AWARD NUMBER: W81XWH-21-1-0104

TITLE: Using Systems Genetics to Probe for Gene Interactions in Congenital Heart Disease

PRINCIPAL INVESTIGATOR: Georg Vogler

CONTRACTING ORGANIZATION: Sanford Burnham Prebys Medical Discovery Institute
La Jolla, CA

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6. AUTHOR(S)
Georg Vogler

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Sanford Burnham Prebys Medical Discovery Institute
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

In this project we are trying to understand the gene networks underlying congenital heart disease, specifically bicuspid aortic valve (BAV) and its relation to hypoplastic left heart syndrome (HLHS). Under this proposal we are using a two-pronged strategy to systematically identify cardiac gene networks: comprehensively identifying genetic interactors of cardiogenic genes *NKX2-5/tinman* and *GATA4/pannier* using the adult *Drosophila* (fruit fly) heart (aim 1), and iPSC-derived cardiac progenitors and cardiomyocytes from two families (parent/proband trios) with BAV+HLHS (aim 2). During year one, we identified ~40 regions in the fly genome that display either synthetic lethality or cardiac phenotypes in conjunction with *tinman/pannier* with about 30% completion of the genetic screen. These regions include several likely candidate genes such as *Muscle-specific protein 300* (*Msp300/Nesprin1*), as well as many new potential loci with currently unknown role in heart development and function. While we are completing the genetic screen to identify all *tin/pnr* interacting loci in the fly genome, we are also setting up the experiments to follow up on identifying the specific gene loci inside the candidate regions responsible for the interaction. For aim 2, we acquired the cell lines (iPSCs) necessary for conducting the proposed research and are currently processing these cells for mass-screening using our collaborators high-throughput assay.

15. SUBJECT TERMS
CHD, congenital heart disease, Drosophila, iPSC, genetics, systems biology, gene networks

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. **INTRODUCTION:**

Our research is focused on the identification of genetic vulnerabilities that might lead to congenital heart defects (CHD) and could affect patient treatment and outcome prognosis. We hypothesize underlying gene networks of cardiac determinants (transcription factors, TFs) and their targets to be affected in cases of complex CHDs, such as hypoplastic left heart syndrome (HLHS). We proposed to build a genetic interaction map of cardiac TFs using the *Drosophila* heart model and analyze congenital heart disease networks in cells obtained from two families with CHD: bicuspid aortic valve (BAV) defects in a parent and child with HLHS.

2. **KEYWORDS:** CHD, congenital heart disease, Drosophila, iPSC, genetics, systems biology, gene networks

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

Under Specific Aim 1, High-throughput screen for genetic interactors of cardiac determinants, we are using the model organism *Drosophila* to study the genetics of heart development and function, ultimately to understand human heart disease. This aim has the objective to systematically test the *Drosophila* genome for loci that interact with the cardiac transcription factors *tinman* and *pannier* (NKX2-5, GATA4/5/6, in humans). Under Specific Aim 2, High-throughput screen for genetic modifiers of BAV/HLHS, we sought to challenge cells (cardiac precursors (CPs) and cardiomyocytes (CMs)) with siRNA for candidate genes, alone and in combination with NKX2-5 and GATA4. Readouts are cardiac differentiation efficacy (for CPs) and CM-proliferation assay.

Specific Aim 1: High-throughput screen for genetic interactors of cardiac determinants	**Timeline**	**Site 1**	**% completion**
Genetic Screen using Drosophila deficiencies	Months		
Ordering of fly stocks	1-3	Dr. Vogler	100
Amplification of sensitized fly line	1-3	Dr. Vogler	100
Deficiency crosses (~470)	3-8	Dr. Vogler	30
Mounting and Filming	4-9	Dr. Vogler	30
Analysis of movies	9-10	Dr. Vogler	30
Prioritization of candidate genes from deficiency hits	10-11	Dr. Vogler	50
Identification of specific interactors within deficiencies			
Ordering of fly stocks	12-24	Dr. Vogler	10
Candidate gene crosses	12-23	Dr. Vogler	10
Mounting and Filming	12-23	Dr. Vogler	
Analysis of movies	12-23	Dr. Vogler	
Confirmatory experiments (qPCR, RNAseq)	12-23	Dr. Vogler	
Milestone(s) Achieved: identified specific interactors	24	Dr. Vogler	
Specific Aim 2: High-throughput screen for genetic modifiers of BAV/HLHS			
hiPSC-derived cardiac precursors and CMs			
Obtaining hiPSC from Mayo Clinic	1-2		100
Generation of cardiac precursors and banking	3-8	Drs. Vogler and Colas	
Generation of cardiomyocytes and banking	3-8	Drs. Vogler and Colas	
siRNA treatments, immunostaining of first gene sets	9-14	Dr. Colas	
Evaluation and repeat experiments	11-16	Dr. Colas	
Test candidates from Specific aim 1	17-23	Dr. Colas	

