

Biomechanical Characterization of β -MHC R403Q Mutation in Atrial and Ventricular Tissues from Minipig HCM Model

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1. Background

R403Q cardiac tissue, premier HCM Model

The beta-myosin heavy chain (β -MHC) mutation, Arg403Gln (R403Q) in cardiac tissue has served as a premier model for studying human Hypertrophic Cardiomyopathy (HCM), a genetic disorder characterized by thickening of the ventricular walls (1).

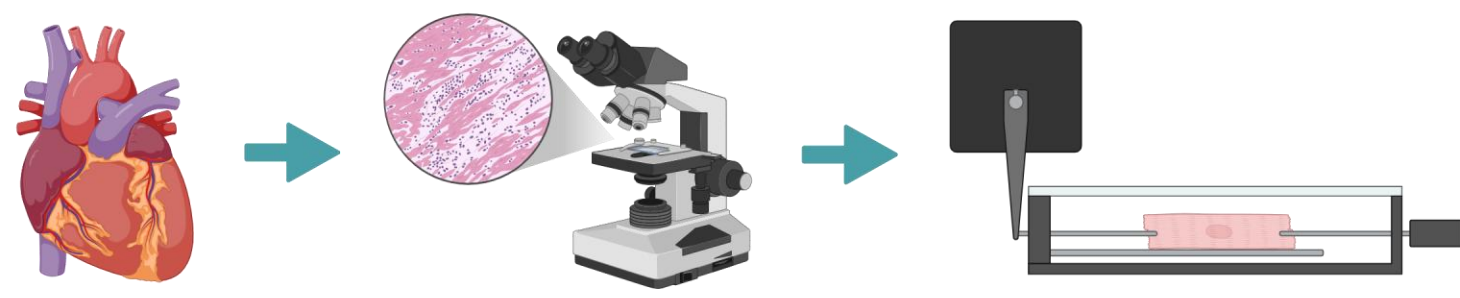
Transgenic pig models with more phenotypic similarity to the human HCM mutation, were used, focusing on the R403Q mutation in the ventricle (2); however, as β -MHC expression in the atria (predominantly α -MHC) increases in HCM, expression of the R403Q mutation in the atria also increases. This coincides with the diastolic dysfunction leading to atrial enlargement and the interaction is not well understood.

In this study, we aimed to characterize the biomechanical role of the R403Q mutation in the atria and explore functional differences of the mutation between the two regions of the heart.

2. Methods

Skinned Fibers – WT Porcine vs R403Q Minipig

- Left Ventricle (LV)
- Left Atria (LA)



The fibers were mounted at resting length (SL 2.0 μ m), and assays were performed in 1% DMSO on Aurora Scientific force transducer rigs.

Multi-Assay Characterization

- Force-pCa
- ATP consumption
- Passive Stretch
- Force-Velocity
- Force-Power

Force-pCa: ratios; 8, 6.5, 6.0, 5.9, 5.8, 5.6, 5.2, 4.5.
Passive stretch: fibers stretched 5% every 30(s), obtaining forces to obtain forces at each stretch. Final stretch confirmed at 2.6 SL.

ATP consumption: observed by running fibers through 0uM and 80uM ATP pCa 6.6 solutions.

Force-velocity: performed by quickly contracting the fibers at increasing intervals (ms); 48, 60, 75, 86, 100, 120, 150, 200, 300, 600, 1200, 2400.

Force-power: collected in tandem with the force-velocity assay.

Statistical significance was set at $P \leq 0.05$ (*denotes significance) using one-way ANOVA, two-way ANOVA, and unpaired t-tests with data reported at mean \pm SEM.

