The Role of FOG2^{S657G}-mediated β1 Adrenergic Receptor Expression in Coronary Microvascular Disease

Neel Pandula[1], Jeff Ren[2], Elissa Williams[3], Sharavana Gurunathan, PhD[4], Donna Conlon, PhD[4], Marie Guerraty, MD/PhD[4]

[1]University of Pennsylvania College of Arts and Sciences (Class of 2027), [2]University of Pennsylvania College of Arts and Sciences, [3]University of Pennsylvania School of Veterinary Medicine, [4]Perelman School of Medicine, Department of Medicine

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Introduction

- Coronary microvascular disease (CMVD) causes 30-50% of ischemic heart disease.[1]
- Cardiomyocyte Friend of GATA 2 (FOG2) is a transcriptional co-regulator crucial for the development and maintenance of the coronary microvasculature.[2]
- Data from the Penn Medicine Biobank revealed an association between a coding variant of *FOG2* (rs28374544, A1969G, Ser657Gly) highly prevalent in patients of African ancestry (minor allele frequency ~20%) and CMVD.[3]
- FOG2 is known to co-regulate and bind directly to GATA4, a transcription factor crucial to proper cardiac morphogenesis and maintenance, repressing GATA4-mediated activity.[4]
- RNA-sequencing and Gene Set Enrichment Analysis data from hearts from a CRISPR/Cas9-edited mouse model with a mutation analogous to human *FOG2^{S657G}*, termed *Fog2^{mut}*, showed increased adrenergic signaling and, specifically, expression of β1 adrenergic receptor (*ADRB1*) at the gene level. Immunoblot showed increased expression of ADRB1 at the protein level.

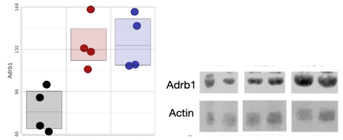

Figure 1: Mice heterozygous or homozygous for *Fog2^{mut}* have increased expression of β1 adrenergic receptor at the gene (left) and protein (right) level.

- **Hypothesis:** FOG2^{S657G} fails to repress GATA4, increasing β1 adrenergic receptor signaling and, subsequently, cardiac work, which promotes CMVD.

Methods

- Luciferase reporter construction
  - *ADRB1* promoter (2893-5375) was PCR-amplified with 3' A overhangs from HEK293-derived human genomic DNA.
  - *ADRB1* promoter was ligated into pCR8/GW/TOPO Gateway Entry vector.
  - *ADRB1* promoter was transferred into pGL4.23-GW Gateway Destination vector upstream of *luc2* gene via LR recombination reaction.

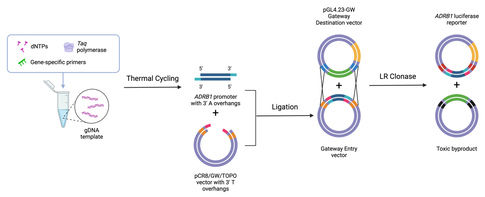

Figure 2: Overview of *ADRB1* luciferase reporter construction.

- Dual-luciferase assay
  - *ADRB1* luciferase reporter was transfected into HEK293 cells via Lipofectamine 2000.
  - Relative luminescence was measured 72 hours post-transfection using Promega Dual-Luciferase Reporter Assay System.
- BP-2000 Blood Pressure Analysis System
  - BP-2000 Blood Pressure Analysis System was used to compare heart rate and blood pressure of *Fog2^{mut}* mice to that of littermate controls (n = 11 WT, 12 MUT) via repeated tail cuff measurements.
- IonOptix System
  - Primary cardiomyocytes (CMs) were isolated from *Fog2^{mut}* and littermate control hearts.
  - IonOptix Multicell High Throughput System was used to compare contractile activity of electrically-paced *Fog2^{mut}* primary CMs to those of littermate controls (n = 1 WT, 1 MUT) both with and without isoproterenol.

Results

Luciferase reporter construction

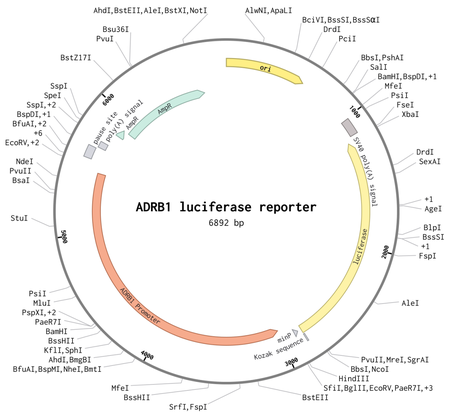

Figure 3: Plasmid map of *ADRB1* luciferase reporter generated via nanopore sequencing.

