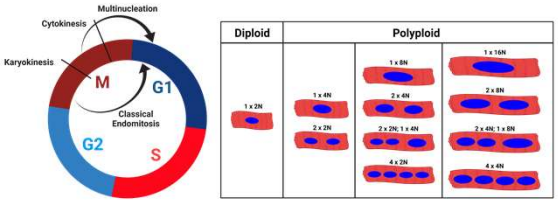
Genetic mapping of cardiomyocyte ploidy phenotypes that influence basal cardiac physiology

Alexandra L Purdy[1], Amirala Bakhshiannik[2,3], Mary Kolell[1], Kaitlyn Andresen[1], Smrithi Chanjeevaram[1], Cheyret Wood[4], Melinda R Dwinell PhD[3], Laura M Saba PhD[4], Caitlin O'Meara PhD[2,3], Michaela Patterson PhD[1,2]

[1]Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI
[2]Cardiovascular Center, Medical College of Wisconsin, Milwaukee, WI
[3]Department of Physiology, Medical College of Wisconsin, Milwaukee, WI
[4]Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado, Aurora, CO

1) Introduction

Heart disease has been the leading cause of death for over a century, yet current therapies only aim to slow disease progression. A multitude of factors can contribute to outcomes and progression to heart failure, including environment, lifestyle, and genetic predisposition. Our interest lies in the latter, where very few genes have successfully been linked to heart failure. Contributors to this low success rate include high genetic diversity of patient populations, ability to control for non-genetic factors, and cellular complexities of disease that contribute to outcomes. We propose utilizing genetically diverse rodents to assess high throughput surrogate phenotypes, which can be measured in the basal state to predict outcomes after injury. This approach can offer a solution to the limitations of association studies for complex diseases like heart failure. The surrogate phenotype I am pursuing is cardiomyocyte (CM) ploidy, where high frequency of diploid cardiomyocytes has been correlated with an improved regenerative competence and functional recovery. Conversely, an increased proportion of polyploid cardiomyocytes is associated with dilated cardiomyopathy and adverse ventricular remodeling following myocardial infarction.



2) Cardiomyocyte ploidy is a highly heritable trait that varies across the genetically diverse rat strains of the Hybrid Rat Diversity Panel

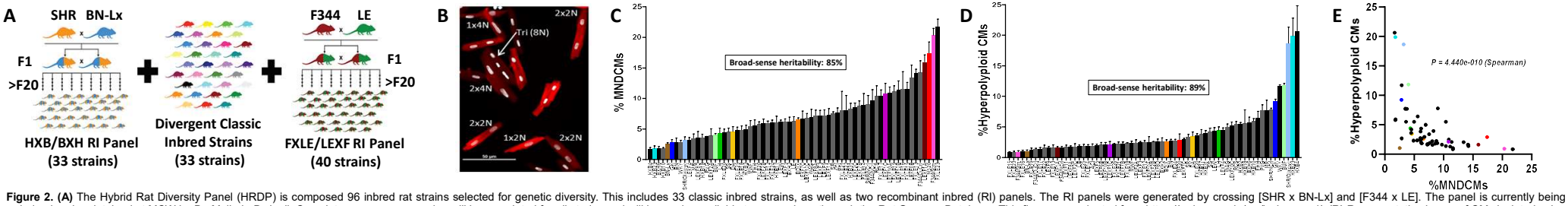


Figure 2. (A) The Hybrid Rat Diversity Panel (HRDP) is composed 96 inbred rat strains selected for genetic diversity. This includes 33 classic inbred strains, as well as two recombinant inbred (RI) panels. The RI panels were generated by crossing [SHR x BN-Lx] and [F344 x LE]. The panel is currently being rederived and maintained at MCW by Dr. Melinda Dwinell. Complete genome sequencing will be completed for all strains and will be made available to researchers through the Rat Genome Database. This figure was adapted from https://rgd.mcw.edu/wg/hrdp_panel/. **(B)** Representative image of CMs isolated using Langendorff perfusion with collagenase II to digest the heart into a single-cell preparation. CMs are labeled by nucleation **(C)** Percent of mononuclear diploid cardiomyocytes (MNDCMs) and **(D)** hyperpolyploid (≥8N) cardiomyocytes across 67 strains of the HRDP (N=3-10). The colored bars represent strains that were selected for further studies to assess baseline function, baseline morphology, and outcomes post-MI. Error bars represent SEM. **(E)** Spearman correlation between the strain means for %MNDCMs and %hyperpolyploid CMs.

3) CM ploidy correlates with baseline cardiac function in select rat strains

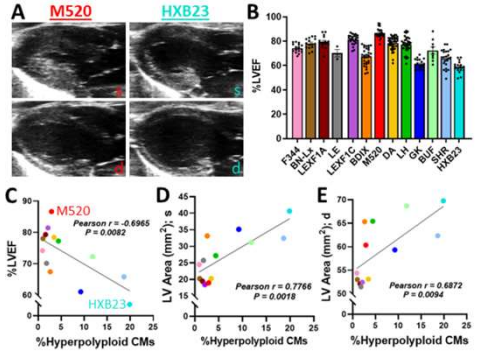


Figure 3. 8-week-old female rats were subjected to echocardiography. **(A)** Example images in systole (s) and diastole (d) for two divergent strains. **(B)** Left Ventricular Ejection Fraction (LVEF) across 13 strains of the HRDP. N=3-20 animals per strain. **(C-E)** Correlation of frequency of hyperpolyploid CM to LVEF **(C)**, LV systolic area **(D)**, and LV diastolic area **(E)**.