3. Results

β -MYHC expression increases in the left atrial tissue compared to wildtype

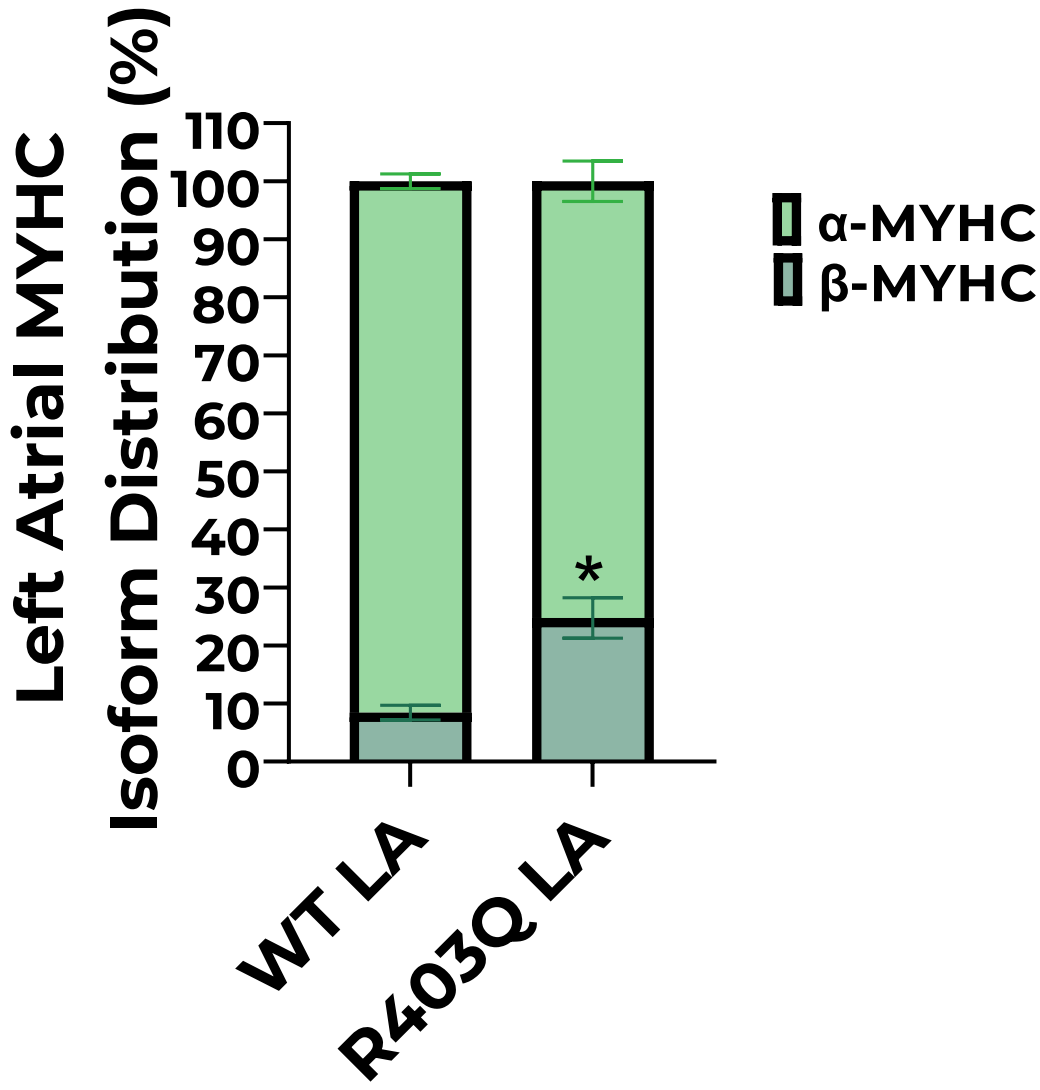


Figure 1: LA β -myosin heavy chain distribution in WT vs R403Q tissue.

β -myosin expression increases in R403Q LA which could impact phenotypic expression of the mutation.

