptibility to cellular deformation in response to mechanical loading, which elevates the open probability of the *PIEZO1* calcium channel in a feed-forward mechanism. Thus, increased *PIEZO1* activity seems to accelerate OA-related disease processes *in vitro.* However, selectively deleting *PIEZO in vivo* in mouse chondrocytes (our work and published data) yielded no statistical difference in OA severity, suggesting that, surprisingly, chondrocyte-expressed *PIEZO1* (and *PIEZO2)* may have little effect on OA development *in vivo*. Our OA-associated *PIEZO1* variants are associated with OA development in the absence of traumatic injury. This is contrary to what *in vitro* (and *in vivo* chemical inhibition) data suggests. Our electrophysiological data indicate that the OA-associated *PIEZO1* alleles have decreased channel activity. Preliminary data indicate that mice expressing one variant *PIEZO1* allele showed significantly increased levels of the inflammatory marker *Il1β* when compared to WT. Taken together, our human genetics and mouse data suggest that *reduced PIEZO1* activity increases susceptibility to OA *in vivo*. Our future work will include performing ACLR on *PIEZO1* variant-expressing mice and assessing the molecular, cellular, and structural response to injury. Furthermore, we are examining the contribution of the OA-associated *PIEZO1* allele to age-associated OA in the mouse.

SIGNIFICANCE: There is value in identifying pathways that confer susceptibility to familial OA. It is essential for 1) understating the mechanisms of disease, 2) the development of therapeutics that could modify the course of OA, 3) development of biomarkers for early detection of OA, 4) prediction of additional genes that likely contribute to susceptibility, and 5) it informs us about common forms of disease. Our research is in contrast with the currently accepted dogma of *PIEZO1* contribution to OA development. Therefore, further characterization of *PIEZO1* variants and mice with non-null human OA alleles will be useful both for understanding the mechanism of disease and therapeutic development.

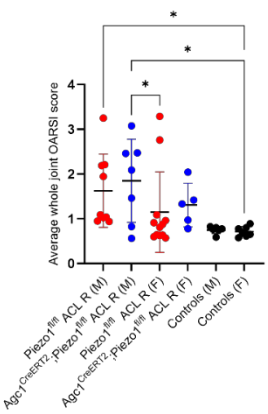

Figure 1. Cartilage specific deletion of *Piezo1* in adult male or female mice (using Agc1*CreERT2*) does not change the response to ACL rupture. Tamoxifen was injected at 14 weeks of age and ACL rupture was performed on 16-week-old mice. OARSI scoring was performed 3 weeks post-ACL rupture.

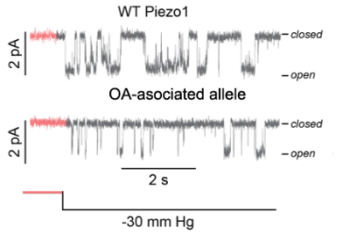

Figure 2. Single channel analysis of one OA-associated *PIEZO1* allele indicates that the mutation reduces the open probability of the channel.

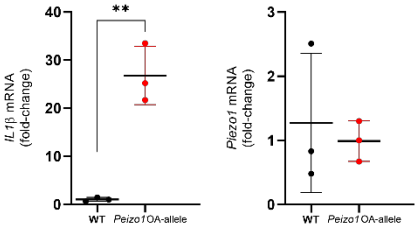

Figure 3. *Il1β* mRNA is upregulated in the knee joints of uninjured young mice (12-14 weeks old) carrying the human OA-associated allele compared to WT controls. *Piezo1* mRNA expression is not altered in the mice carrying the OA-associated allele.