PIEZO1 Variation in Myofibroblasts Obtained at Open Heart Surgery

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Introduction

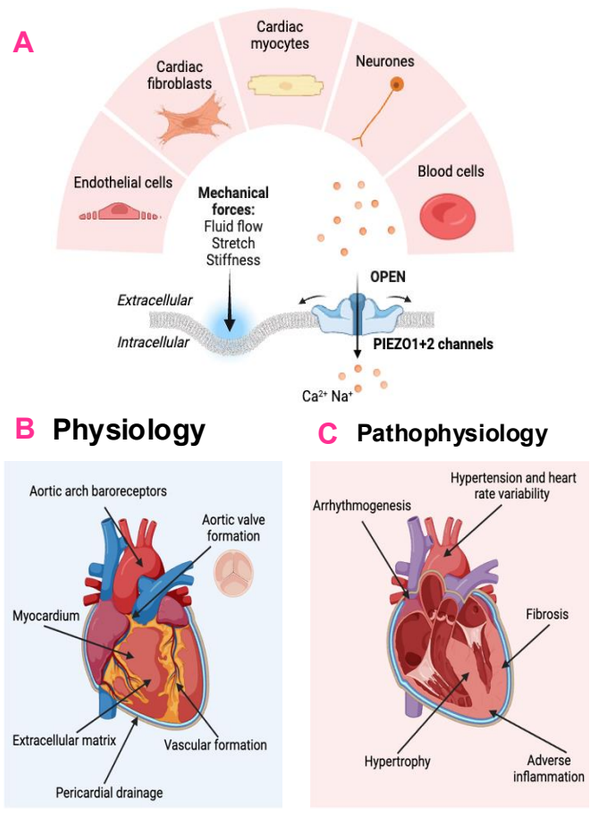


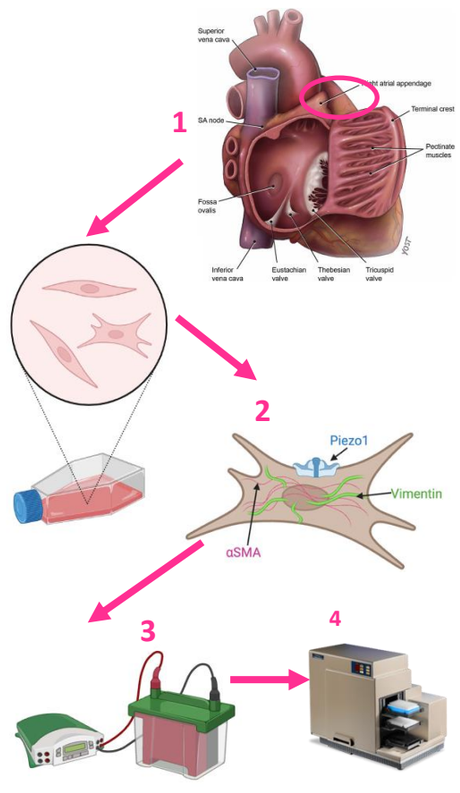
Fig. 1 Project background
PIEZOs are present throughout the CV system. They have distinct roles in physiology and disease.

The mechanosensitive, Ca^{2+}-permeable ion channel PIEZO1 is present throughout the cardiovascular system and has distinct roles in physiology and disease. PIEZO1 expression in cardiac physiology is relatively low but upregulated in disease when the structure and mechanical properties of the heart often change. Experimentally-induced aortic constriction in mice results in a 6-fold increase in PIEZO1 mRNA expression (Yu et al, 2022).

PIEZO has distinct roles in cardiac fibroblasts (CF), regulating differentiation, fibrotic remodelling and inflammatory signalling (Blythe *et al*. 2019, McGrane *et al*. 2025). Overexpression of PIEZO1 is associated with increased atrial stiffness (Jiang *et al*. 2021; McGrane *et al*. 2025; Jakob *et al.* 2021).

Methods

1. CF were isolated from right atrial appendage (RAA) biopsies from patients undergoing surgery for aortic stenosis (AS), coronary artery disease (CAD), or both (comorbid). Ethics IRAS Project ID: 200339.
2. Cell identity was determined using immunohistochemistry.
3. Western blotting was conducted to quantify protein abundance.
4. PIEZO1 channel function was measured following Yoda2 application (PIEZO1 agonist) in isolated CF loaded with Ca^{2+} indicator dye, Fura2, using Flexstation III device. Dose response analysis was conducted for agonist sensitivity analysis (EC_{50}).



Statistical analysis was performed using Student's *t*-test to compare two groups, and two-way ANOVA with post-hoc Tukey analysis for multiple comparison.

Aim: Characterize PIEZO1 expression and function in isolated CF and RAA tissue from patients undergoing cardiac surgery, with or without associated T2D.