3. Results (Continued)

R403Q fibers are less robust with a loss in visible sarcomeres

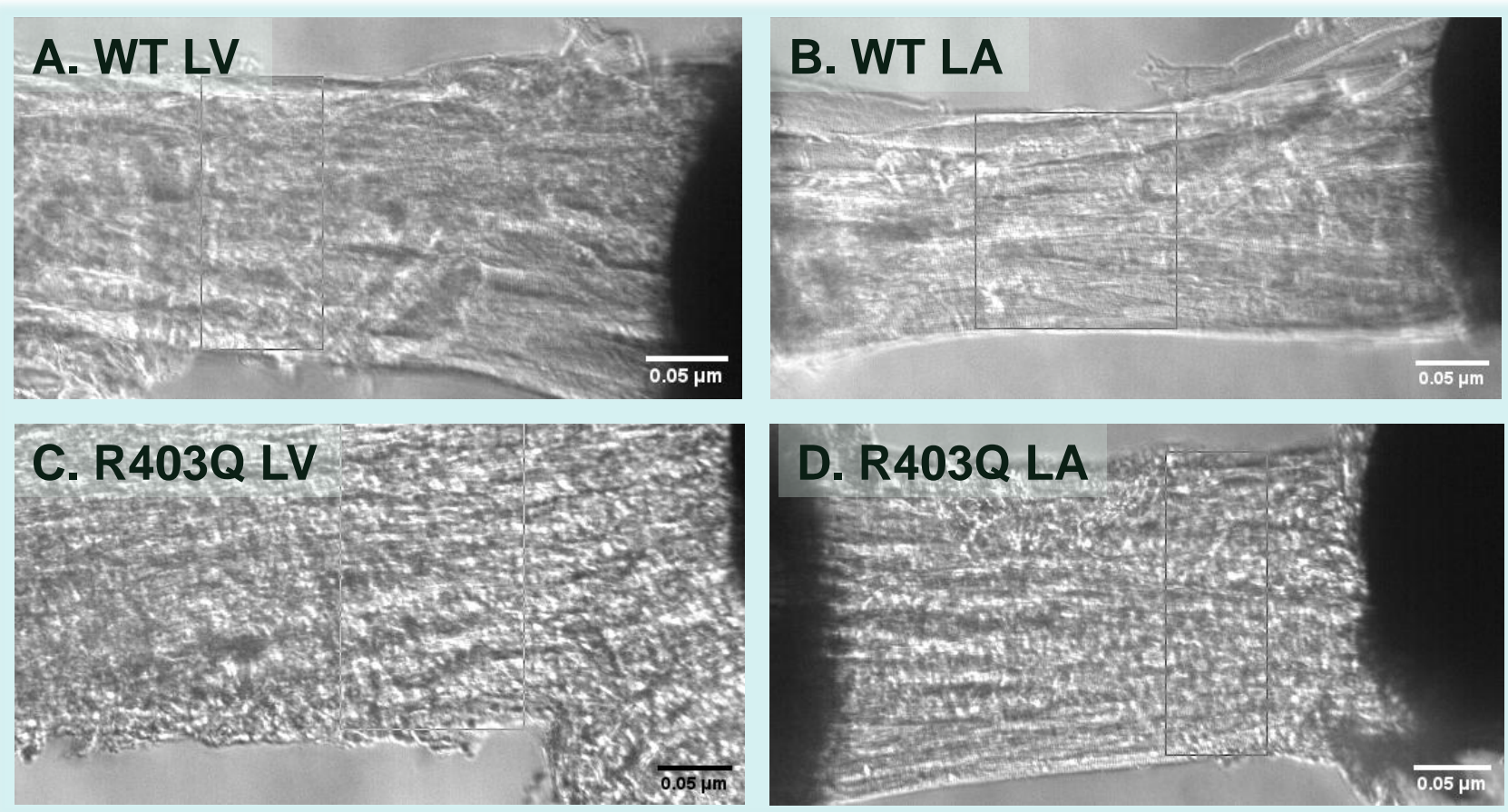


Figure 2: Images taken at 20x on Olympus TH4-100 A. Wildtype LV. B. Wildtype LA. C. R403Q LV. D. R403Q LA.

