Disease Mechanisms of Arrhythmogenic Cardiomyopathy

Emily Maglin,[1] Morgan Engel, BS[2], Elisa N. Cannon, BA[2], Stephen P. Chelko, Ph.D.[2,3]

Florida State University, College of Arts and Sciences;[1] Florida State University, College of Medicine;[2] Johns Hopkins University, School of Medicine[3]

Introduction

- Arrhythmogenic Cardiomyopathy (ACM) is an inherited heart disease and a leading cause of sudden cardiac death (SCD) in the young; where exercise potentiates disease progression.
- ACM is brought on by mutations in genes that encoded the cardiac desmosome (**Figure 1**). This region is what anchors cell-cell connections and cardiac skeletal fibers within cells. Disruption of the desmosome results in myocardial inflammation, fibrotic remodeling, impaired heart contractility, and in severe cases, SCD.

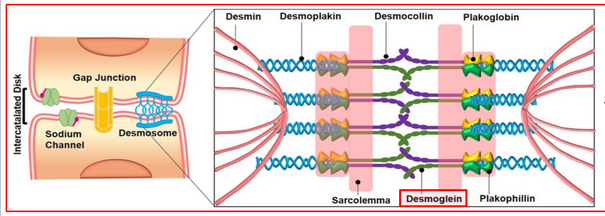

Figure 1. The Cardiac Desmosome.

Mutations in desmosomal genes, such as *Desmoglein-2* (*Dsg2*), leads to loss in cardiomyocyte connection, mechanical disruption of the myocardium, and reduced electrical conduction.

- Prior research, utilizing mice that harbor the desmosomal gene mutation – *Desmoglein-2* (*Dsg2*$^{mut/mut}$ mice) – investigated the impact of forced-swim exercise (90mins/day, 5days/week, for 10 weeks) on disease progression.[1,2] However, the results of these studies do not accurately represent the profound impact that prolonged running exercise can have on the progression and presentation of ACM.
- Currently, Dr. Chelko's laboratory is investigating the impact of voluntary running-based exercise on ACM. Specifically, using StarrLife Sciences' In-Cage Running Wheels will allow for tracking exercise intensity (i.e., speed), duration (i.e., time on wheel), and total distance run over 10 weeks in order to correlate these exercise parameters against ACM functional and pathological phenotypes.

Methods

- The current study utilized *Dsg2*$^{mut/mut}$ mice, as well as age-matched wild-type (WT) mice and *Dsg2*$^{mut/mut}$ mice with germline deletion of *Colony Stimulating Factor-2* (*Csf2*). When translated, CSF2 is a potent cytokine and was observed to be a central component for myocardial inflammatory signaling in ACM.[3]
- Therefore, the latter Double Mutant mice (i.e., *Dsg2*$^{mut/mut}$;*Csf2*$^{-/-}$ mice) harbor an ACM mutation and are unable to generate CSF2 protein.
- We compared running capacity and heart function (echocardiography and ECG telemetry) in all three cohorts: WT, *Dsg2*$^{mut/mut}$ and *Dsg2*$^{mut/mut}$;*Csf2*$^{-/-}$ mice.

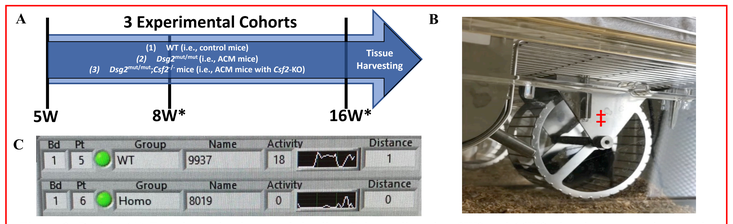

Figure 2. Design. (**A**) Experimental cohorts and timeline. Echos collected at 8W* and 16W*, then hearts were harvested at endpoint (i.e., 16W). (**B**) StarrLife Sciences' in-cage running wheels. Note (‡), sensor that continuously records each wheel revolution; which (**C**) converts revolutions to total distance in miles.

- Mice were individually housed in In-Cage Running Wheels with sensors to record indefinite measures of exercise starting at 5 weeks of age (**Figure 2**).
- Echocardiographic and ECG data were obtained at 8 and 16 weeks of age, and compared against differences in systolic function, ventricular sizes, and heart rhythm disorders [i.e., arrhythmias].

Hypothesis

- We hypothesize there will be a considerable difference in the amount of fibrotic scarring, cardiac dysfunction and arrythmias in *Dsg2*$^{mut/mut}$ mice compared to both WT and *Dsg2*$^{mut/mut}$;*Csf2*$^{-/-}$ mice after endurance running exercise.

Current Results

Reduced Running Capacity in ACM mice.

