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

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Background

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 Ca2+ 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 Ca2+ 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.

Proposed role of PIEZO1 in cartilage

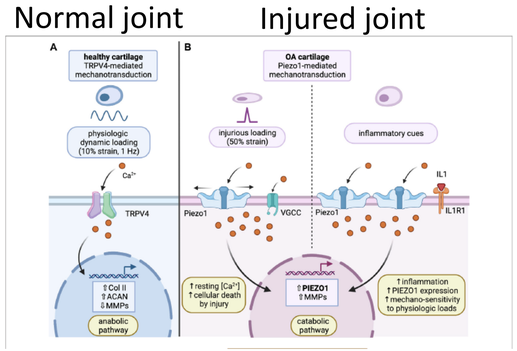


Figure from Gao et al, 2022

PIEZO1 is thought to have a role primarily in the response to traumatic injury where it regulates inflammatory signaling to promote degradation of cartilage.

Identification of families with PIEZO1 mutations

Table 1. PIEZO1 Variants Identified in Independent Osteoarthritis Families and through GWAS

OA Phenotype (Family)	Human Variants (Mouse amino acid)	Minor Allele Frequency	Functional Effect
Finger Interphalangeal Joint OA (FIJ876)	p.R531C (p.R537)	0.0005	Loss-of-function
Erosive Hand OA (ERO13)	p.K2502R (p.K2528)	0.006	Loss-of-function
Erosive Hand OA (ERO15)	p.P2510L (p.P2536)	0.006	Loss-of-function
Finger Interphalangeal Joint OA (FIJ126)	p.R1404W (p.R1398)	0.0007	Loss-of-function
Reduced Hip OA Progression (GWAS)	p.F2458L (p.F2484)	0.001	Gain-of-function

Table 1. We identified 4 *PIEZO1* mutations that segregate with familial forms of OA. A rare coding variant was identified in a GWAS that was associated with individuals who have OA, but do not progress to need a joint replacement (Henkel, et al, 2023).

Functional effect was determined by electrophysiological studies in **Figure 2**.

Location of PIEZO1 mutations

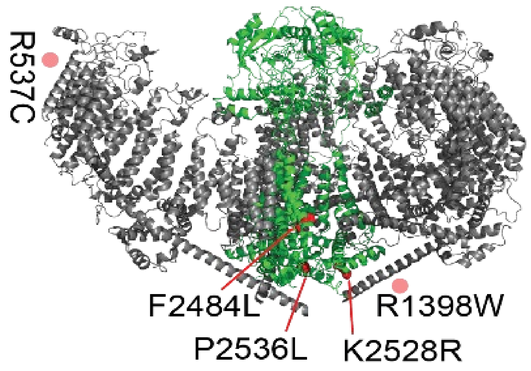
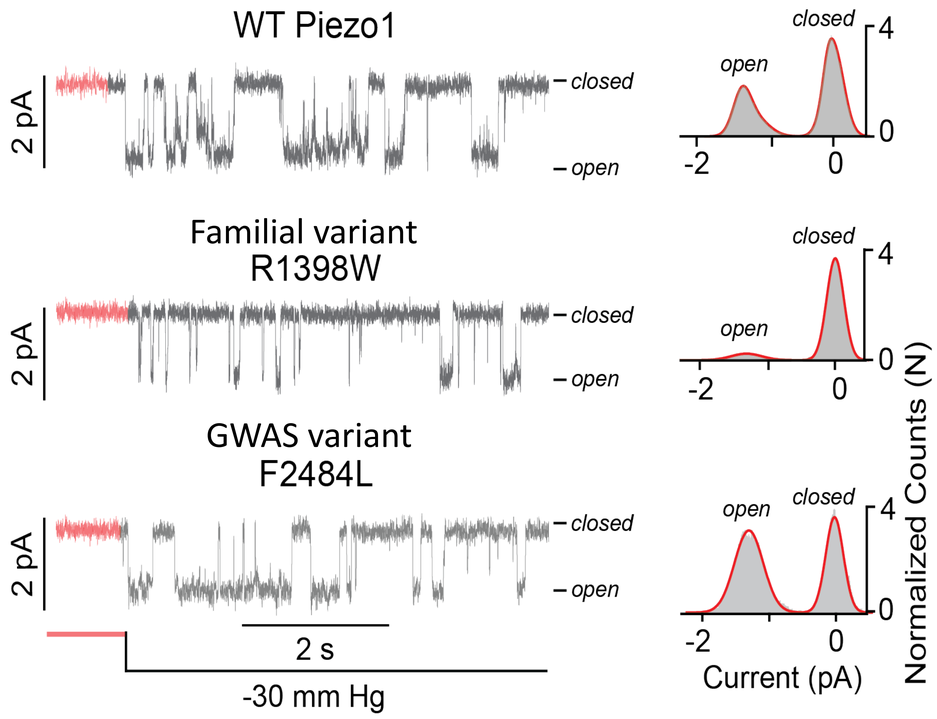