R403Q tissues have lower max forces and higher sensitivity towards calcium

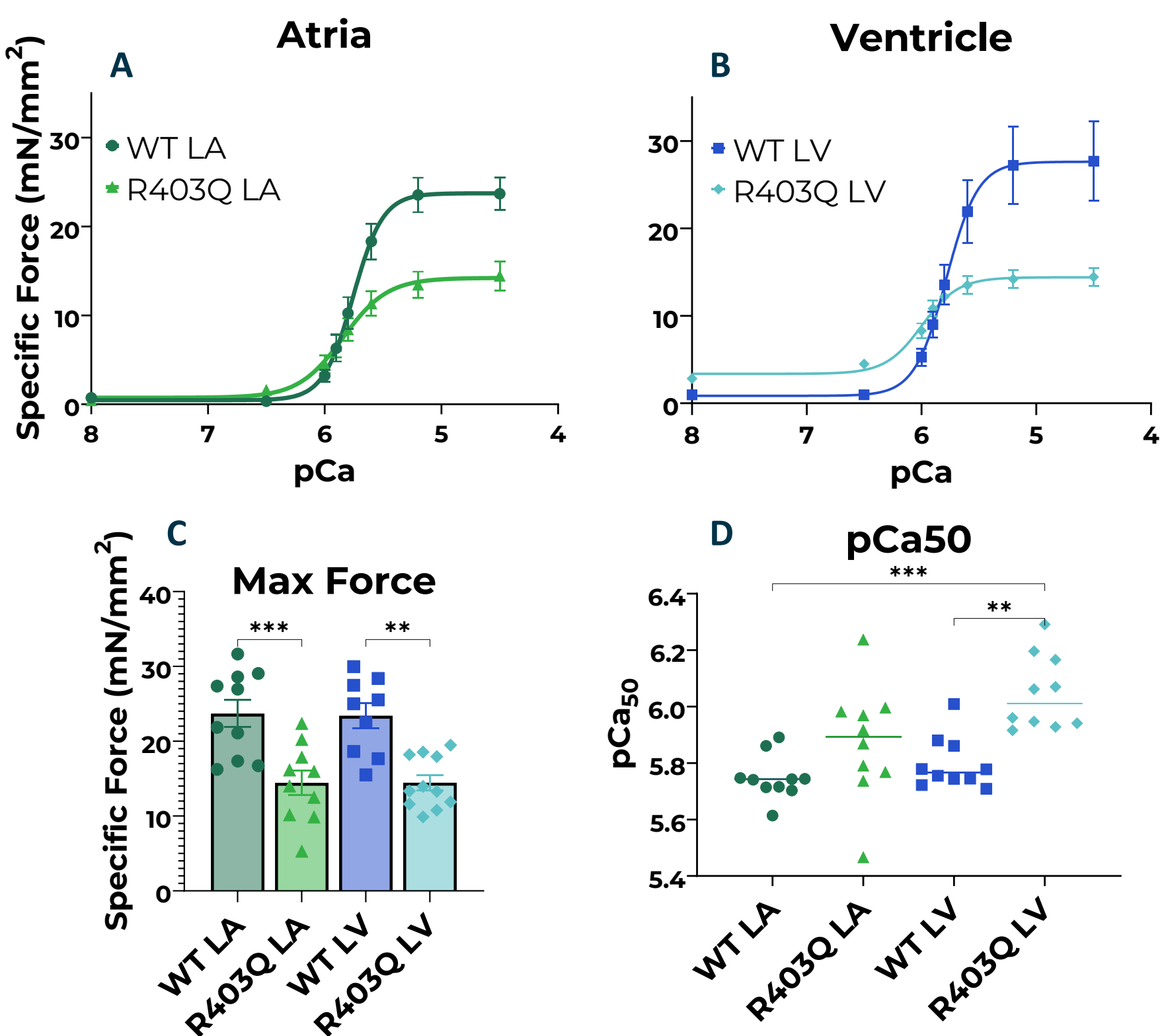


Figure 3: A. Specific force-pCa WT vs R403Q LA. B. Specific force force-pCa WT vs R403Q LV. C. Specific Max Force for each tissue type. D. pCa_{50} for each tissue type

Compared to WT, both R403Q tissues show lower max forces* (3). R403Q LV showed higher calcium sensitivity* while R403Q LA trended towards higher calcium.

Tissue Source	Specific Max Force (mN/mm ²)	pCa_{50}
WT LA	23.7 \pm 1.8	5.75 \pm 0.02
WT LV	23.4 \pm 1.7	5.80 \pm 0.03
R403Q LA	14.4 \pm 1.6*	5.87 \pm 0.06
R403Q LV	14.5 \pm 1.0*	6.05 \pm 0.04*

R403Q LV increases passive stiffness in fibers, while R403Q LA exhibited an inverse effect