Hypothesis: Aortic or coronary artery restriction causes PIEZO1 upregulation in human cardiac fibroblasts that is adverse for the human heart, and which might be targeted for therapeutic benefit

Results

Cellular area is similar between AS- and CAD-isolated CF at early passage

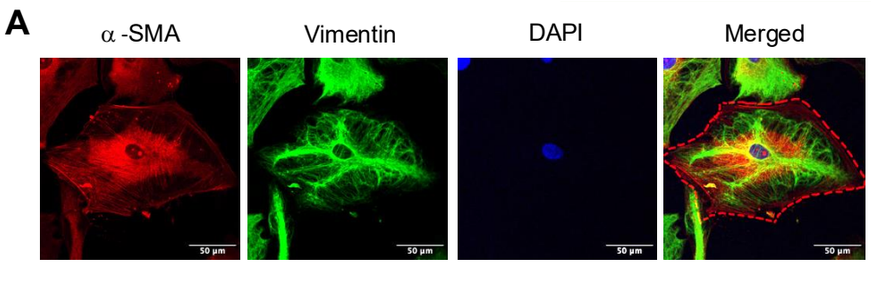


Fig. 2 Assessment of mean cell area of AS and CAD at Passage (P)2.
A Example of staining used for determining cell area. Staining for MyoCF (α–SMA (alpha smooth muscle actin), red), CF (vimentin, green), nuclei (DAPI, blue) and merged image. Cells exhibit a myofibroblast phenotype. Scale bar 50 µm.
B Quantification of cell area in the AS and CAD cohorts using images of the type shown in A. N=3 per group. Values are mean ± S.D. There is no difference in cell area between the cohorts.

PIEZO1 channels are expressed in patient-derived CF

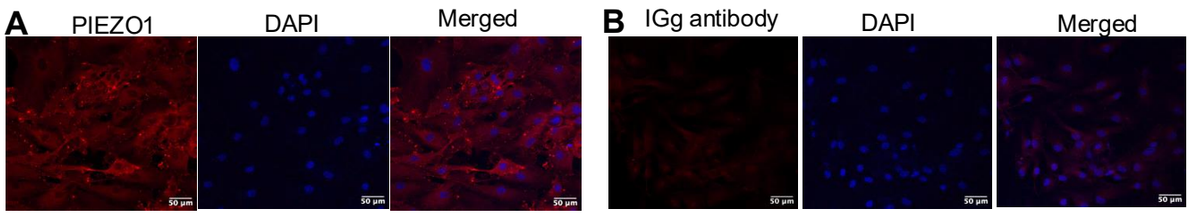


Fig. 3 Immunostaining analysis of PIEZO1 on ibidi slide preparations in patient-derived cardiac fibroblasts (N=1).
A PIEZO1 is expressed in patient-derived cardiac fibroblasts by immunostaining analysis. PIEZO1 (red), nuclei (DAPI, blue) and merged image. Scale bar 50 µ m.
B Negative staining with IGg non-specific antibody (N=1). Scale bar 50 µm.

No changes in PIEZO1 protein abundance or glycosylation

Antibody validation

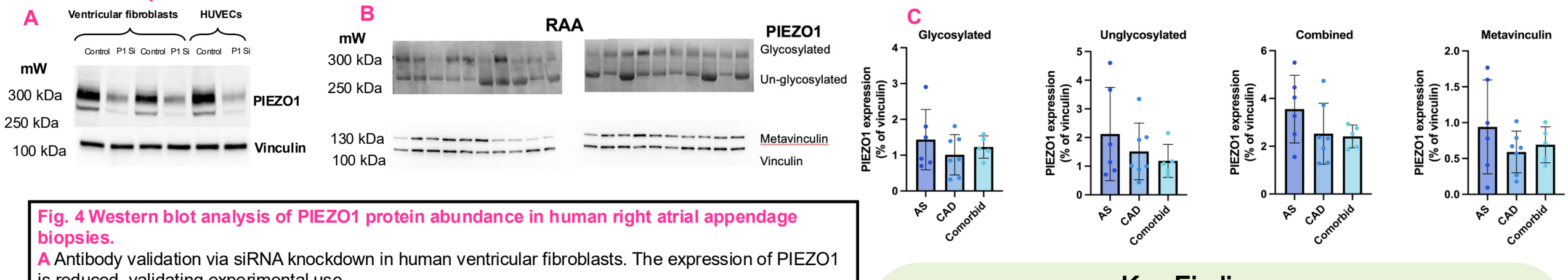


Fig. 4 Western blot analysis of PIEZO1 protein abundance in human right atrial appendage biopsies.
A Antibody validation via siRNA knockdown in human ventricular fibroblasts. The expression of PIEZO1 is reduced, validating experimental use
B Western Blot analysis of PIEZO1 in human right atrial appendage biopsy lysates. The glycosylated and unglycosylated forms are present. The housekeeper vinculin was used to normalise PIEZO1 expression. The mechanosensitive protein, metavinculin, was also assessed.
C Quantification of band intensity and stratification based on patient cohort.
AS = aortic stenosis, CAD = coronary artery disease, comorbid = combines AS and CAD.