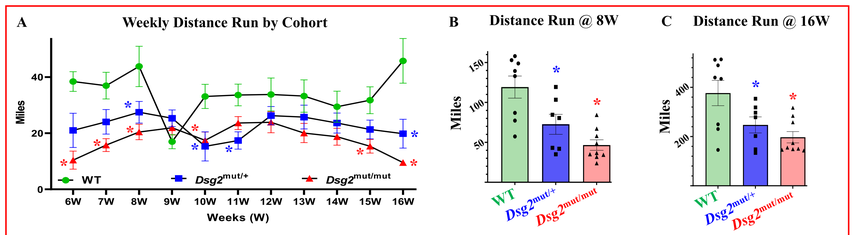

Figure 3. Weekly and Total Distance Run. (**A**) Distance run per week, by cohort. Note, WT mice show increased distance run (**B**) at both 8W and (**C**) 16W of age compared to *Dsg2*$^{mut/+}$ and *Dsg2*$^{mut/mut}$ mice. */*P<0.01 any cohort (regardless of time-point) vs WT mice; via 2-Way ANOVA with Tukey's post-hoc analysis.

Voluntary wheel exercise results in exercise-induced disease penetrance in Dsg2-heterozygotes (Dsg2$^{mut/+}$*).*

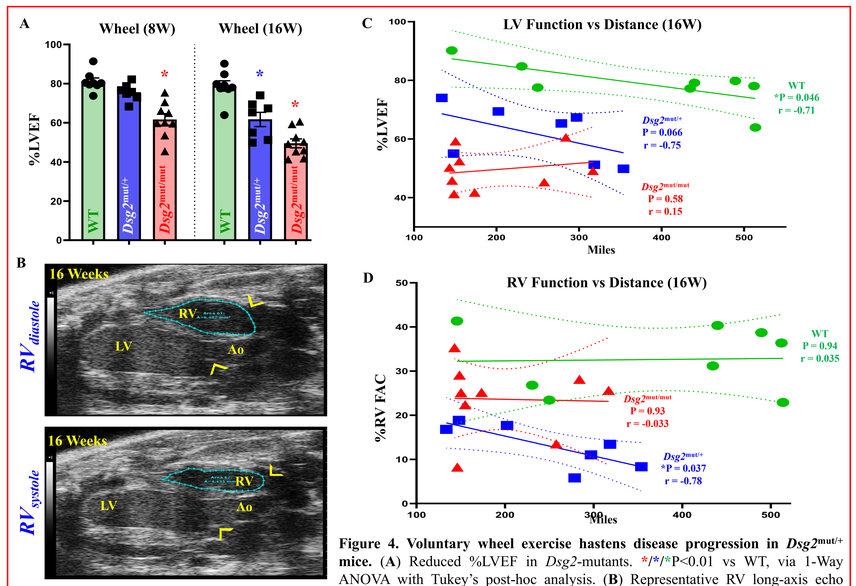

Figure 4. Voluntary wheel exercise hastens disease progression in *Dsg2*$^{mut/+}$ mice. (**A**) Reduced %LVEF in *Dsg2*-mutants. */*/*P<0.01 vs WT, via 1-Way ANOVA with Tukey's post-hoc analysis. (**B**) Representative RV long-axis echo from *Dsg2*$^{mut/+}$ mice. Yellow arrows, valves; Ao, Aorta. (**C**) %LVEF and (**D**) %RV fractional area change (%RV FAC) was utilized to assess cardiac function against distance run using Spearman's rho (r) correlation analyses.

Discussion of Current Results

- Temporal analysis demonstrated homozygous-*Dsg2* mice display reduced running capacity and earlier onset of ACM disease phenotypes in response to exercise (**Figure 3**).
- Interestingly, voluntary-wheel exercise also demonstrated prolonged running led to disease activation in heterozygote-*Dsg2;* a finding not previously observed in age-matched sedentary mice and in heterozygotes in response to swimming (**Figure 4**).
- These results provide evidence for a direct, causal relationship between the harmful effects of endurance running on ACM disease progression.
- The outcomes of running-based exercise on disease progression in ACM mice is, therefore, of monumental importance in understanding these harmful effects in patients with ACM. Particularly, since it is unethical to design such a study in patients with ACM.
- These translational findings finally prove the causal relationship of exercise-induced cardiac dysfunction in ACM. Currently, the only correlative analysis conducted in this vulnerable patient population is comparing retrospective, self-administered exercise-history reports with functional phenotypes at last clinical follow-up.[4-6]
- Thus, these past patient studies relied heavily on ACM patients' recalling of decades long exercise histories. A correlative analysis at best, but not a cause-and-effect comparison as performed in these animal studies.
- Lastly, these findings demonstrates prolonged, strenuous physical activity potentiates disease phenotypes in asymptomatic ACM heterozygotes.