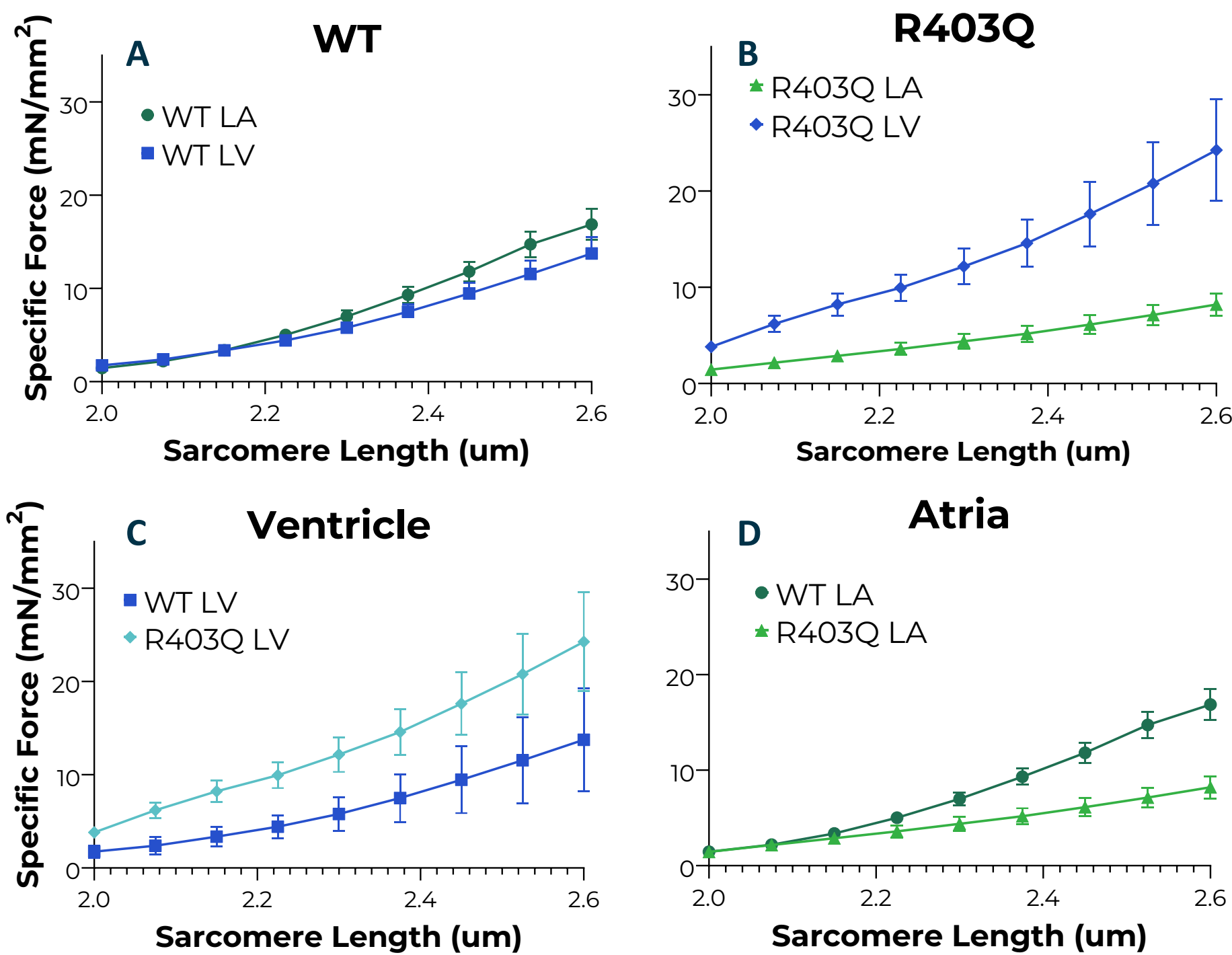


Figure 4: Passive force of A. WT LA vs LV. B. R403Q LA vs LV. C. WT LV vs R403Q LV D. WT LA vs R403Q LA.

Passive forces were higher in R403Q LV tissues at SL 2.0-2.3*. While R403Q LA had less force and SL 2.4-2.6* compared to WT. This reduced force correlates with the increased atrial volume in R403Q hearts.