Milestone(s) Achieved: identified patient-specific pathways affected	24	Dr. Vogler	

- **What was accomplished under these goals?**

To probe the *Drosophila* genome for loci that are critical for heart development and function (aim 1) we proposed to screen for deficiencies (deletions in the genome that remove a defined set of genes) which, when placed *in trans* to a *tinman/pannier* double mutant condition, would alter the mild cardiac phenotype of *tin/pnr* alone. We first amplified the pnr^{VX6}, tin^{346} sensitizer fly line to be able to continuously collect flies for subsequent crosses. This sensitizer lines also carries a cardiac-specific red-fluorescent protein (tandem-tomato, tdtK) that we use for high-speed video recording of heart structure and function (see also Figure 2 B,C). Fly stocks are made available by the Bloomington Drosophila Stock Center (BDSC, Indiana, USA) as a 'deficiency kit' containing 471 fly lines that cover 16895 genes (95% of the fly genome; Figure 1 bottom row). We first tested genes of the X and 2nd chromosome by crossing flies of the deficiency to flies that carry pnr^{VX6}, tin^{346} (loss-of-function alleles for *pannier* and *tinman*). For the X chromosome, we collected virgin flies from the deficiency stock due to their associated lethality in hemi-zygotic males and crossed these to pnr^{VX6}, tin^{346} male flies. For all other chromosomes, we collected pnr^{VX6}, tin^{346} virgin flies and crossed them to deficiency carrying male flies. At this time, we have crossed 150 deficiency lines, covering most of the X and 2nd chromosome (4801 genes) and evaluated 76 deficiencies (Figure 1).

Among the deficiencies tested, we identified 14 that caused synthetic lethality when placed *in trans* to pnr^{VX6}, tin^{346} (Figure 1; black bars). While not heart-specific, these loci contain one or more genes that strongly interact and now become haplo-insufficient when placed together with pnr^{VX6}, tin^{346} resulting in developmental lethality. We prioritized the identification of the specific genes inside these lethal deletions that are interacting *with* pnr^{VX6}, tin^{346} due to the relative ease of simply testing for lethality as readout. While all lethal deficiencies cover a total of 654 genes thus far, their number can be readily reduced using overlapping deficiencies (e.g., *Df(2L) BSC454* / *ED19* / *BSC106*, from 34 genes to 12 candidates, including JAK/STAT receptor *Hopscotch*). Going forward, we will gather smaller subsets of deficiencies to narrow down the lethal region to then test individual candidate genes finally. Due to the developmental lethality, we will analyze embryos and embryonic hearts triple-heterozygous for the candidate gene and pnr^{VX6}, tin^{346} for developmental defects associated with the observed lethality.