Key Findings

- Isolated cells from right atrium exhibit a homogeneous myoCF phenotype, in accordance with literature (Mughal *et al*. 2009).
- PIEZO1 channels are expressed in patient-derived myoCF
- The sensitivity of PIEZO1 channels to chemical activation is lower in comorbid cardiovascular disease but this is not due to reduction in protein abundance

Conclusion

Despite animal studies suggesting adverse PIEZO1 upregulation in heart failure models, in human comorbid pre-stage heart failure phenotype, a combination of low PIEZO1 expression and low sensitivity may reduce the ability of myocardial cells to sense their mechanical environment, weakening structures and accelerating heart failure progression.

Reduced PIEZO1 sensitivity in comorbid cardiovascular disease

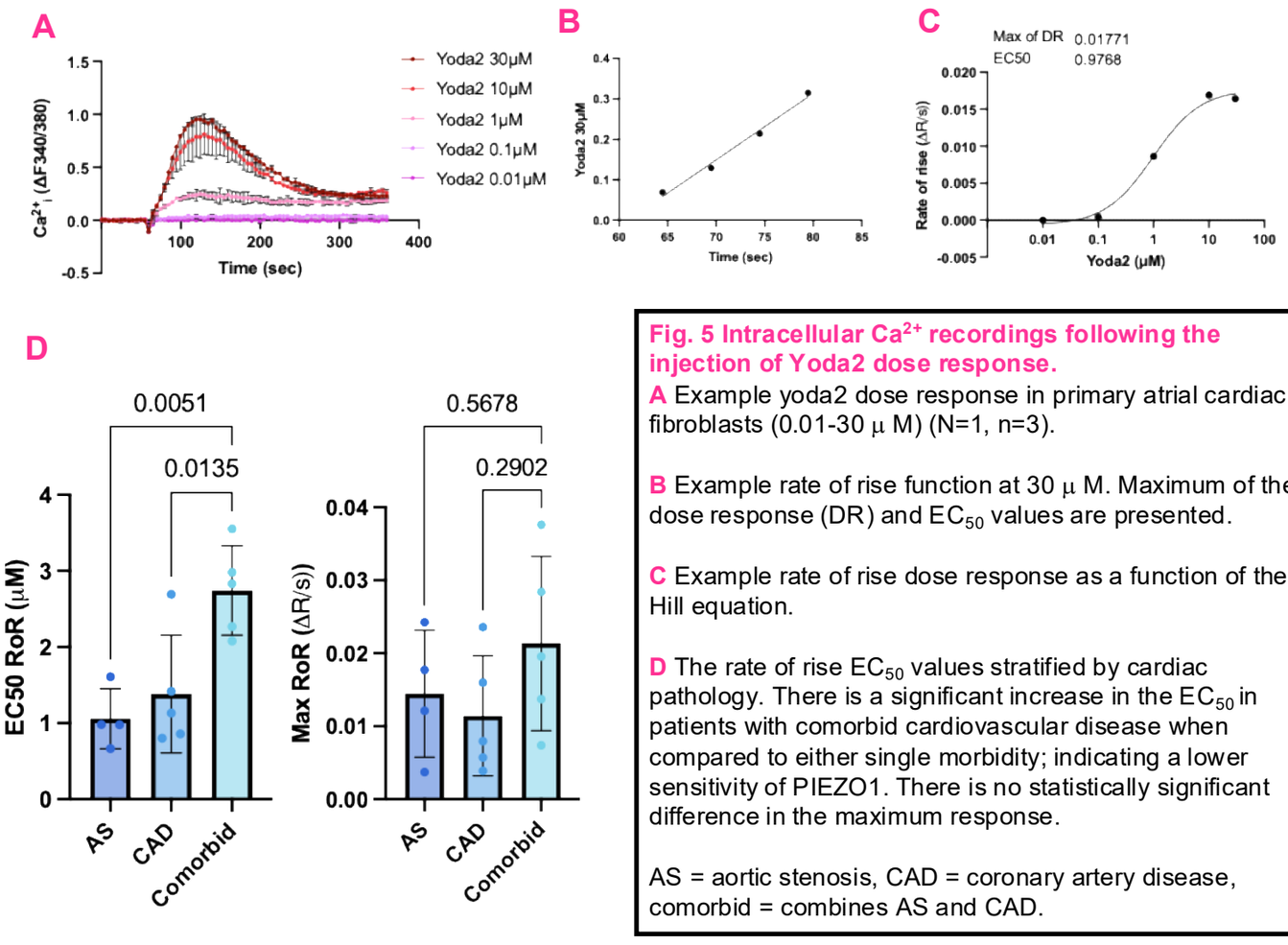


Fig. 5 Intracellular Ca^{2+} recordings following the injection of Yoda2 dose response.
A Example yoda2 dose response in primary atrial cardiac fibroblasts (0.01-30 µ M) (N=1, n=3).

B Example rate of rise function at 30 µ M. Maximum of the dose response (DR) and EC_{50} values are presented.

C Example rate of rise dose response as a function of the Hill equation.

D The rate of rise EC_{50} values stratified by cardiac pathology. There is a significant increase in the EC_{50} in patients with comorbid cardiovascular disease when compared to either single morbidity; indicating a lower sensitivity of PIEZO1. There is no statistically significant difference in the maximum response.

AS = aortic stenosis, CAD = coronary artery disease, comorbid = combines AS and CAD.

Original hypothesis

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