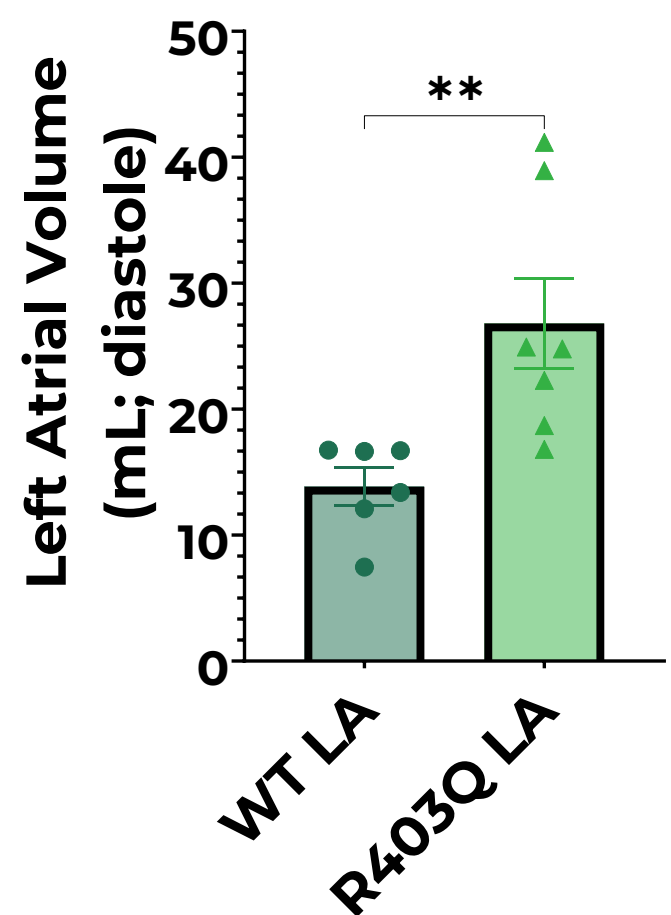


Figure 5: LA diastolic volume WT vs R403Q tissue

3. Results (Continued)

R403Q LA consumes ATP at a faster rate compared to WT LA

There was no difference between WT LA and LV basal ATP consumption (4). R403Q LA was faster (9.36 \pm 1.94uM/sec)* compared to WT LA (2.46 \pm 0.57uM/sec).

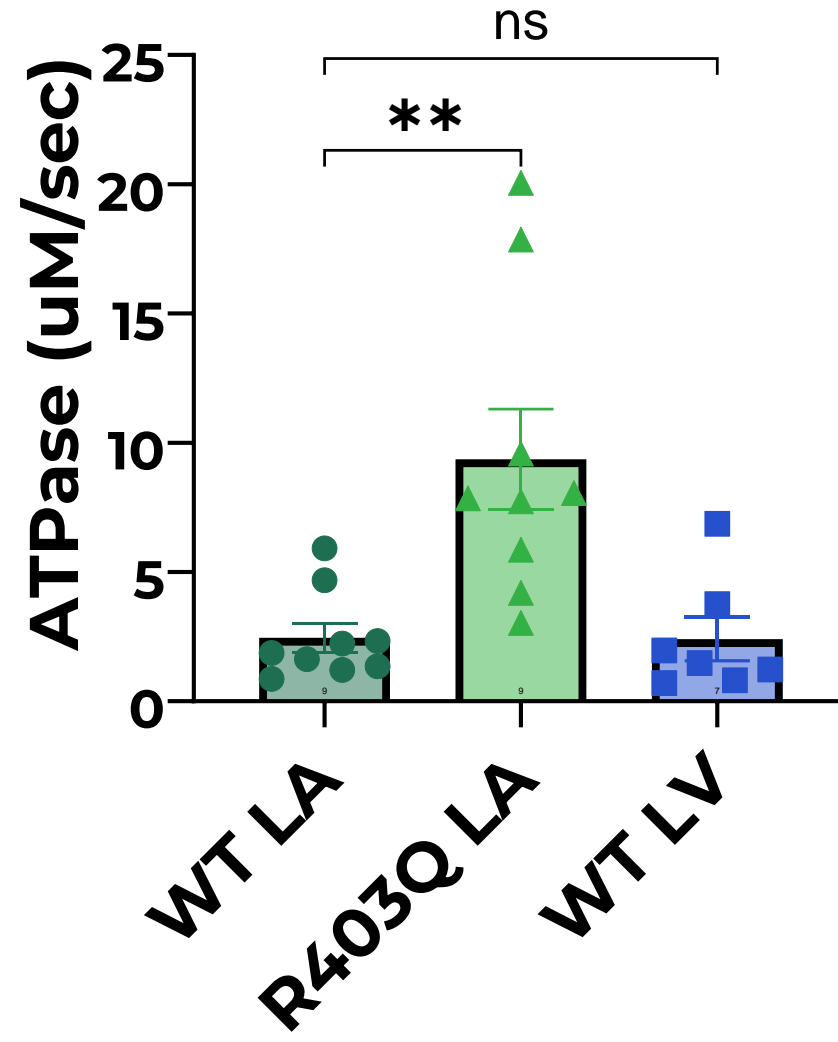


Figure 6: Fiber ATP consumption rates for WT LA, R403Q LA, and WT LV. Run at pCa 6.6. ATP consumption of R403Q LV was not captured in this window.

