**ONLINE DATA SUPPLEMENT**

# Enhanced transmural fiber rotation and Cx43 heterogeneity are associated with an increased upper limit of vulnerability in a transgenic rabbit model of human hypertrophic cardiomyopathy

Short title: Increased ULV in a transgenic rabbit model of HCM

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## MATERIALS AND METHODS

### Optical Mapping Experiments

Hearts were stained by a gradual injection of 50 μL of stock solution (1.25 mg/ml) of the voltage-sensitive dye di-4-ANEPPS (Molecular Probes, Eugene, OR) diluted in dimethylsulfoxide (Sigma Aldrich, Inc., St. Louis, MO). To mimic the electrode configuration of external defibrillation, two stainless steal mesh electrodes were placed into the solution chamber with one mesh facing the lateral right ventricular (RV) wall and the other mesh facing the lateral left ventricular (LV) wall with 10 cm spacing between the meshes.

### Diffusion-Weighted Magnetic Resonance Imaging

Within the slice, the helix angle ($\alpha$) was calculated at 10 steps across the LV wall from epi- to endocardium in four 20°-wide sectors at the anterior, lateral (between the papillary muscles), inferior, and septal regions. The fiber angle for the entire slice was then calculated as the average of these four regions. To account for inherent geometrical differences between WT and TG hearts, a local coordinate system was used for calculation of $\alpha$. First described by Streeter,[1-3] this coordinate system is known to be the least sensitive to geometrical changes compared to other coordinate systems reported in DTMRI studies.

### Data Analysis

#### *Optical Mapping Data Analysis*

From the S1-S2 data (20 S1 pacing stimuli at 300 ms followed by an S2), the control APD ($APD_c$) was defined as $APD_{80}$ of the $19^{th}$ paced beat, as opposed to the $20^{th}$, to avoid erroneous calculation of the APD during short S2 intervals. The test APD ($APD_t$) was defined as $APD_{80}$ of

the S2 action potential. The control diastolic interval ($DI_c$) was defined as the time between $APD_{80}$ of the 19th paced beat and activation of the 20th paced beat. Test DI ($DI_t$) was defined as the time between the predicted $APD_{80}$ of the 20th paced beat and the activation time of the S2 action potential. To account for inherent differences in APD and DI among the different PDA channel locations as well as between animals, normalized restitution plots were obtained by dividing $APD_t$ by $APD_c$ and $DI_t$ by $DI_c$ for each PDA channel. Restitution plots were generated by plotting normalized APD against the corresponding normalized DI for each PDA channel. Figures 3A and 3B show an example restitution plot before and after normalization.

The maximum slope of the normalized restitution data was estimated with a linear fit by including points at the steepest part of the curve (shortest DIs) (Figure 3C). For those animals in which a full APD restitution curve was performed, the normalized data was also fit using non-linear least-squares regression to a biexponential function[4] according to the following equation:

$$APD_{fit} = B_1 - A_1 e^{-DI/\tau_1} - A_2 e^{-DI/\tau_2} \qquad \text{Eq (1)}$$

where $APD_{fit}$ is the APD at the corresponding $DI$ and $A_1$, $A_2$, $\tau_1$, $\tau_2$, and $B$ are the fitting parameters. A biexponential was chosen to accommodate the shortening of APD at very long DIs that is sometimes observed in rabbit myocardium[4] (Figure 3B). The dispersion of $APD_{80}$ during pacing at a cycle length of 300 ms was calculated according to the following equation:

$$disp = StDev(APD_{80}) / Mean(APD_{80}) * 100 \qquad \text{Eq (2)}$$

Conduction velocity vector fields were calculated from activation maps using a polynomial fitting method developed by Bayly et al.[5] Activation maps of paced data were used to estimate the longitudinal ($C_l$) and transverse ($C_t$) conduction velocities by calculating the velocity vectors

during the time period from approximately 5 to 15 ms after the earliest activation. The magnitude of each velocity vector was then plotted against the angle of the vector and the resulting points were fit using a non-linear least-squares regression to the sum of two sinusoids according to the following equation:

$$CV_{fit} = A_1 \sin(x - x_1) + A_2 \sin(2x - x_2) + B \qquad \text{Eq (3)}$$

where $CV_{fit}$ is the amplitude of the sinusoid at the corresponding angle $x$ and $A_1$, $A_2$, $x_1$, $x_2$, and $B$ are the fitting parameters. The maximum and the minimum points of the sinusoidal fit were deemed $C_l$ and $C_t$, respectively. This procedure was performed for paced beats at a basic cycle length of 300 ms as well as for an S2 at 180 ms. An example activation map and corresponding velocity vectors are shown in Figure 4A and the magnitude and angle of the velocity vectors plotted with the sinusoidal fit are shown in Figure 4B.