Future Directions

- The current study is still undergoing and is investigating a variety of outcomes. Currently, hearts from running-based studies have been collected and are undergoing immunohistochemical analyses in order to examine the pathological impact exercise imparts in ACM mice on an anatomical layer (**Figure 5**).
- Hearts have been carefully sliced into thin layers (5µm) that can easily undergo a variety of staining procedures. The next aim of the current study is to immunostain heart slices from three different, age-matched mouse cohorts: (1) **WT mice** and (2) ***Dsg2*$^{mut/mut}$ mice**, and (3) *Dsg2*$^{mut/mut}$ mice in which *Colony Stimulating Factor-2* (*Csf2*) has been knocked-out (***Dsg2*$^{mut/mut}$;*Csf2*$^{-/-}$ mice**).

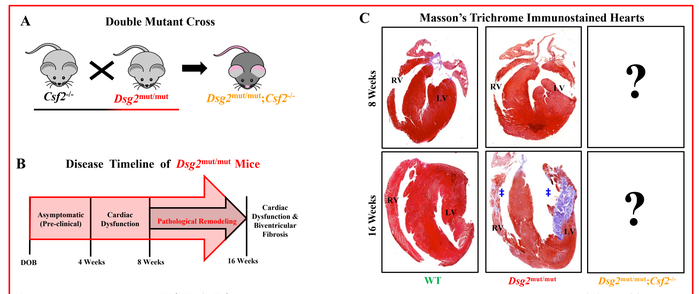

Figure 5. Assessing *Dsg2*$^{mut/mut}$;*Csf2*$^{-/-}$ mice disease phenotypes, at rest and in response to exercise. (**A**) Double mutant breeding. (**B**) *Dsg2*$^{mut/mut}$ disease timeline. (**C**) Representative Masson's Trichrome immunostained hearts from WT and *Dsg2*$^{mut/mut}$ mice. These hearts were taken from Dr. Chelko's exercise studies and will be compared against *Dsg2*$^{mut/mut}$ mice with complete removal of *Csf2* gene (i.e., ***Dsg2*$^{mut/mut}$;*Csf2*$^{-/-}$ mice**) at rest and in response to endurance exercise. RV, right ventricle; LV, left ventricle, ‡, note, presence of diffuse RV scarring and large epi-to-endocardial scarring of the LV.

- Chelko et al previously demonstrated elevated levels of myocardial CSF2,[3] a pro-inflammatory protein (i.e., cytokine). Thus, we hypothesize ***Dsg2*$^{mut/mut}$;*Csf2*$^{-/-}$ mice** will display reduced fibrotic scarring compared to their aged-matched counterparts.

Anticipated Results

- Functional (via Echocardiography): We expect to observe a considerable preservation of cardiac function in ***Dsg2*$^{mut/mut}$;*Csf2*$^{-/-}$** mice compared to **WT** and ***Dsg2*$^{mut/mut}$** mice at 8 and 16 weeks of age.
- Pathological (via Masson's Trichrome): We anticipate fibrotic scarring in ***Dsg2*$^{mut/mut}$** mice and absence of pathological remodeling in ***Dsg2*$^{mut/mut}$;*Csf2*$^{-/-}$** mice at 16 weeks of age.
- Furthermore, we are currently processing the *Dsg2*-heterozygote mouse hearts and expect these hearts will present with noticeable fibrotic scarring as compared to sedentary, age-matched *Dsg2*-heterozygotes, because functional phenotypes declined during prolonged exercise.
- Regardless, scarring is likely to be more severe in all ACM cohorts due to prolonged running-based exercise exposure, a known cause of early disease onset in ACM.[4-6]

Acknowledgements

- I would like to give my sincerest "thank you" to Dr. Stephen Chelko for providing me this research opportunity and witness this truly amazing work being done in his laboratory. Dr. Chelko is a phenomenal scientist and mentor, and I could not be more grateful to be a member of his research team this year. I look forward to future work under his direction and the results of our final analysis. I would also like to thank the members of Dr. Chelko's lab, both current and graduate/medical students, who took me under their wing and helped me learn and practice a variety of important laboratory techniques.

References

[1]Chelko et al, *Journal of Clinical Investigation Insight*, 2016.
[2]Chelko et al, *Science Translational Medicine*, 2021.
[3]Chelko et al, *Circulation*, 2019
[4]James et al, *Journal of the American College of Cardiology*, 2013
[5]Sawant et al, *Journal of the American Heart Association,* 2014
[6]Lie et al, *Journal of the American College of Cardiology Clinical Electrophys.*, 2018