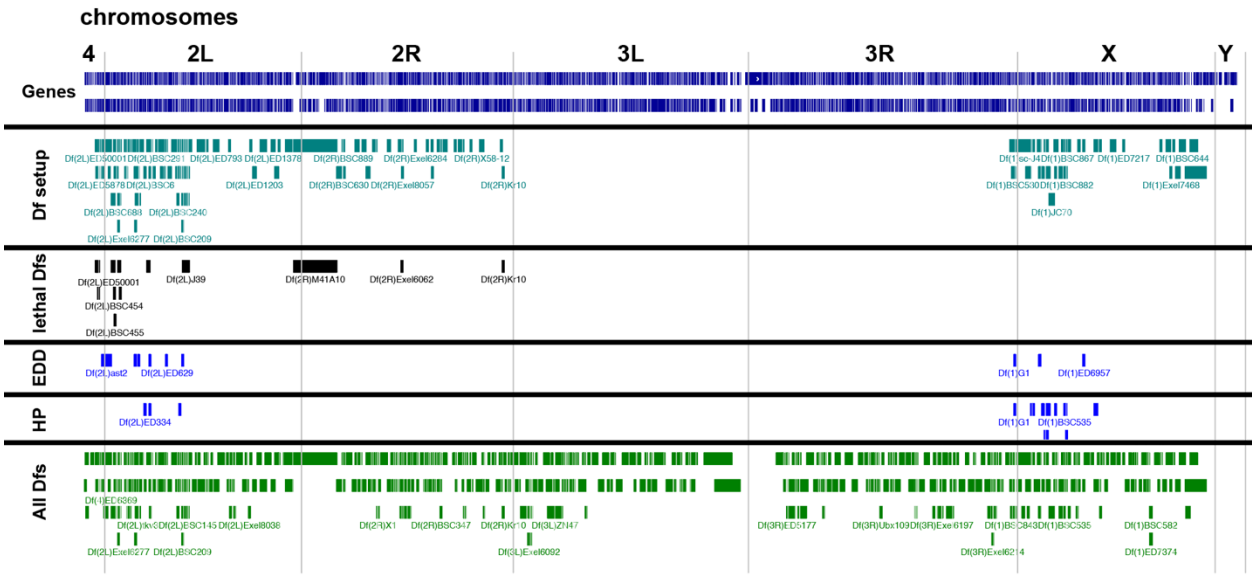

Figure 1. Map of the Drosophila genome and fly screen progress. Chromosomes and chromosome arms are shown on top. Progress on deficiency (Df) crosses is measured as Df setup (top row; 150) / All Dfs (bottom row; 471). Dfs that cause synthetic lethality are shown in black. Df with cardiac phenotypes (examples: end-diastolic diameter, EDD; heart period, HP) are shown in blue.

For all crosses, we aimed at collecting 20 female F1 flies per cross, to be analyzed along a control cross (*pnr^{VX6}, tin^{346}* x *w^{1118}*). Flies were mounted and imaged using our established pipeline and analyzed using our custom software. We then used several parameters describing heart structure (end-diastolic and systolic diameters (EDD, ESD)) and heart function, e.g., contractility (fractional shortening FS)), heart rate (HR), heart period (HP), rhythmicity (arrhythmia index and MAD indices), contraction time (systolic interval (SI)) and cardiac output (CO). If a deficiency crossed to *pnr^{VX6}, tin^{346}* shows deviation of one or more heart parameters compared to the control cross it indicates a potential underlying genetic interaction of one or more loci inside the deficiency with *pnr^{VX6}, tin^{346}*. In contrast to synthetic lethality, were only the combined genotype is lethal whereas neither the deficiency nor *pnr^{VX6}, tin^{346}* alone are lethal, for all other phenotypes we need to consider that the phenotype is a result of an additive effect of the single genotypes. For all phenotypes presented below we will test this by subsequently evaluating the deficiency by itself, without *pnr^{VX6}, tin^{346}*.

Furthermore, while we originally designed the experiment to analyze 3-week-old F1 flies, we found that both, triple heterozygous genotypes (Df, *pnr^{VX6}, tin^{346}*) but also control crosses deteriorate significantly (die-off, severe constriction). We therefore focused on imaging at 1 week of age resulting in improved assay robustness.