Significant chamber difference; R403Q LV could not produce similar forces to WT at the same velocity

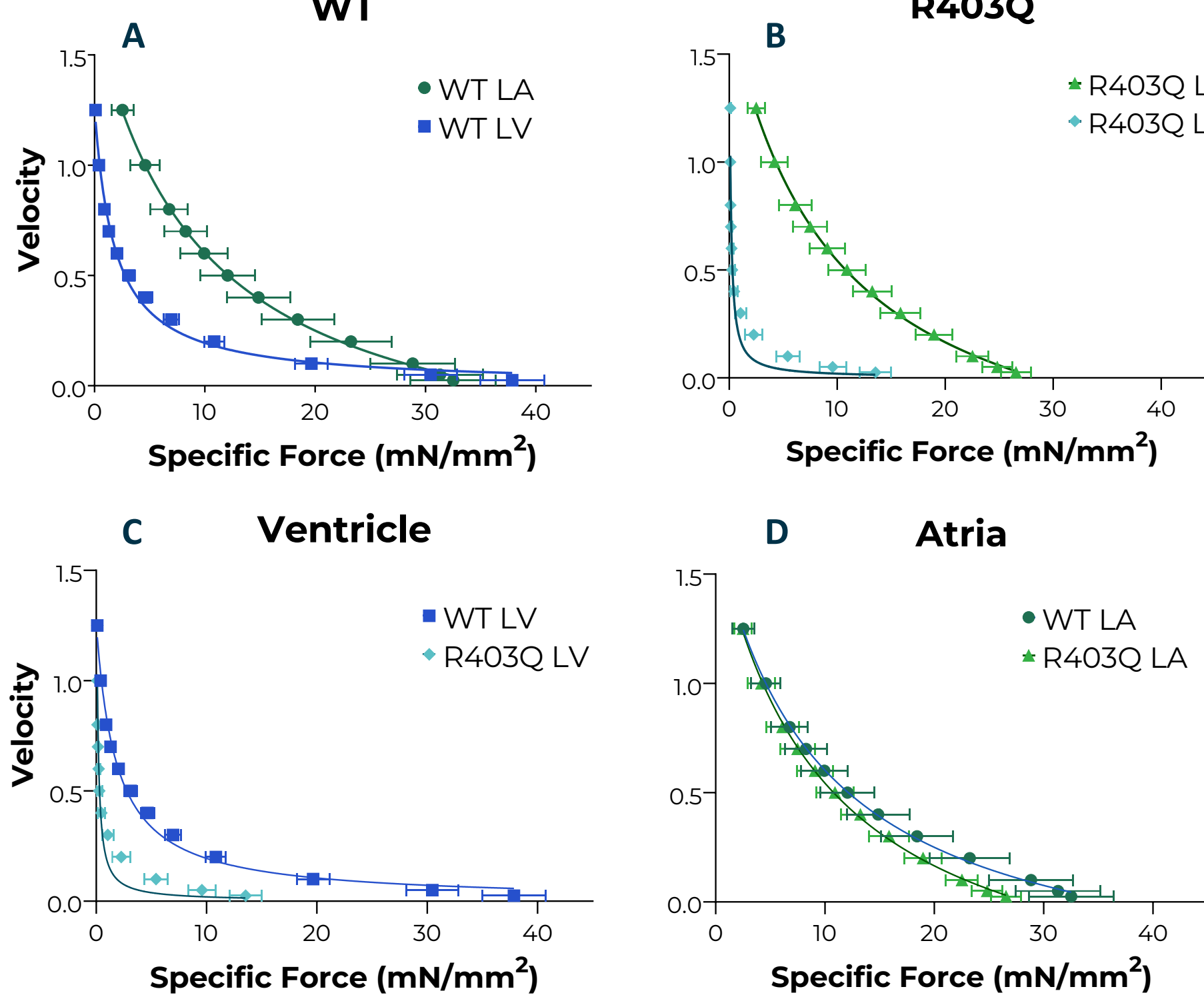
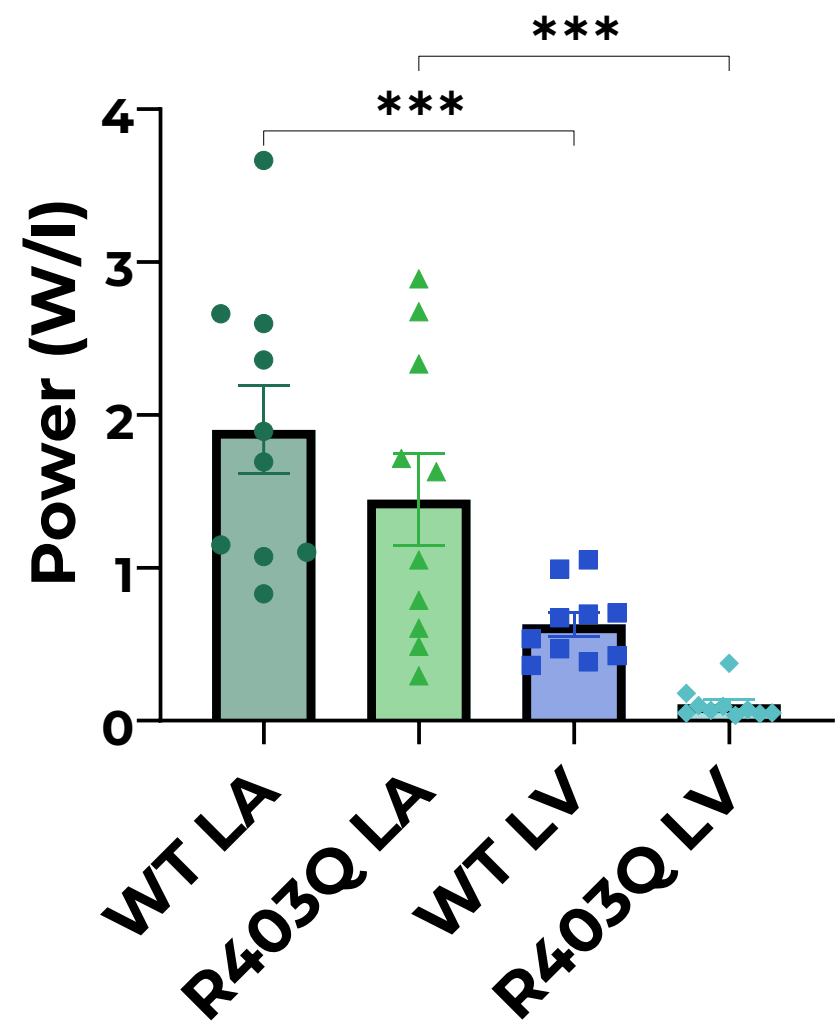