BP-2000 heart rate and blood pressure measurements

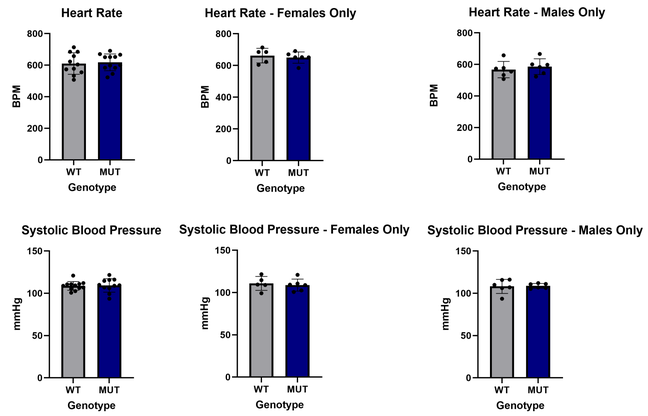

Figure 4: No significant difference in heart rate or blood pressure between *Fog2^{mut}* mice and littermate controls regardless of sex. n = 11 WT, 12 MUT

IonOptix primary cardiomyocyte measurements

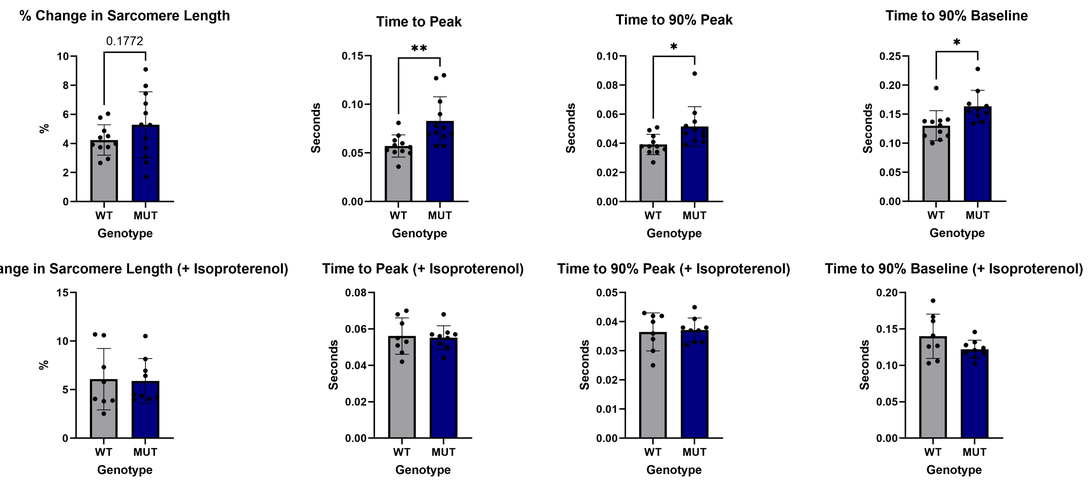

Figure 5: Prior to treatment with isoproterenol, *Fog2^{mut}* CMs exhibited significantly greater time to 90% peak, time to 90% baseline, and time to peak as well as increased percent change in sarcomere length relative to littermate control CMs, indicating larger contractions with slower contraction times. Following addition of isoproterenol, these differences were muted. n = 1 WT, 1 MUT. *p<0.05

Results

Dual-luciferase assay

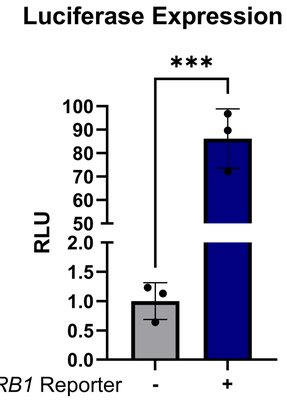

Figure 6: HEK293 cells transfected with *ADRB1* reporter have greatly increased activity relative to negative controls. n = 3 -, 3+

Summary

- Successful assembly of a novel *ADRB1* luciferase reporter confirmed by nanopore sequencing. Function of reporter confirmed by dual-luciferase assay.
- No significant difference in heart rate or blood pressure between *Fog2^{mut}* mice and littermate controls.
- *Fog2^{mut}* primary CMs showed larger contractions with slower contraction times relative to littermate control primary CMs, with the addition of isoproterenol muting these differences.

Future Directions

- Conducting dual-luciferase assays using *ADRB1* luciferase reporter to determine the mechanism by which FOG2^{S657G} increases expression of *ADRB1*.
- Assessing differences in the phosphorylation of downstream proteins in the β1 adrenergic receptor signaling pathway between *Fog2^{mut}* and littermate control hearts.
- Assessing differences in calcium flux across the sarcoplasmic reticulum between *FOG^{2S657G}* human induced pluripotent stem cell-derived CMs and control using IonOptix Multicell High Throughput System.

References

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