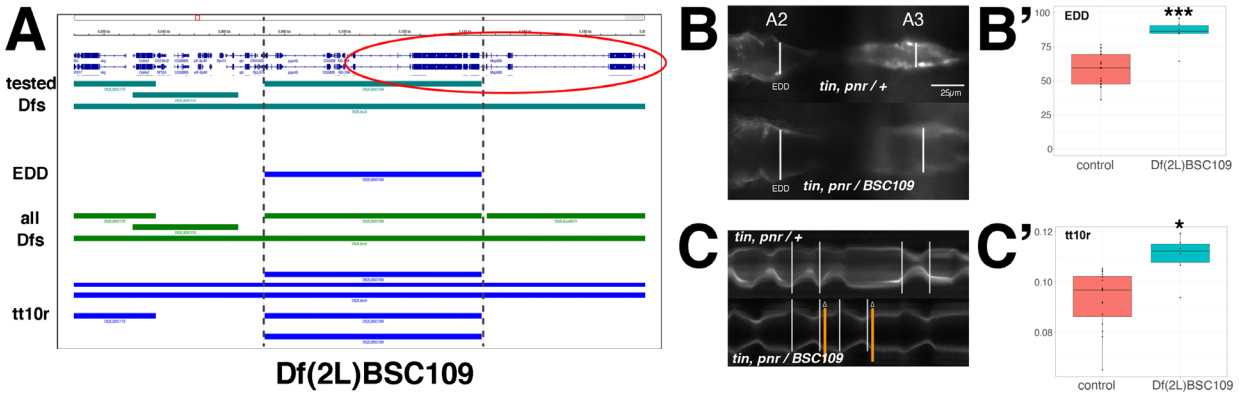

Figure 2. Example of a potential genetically interacting deficiency, *Df(2L)BSC109*. (**A**) Genome map of a locus covered by several deficiencies, some of which overlap the *Msp300* locus (dashed lines and red circle). Two phenotypes are highlighted, EDD (end-diastolic diameter), and tt10r (time-to-10-percent-return; systolic interval approximation). (**B, B'**) Heart size difference between *pnr^{VX6}, tin^{346}* and *pnr^{VX6}, tin^{346}* x *Df(2L)BSC109*. EDD is measured in two chambers, A2 and A3. With *BSC109*, the hearts are significantly enlarged compared to *pnr^{VX6}, tin^{346}* . Wilcoxon test; $p < 0.0001$. (**C, C'**) Heart beat lengths (tt10r) is significantly increased in *pnr^{VX6}, tin^{346}* x *Df(2L)BSC109* compared to *pnr^{VX6}, tin^{346}* alone (prolonged time interval-Δ is indicated by orange line). Wilcoxon test; $p < 0.05$.

Of 62 deficiencies crossed to *pnr^{VX6}, tin^{346}* with viable progeny, we identified 9 combinations with changes in EDD, 10 in ESD, 7 in FS, 12 in SI, 18 in tt10r (90% of SI length), 14 in HP, 13 in MAD_HP, and 10 with changed cardiac output (CO). In total, 41 deficiencies showed a deviation from one or more of the baseline parameters (68%; Figure 1, blue bars). For structural phenotypes (heart size and contractility (EDD, ESD, FS)), we found 15 deficiencies with significantly different values compared to baseline. One example is *Df(2L)BSC109*, a small deficiency on chromosome arm 2L (Figure 2A). When crossed to *pnr^{VX6}, tin^{346}*, this deficiency shows increased heart size (EDD) as well as longer contraction time (tt10r; Figure 2). We are currently testing a neighboring deficiency that also covers *Msp300* and test those deficiencies by themselves to account for the possibility that the phenotype is independent of *pnr^{VX6}, tin^{346}*. If the interaction is confirmed, we will order null alleles for *Msp300* and test again against *pnr^{VX6}, tin^{346}*, and if no interaction is found with this gene, we will continue with the other 4 genes covered by *BSC109*. This strategy will also be employed with all other hits of this screen (current and future).

With respect to the analysis of iPSC-derived cells, we obtained clones for both families (7H and 158H) from our collaborators at Mayo Clinic, Rochester. We are now in the process of generating the cells necessary for the subsequent experiments (cardiac progenitors and cardiomyocytes).