Figure 7: Force-Velocity of A. WT LA vs LV. B. R403Q LA vs LV. C. WT LV vs R403Q LV D. WT LA vs R403Q LA.

LA tissues had higher velocity compared to LV. Using 0.05 ML/s velocity for comparison, R403Q LV (9.58 \pm 1.2mN/mm²)* exhibited lower forces vs WT (30.5 \pm 2.4mN/mm²). In LA, there was no difference.

Power production is significantly different between chambers



There was a significant decrease in power output from both LV tissues. R403Q LV (0.11 \pm 0.03)* had lower power compared to WT (0.63 \pm 0.08). R403Q LA trended lower compared to WT LA.

Figure 8: Peak-Power of WT LA, R403Q LA, WT LV, and R403Q LV.

4. Conclusions

The R403Q LA trends towards hypercontractility, increased ATP consumption, and lower passive force. Although these effects were less pronounced compared to those observed in R403Q LV, our findings suggest that the shift towards a higher β -MHC expression in LA may play a role within HCM patients.

References

- (1) Nag S, et al. *Sci Adv* 2015;1(9)
- (2) Lowey S, et al. *PNAS* 2018;115(44)
- (3) Anderson R.L., et al. *PNAS* 2018;115(35)
- (4) Narolska N.A, et al. *Card Res* 2005;65(221)