## SUPPLEMENTAL TABLES

| | WT | | TG | |
|---|---|---|---|---|
| | **RV-** | **LV-** | **RV-** | **LV-** |
| **%APD** | 77.5 ± 6.0 | 79.2 ± 3.1 | 82.0 ± 3.5 | 81.3 ± 3.1 |
| **$VEP_{max}$ (mV)** | 34.5 ± 14.5 | 84.4 ± 4.2 | 29.1 ± 8.7 | 80.5 ± 21.7 |
| **$VEP_{min}$ (mV)** | -44.2 ± 5.5 | -26.1 ± 5.5 | -33.9 ± 6.2 | -23.3 ± 4.0 |
| **$VEP_{max}$ − $VEP_{min}$ (mV)** | 78.7 ± 15.0 | 110.4 ± 6.5 | 63.1 ± 13.4 | 103.8 ± 19.6 |

**Online Table 1:** Post-shock maximum and minimum virtual electrode polarization (VEP) in response to an 8 V/cm shock applied at approximately 80% APD. Comparisons between transgenic (TG) and wild-type (WT) animals revealed no significant differences for any parameter.

| | **WT** | **TG** |
|---|---|---|
| Slope (Linear) | 0.424 ± 0.262 | 0.472 ± 0.182 |
| **$\tau_1$*** (ms) (Biexponential) | 0.342 ± 0.209 | 0.281 ± 0.068 |
| Mean $APD_{80}$ (ms) | 150.8 ± 13.2 | 154.0 ± 13.9 |
| $APD_{80}$ Dispersion | 6.82 ± 4.21 | 6.80 ± 2.96 |
| ERP (ms) | 171.7 ± 12.11 | 174.2 ± 13.6 |

* $\tau_1$ calculated from 6 of 12 animals

**Online Table 2:** Action potential duration (APD) parameters. Mean and standard deviation of several parameters relating to the APD for wild-type (WT) and transgenic (TG) hearts. $\tau_1$: time constant of the rising phase of the biexponential fit, ERP: effective refractory period.

**Conduction Velocity (m/s)**

**S2 = 180 ms**

| | WT | TG |
|---|---|---|
| $C_l$ (m/s) | 0.577 ± 0.104 | 0.544 ± 0.045 |
| $C_t$ (m/s) | 0.212 ± 0.033 | 0.194 ± 0.024 |
| ratio | 2.712 ± 0.147 | 2.851 ± 0.540 |

**S1 = 300 ms**

| | WT | TG |
|---|---|---|
| $C_l$ (m/s) | 0.593 ± 0.092 | 0.565 ± 0.055 |
| $C_t$ (m/s) | 0.238 ± 0.027 | 0.220 ± 0.034 |
| ratio | 2.494 ± 0.427 | 2.601 ± 0.314 |

**Online Table 3:** Epicardial conduction velocities. Mean and standard deviation of longitudinal ($C_l$) and transverse ($C_t$) conduction velocities for wild-type (WT) and transgenic (TG) hearts at two different pacing intervals. The anisotropic ratio ($C_l / C_t$) of conduction velocity is also listed. Comparisons between TG and WT animals revealed no significant differences for any parameter.

| Percent Cx43 | WT endo | WT mid | WT epi | TG endo | TG mid | TG epi |
|---|---|---|---|---|---|---|
| **Mean ± StDev** | 5.31 ± 2.46 | 2.68 ± 0.77* | 4.23 ± 1.00 | 3.92 ± 2.48 | 5.46 ± 2.44 | 4.92 ± 1.51 |

*$p < 0.05$ vs TG mid and $p < 0.05$ vs WT endo

**Online Table 4:** Connexin 43 (Cx43) densities. Table of Cx43 densities from the graph shown in Figure 6D. p-values reflect comparison between WT and TG hearts obtained with Fisher's protected t-test.

## References

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