- **What opportunities for training and professional development has the project provided?**
  Nothing to report
- **How were the results disseminated to communities of interest?**

  Nothing to report

- **What do you plan to do during the next reporting period to accomplish the goals?**

  Like shown in the progress section, we will continue to identify loci that indicate interaction with pnr^{VX6}, tin^{346} and follow-up with detailed analysis for each locus. Since Tinman and Pannier are both cardiac transcription factors (cTFs), we will corroborate the most direct hypothesis of the basis of interaction, i.e., the interacting gene being a direct target of cTFs, by spatially resolved quantitative transcriptomics analysis: RNA probes for the candidate will be designed and heterozygous pnr^{VX6}, tin^{346} embryos will be stained by quantitative fluorescent in-situ hybridization. In parallel, we will build a gene network of cTFs together with identified interactors. For aim 2, we will expand cardiac progenitors and cardiomyocytes and screen as proposed and including newly identified targets from aim 1.

4. **IMPACT:**

   - **What was the impact on the development of the principal discipline(s) of the project?**

     Nothing to report

     What was the impact on other disciplines?

     Nothing to report

   - **What was the impact on technology transfer?**

     Nothing to report

   - **What was the impact on society beyond science and technology?**

     Nothing to report.

5. **CHANGES/PROBLEMS:**

   - **Changes in approach and reasons for change**
     Nothing to Report
   - **Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.**
     Nothing to Report
   - **Actual or anticipated problems or delays and actions or plans to resolve them**
     Nothing to Report
   - **Changes that had a significant impact on expenditures**
     Nothing to Report
   - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
     Nothing to Report
   - **Significant changes in use or care of human subjects**
     Nothing to Report

- **Significant changes in use or care of vertebrate animals.**
  Nothing to Report

- **Significant changes in use of biohazards and/or select agents**
  Nothing to Report

6. **PRODUCTS:**

   - **Publications, conference papers, and presentations**

     - **Journal publications.** Nothing to Report
     - **Books or other non-periodical, one-time publications.** Nothing to Report
     - **Other publications, conference papers, and presentations.** Nothing to Report

   - **Website(s) or other Internet site(s)**
     Nothing to Report

   - **Technologies or techniques**
     Nothing to Report

   - **Inventions, patent applications, and/or licenses**
     Nothing to Report

   - **Other Products**
     Nothing to Report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

   - **What individuals have worked on the project?**

Name:	Georg Vogler
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-8303-3531
Nearest person month worked:	5
Contribution to Project:	Dr. Vogler has designed the experiments, guided data acquisition and troubleshooting, and performed data analysis.
Funding Support:	N/A

Name:	Marco Tamayo
Project Role:	Lab Coordinator
Researcher Identifier (e.g. ORCID ID):	0000-0001-9891-0755
Nearest person month worked:	3

Contribution to Project:	Mr. Tamayo obtained and is maintaining all necessary fly stocks, performs fly husbandry, and data acquisition.
Funding Support:	N/A

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?** See below

Name of Individual: Vogler, Georg
Current Appointments: 09/2007 Research Assistant Professor

CURRENT

This award

Title: Using Systems Genetics to Probe for Gene Interactions in Congenital Heart Disease

Major Goals: The goal of this application is to establish an experimental paradigm that uses Systems Genetics to study the oligogenic basis of congenital heart disease (such as Hypoplastic Left Heart Syndrome (HLHS)), employing complementary models (Drosophila adult in vivo hearts and cardiomyocytes derived from human induced pluripotent stem cells, hiPSC-CMs), in conjunction with genomics data from HLHS patients.

Specific Aims:
1. High-throughput screen for genetic interactors of cardiac determinants.
2. High-throughput screen for genetic modifiers of BAV/HLHS.

Project Number: W81XWH-21-1-0104
Name of PD/PI: Vogler, G.
Source of Support: Department of the Army
Project Performance Period: 02/2021 - 02/2023
Total Award Amount:

Time Commitment per Budget Period:

Year	Person Months
1. 2021	2.40 calendar months
2. 2022	2.40 calendar months

Grants Management Officer: Rahul G. Thakar,, rahul.g.thakar.ctr@mail.mil
Overlap: None

New Award

Title: Genetic Control of Cardiac Development: Congenital Heart Disease Gene Discovery in Drosophila

Major Goals: The overall goal is to use our heart assays as an efficient throughput in vivo discovery tool for systematically identifying the ~90% as of yet unknown CHD genes.