Figure 1. Cryo-EM structure of Piezo1. Piezo1 blades (grey) from residues 577 to 2123. Pore domain (green) spans from 2124 to 2547. Human OA-variants are shown in red. Amino acids 1-576 are not structurally resolved. R537C is predicted to be in TM 9 and 10.

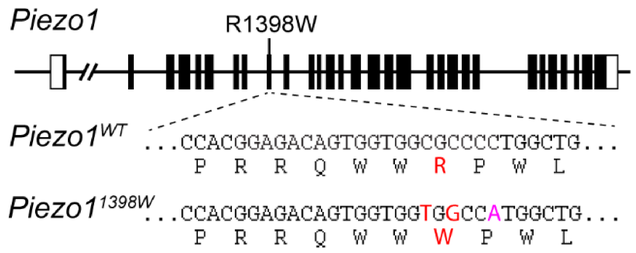
The familial PIEZO1 alleles are hypomorphic while the GWAS allele is hypermorphic

Figure 2. Single-channel currents of WT, familial, and GWAS 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.



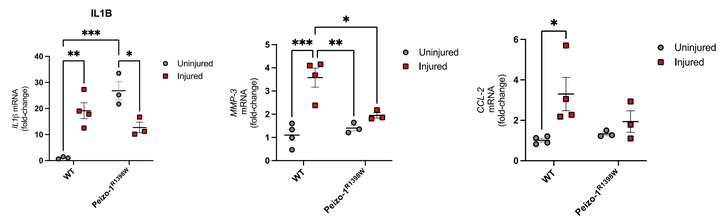
Generation of a mouse containing a human OA-associated PIEZO1 R1398W allele

Figure 3. We used CRISPR/Cas9 to generate a mouse that harbors a human OA-associated *PIEZO1*^{R1398W} allele. Restriction enzyme digest is performed on the PCR product. All animals express PIEZO1, but only animals successfully expressing the PIEZO1 disease allele will have successful digest.



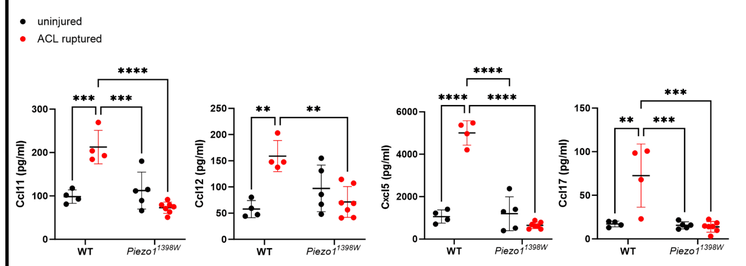
Gene expression in uninjured and injured PIEZO1^{R1398W} knee joints

Figure 4. We ruptured the ACL of both WT and *Piezo1*^{1398W} mice. Whole joints were collected 5 days post-injury and qPCR analysis was performed.



Systemic response to injury in WT and PIEZO1^{R1398W} mice

We collected serum from both WT and *Piezo1*^{1398W} mice 5 weeks following ACL rupture. Subsequently, we analyzed this serum for changes in the level of 44 different cytokines.



Conclusions

- Published *in vitro* data shows increased Ca^{2+} conductance in chondrocytes leads to weakened cytoskeleton, which leads to further injury susceptibility and open channel probability.
- Familial OA-associated *PIEZO1* alleles show decreased open channel probability, while the GWAS allele appears to increase the open probability.
- Preliminary data show that uninjured *Piezo1*^{R1398W} mice express high levels of inflammatory cytokines, which is reduced following injury.
- The systemic response to injury is reduced in *Piezo1*^{R1398W} mice.
- Taken together, we hypothesize that the hypomorphic *Piezo1* alleles are acutely protective after injury but confers long-term susceptibility to development of OA.
- Baseline Piezo1 activity may contribute to long-term maintenance of articular cartilage.

Acknowledgements

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