4) QTL mapping of CM ploidy phenotypes

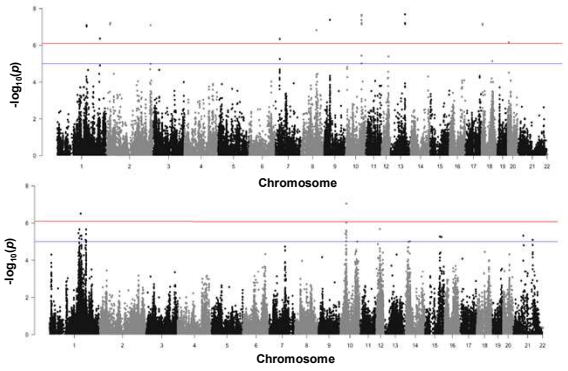


Figure 4. Genetic mapping of 63 strains analyzed for CM ploidy phenotypes was conducted using a genomic marker dataset that can be accessed via the PhenoGen website (https://phenogen.org). Manhattan plots for **(A)** %MNDCMs and **(B)** %hyperpolyploid CMs represent loci that pass either suggestive (blue) or significant (red) thresholds. Prior to mapping, data was transformed using arcsine transformation.

5) *Shroom3* is a candidate from the hyperpolyploid locus on chromosome 14

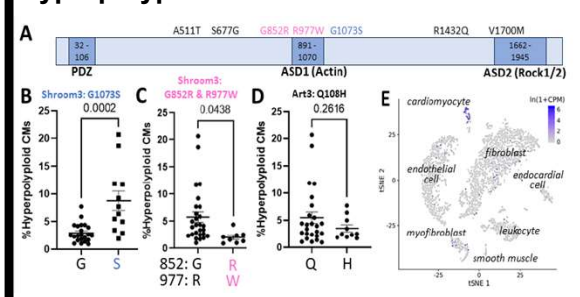


Figure 5. (A) Schematic of SHROOM3 protein annotated with the 7 predicted to be damaging variants noted across the top. **(B)** Dot plot depicting the distribution of strains and their frequency of hyperpolyploid CM at single nucleotide variant G1073S. **(C)** Distribution of the G852R and R977W variants (inherited together) across the HRDP. Highlighted in pink because the predicted-to-be-damaging variants. **(D)** Distribution of a predicted-to-be-damaging variant of Art3, which do not distribute across the HRDP as anticipated. **(E)** tSNE plot of Shroom3 expression across the heart collected from Tabula Muris.

6) CM ploidy is altered in *Shroom3* cKO mice

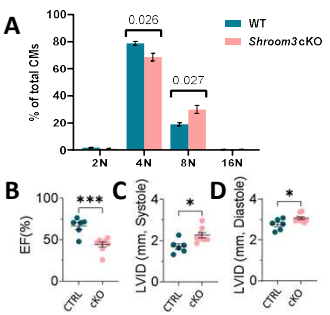


Figure 6. (A) Frequency of each CM population accounting for both nucleation and nuclear ploidy (i.e. 4N = 1x4N CMs and 2X2N CMs). N=4 for both WT and *Shroom3* cKO. Echocardiography was performed on obtain left ventricular ejection fraction (EF) **(B)** and left ventricular internal diameter (LVID) in systole **(C)** and diastole **(D)**.

7) Conclusions

- CM ploidy varies across the genetically diverse rat strains of the HRDP surveyed thus far and it is a highly heritable trait.
- CM ploidy correlates with %LVEF, %LVFS and LV area, suggesting a potential role for CM ploidy in baseline heart function.
- *Shroom3* is a candidate gene with 7 potentially damaging variants in the HRDP rats, is expressed in CMs, and influences CM hyperploidy.
- Echocardiography results from *Shroom3* cKO mice recapitulate the phenotypes of HRDP rat strains with increased amounts of hyperpolyploid CMs.

8) Future directions

- Perform QTL mapping using whole genome sequence data once it becomes available for all HRDP rat strains.
- Investigate the impact of novel *Shroom3* variants (G852R and R977W) on the protein's ability to bind to actin and ROCK.
- Leverage custom adenovirus to determine the ability of the G1073 (reference) and S1073 *Shroom3* variant to prevent increased CM hyperpolyploidy.

Funding and Acknowledgements:

- R21HL156022 (Dr. Michaela Patterson and Dr. Caitlin O'Meara)
- 2T32HL007852 (Alexandra Purdy)
- MCW CVC pilot award (Dr. Michaela Patterson and Dr. Caitlin O'Meara)
- R01HL155085 (Dr. Michaela Patterson)
- R01HL170547 (Dr. Caitlin O'Meara and Dr. Brian Link)
- Hybrid Rat Diversity Program (Dr. Melinda Dwinell, Allison Zappa, and Lynn Malloy)
- Echocardiography resources (Dr. John Auchampach and Dr. Tina Wan)