Specific Aims:
1. Define and functionally test cardiogenic gene regulatory networks identified by scRNAseq of embryonic Drosophila heart cells.
2. Determine the cardiogenic function of CHD gene candidates identified from patient derived WGS data, focusing on ribosomal protein genes (RpL13) and MICOS complex genes.

Project Number: R01 HL054732
Name of PD/PI: Bodmer, Rolf
Source of Support: NIH/NHLBI
Project Performance Period: 07/2021 – 06/20205
Total Award Amount:

Time Commitment per Budget Period:

Year	Person Months
1. 2021	2.40 calendar months
2. 2022	2.40 calendar months
3. 2023	2.40 calendar months
4. 2024	2.40 calendar months

5. 2024	2.40 calendar months

Grants Management Officer: E-Bijan Elaya Cox, e-bijan.cox@nih.gov,
Overlap: None

Name of Individual: Colas, Alexandre
Current Appointments: 01/2008 Assistant Professor

CURRENT

This award

Title: Using Systems Genetics to Probe for Gene Interactions in Congenital Heart Disease

Major Goals: The goal of this application is to establish an experimental paradigm that uses Systems Genetics to study the oligogenic basis of congenital heart disease (such as Hypoplastic Left Heart Syndrome (HLHS)), employing complementary models (Drosophila adult in vivo hearts and cardiomyocytes derived from human induced pluripotent stem cells, hiPSC-CMs), in conjunction with genomics data from HLHS patients.

Specific Aims:
3. High-throughput screen for genetic interactors of cardiac determinants.
4. High-throughput screen for genetic modifiers of BAV/HLHS.

Project Number: W81XWH-21-1-0104
Name of PD/PI: Vogler, G.
Source of Support: Department of the Army
Project Performance Period: 02/2021 - 02/2023
Total Award Amount:

Time Commitment per Budget Period:

Year	Person Months
1. 2021	0.60 calendar months
2. 2022	0.60 calendar months

Grants Management Officer: Rahul G. Thakar, , rahul.g.thakar.ctr@mail.mil
Overlap: None

New Award

Title: Genetic Control of Cardiac Development: Congenital Heart Disease Gene Discovery in Drosophila

Major Goals: The overall goal is to use our heart assays as an efficient throughput in vivo discovery tool for systematically identifying the ~90% as of yet unknown CHD genes.

Specific Aims:
3. Define and functionally test cardiogenic gene regulatory networks identified by scRNAseq of embryonic Drosophila heart cells.
4. Determine the cardiogenic function of CHD gene candidates identified from patient derived WGS data, focusing on ribosomal protein genes (RpL13) and MICOS complex genes.

Project Number: R01 HL054732
Name of PD/PI: Bodmer, Rolf
Source of Support: NIH/NHLBI
Project Performance Period: 07/2021 – 06/2025
Total Award Amount:

Time Commitment per Budget Period:

Year	Person Months
1. 2021	0.60 calendar months
2. 2022	0.60 calendar months
3. 2023	0.60 calendar months
4. 2024	0.60 calendar months
5. 2024	0.60 calendar months

Grants Management Officer: E-Bijan Elaya Cox, e-bijan.cox@nih.gov, None
Overlap:

New Award

Title: Discovery of Small Molecule Regulators of Atrial Cardiomyocyte Action Potential Duration to Restore Normal Cardiac Rhythm in Atrial Fibrillation

Major Goals: The goal of this proposal is to identify or create chemical biology tools and starting points for therapeutics that will modulate the proteins specifically disrupting the synchronized, regular electrical conductance of human atrial cardiomyocytes derived from stem cells.

Specific Aims:
1. Perform HTS screening with a large chemical library using cardiomyocyte rhythm assay.
2. ADME and SAR characterization of candidate probes.
3. Identify pathways regulated by cardiomyocyte rhythm probes.

Project Number: R01 HL153645
Name of PD/PI: Colas, A.
Source of Support: NIH/NHLBI
Project Performance Period: 06/2021 – 06/2025
Total Award Amount:

Time Commitment per Budget Period:

Year	Person Months
1. 2021	2.40 calendar months
2. 2022	2.40 calendar months
3. 2023	2.40 calendar months
4. 2024	2.40 calendar months

Grants Management Officer: Ravi C. Balijepalli ravi.balijepalli@nih.gov
Overlap: None

Title: *Discovery of Small Molecule Promoters of Cardiomyocyte Proliferation to Restore*

Major Goals: To identify compounds that can stimulate endogenous cardiomyocyte proliferation through use of a high throughput screen of a large chemical library against human cardiomyocytes matured from pluripotent stem cells; to explore the activity of these chemical probes in cellular systems to provide insight into the biology of this important process; and to advance our long-term goal of developing a disease modifying treatment for cardiovascular diseases.

Specific Aims:
1. Perform HTS screening with a large chemical library using cardiomyocyte proliferation assay.
2. Perform hit confirmation, validation, characterization, and probe identification.
3. ADME and SAR characterization of candidate probes.
4. Identify pathways regulated by cardiomyocyte proliferation probes.

Project Number: R01 HL148827
Name of PD/PI: Colas, A.
Source of Support: NIH/NHLBI
Project Performance Period: 06/2019 – 04/2023
Total Award Amount:

Time Commitment per Budget Period:

Year	Person Months
3. 2021	3.00 calendar months
4. 2022	3.00 calendar months

Grants Management Officer: Karen Brummett, brummettk@gmail.nih.gov
Overlap: None

New Award

Title: *Discovery of Small Molecule Promoters of Cardiomyocyte Proliferation to Restore Cardiac Performance in Disease*

Major Goals: (1) to identify prototype drugs (i.e. compounds / probes) that can stimulate endogenous cardiomyocyte proliferation through use of a high throughput

screen of a large chemical library against human cardiomyocytes matured from pluripotent stem cells; (2) to explore the activity of these chemical probes in cellular systems to provide insight into the biology of this important process; and (3) to advance our long-term goal of developing a disease modifying treatment for cardiovascular diseases.

Specific Aims:
1. Perform HTS screening with a large chemical library using cardiomyocyte proliferation assay.
2. Perform hit confirmation, validation, characterization, and probe identification.
3. ADME and SAR characterization of candidate probes.
4. Identify pathways regulated by cardiomyocyte proliferation probes.

Project Number: R01 HL148827S1
Name of PD/PI: Colas, A.
Source of Support: NIH/NHLBI
Project Performance Period: 02/2021 – 04/2023
Total Award Amount:

Time Commitment per Budget Period:

Year	Person Months
2. 2021	0.00 calendar months
3. 2022	0.00 calendar months

Grants Management Officer: Kristen Williams, , Kristen.williams@nih.gov
Overlap: None

Title: Genetic Pathways in Ceramide-Associated Lipotoxic Cardiomyopathy and Heart Failure

Major Goals: In this proposal, we will examine the role of ceramide-interacting proteins in modulating sarcomeric structure in ceramide/HFD-induced LCM in the *Drosophila* heart and in hiPSC-CMs and examine the role of ceramide-interacting proteins in modulating metabolic components in ceramide/HFD-induced LCM in the *Drosophila* heart and in hiPSC-CMs

Specific Aims:
1. Role of Ceramide-Interacting Proteins in Modulating Sarcomeric Structure in ceramide/HFD-induced LCM in the *Drosophila* heart and in hiPSC-CMs.
2. Role of -Interacting Proteins in Modulating Metabolic Components in Ceramide/HFD-Induced LCM in the *Drosophila* heart and in hiPSC-CMs.
3. Role of Ceramide-Interacting Proteins in Modulating Apoptosis and DNA Damage.

Project Number: R01 HL149992
Name of PD/PI: Bodmer, R
Source of Support: NIH/NHLBI
Project Performance Period: 12/2019-11/2023
Total Award Amount:

Time Commitment per Budget Period:

Year	Person Months
3. 2021	1.20 calendar months
4. 2022	1.20 calendar months

Grants Management Officer: Renee Wong; Phone: Email: wongr2@nhlbi.nih.gov None
Overlap:

- **What other organizations were involved as partners?**
  - **Organization Name: Mayo Clinic**
  - **Location of Organization:** Rochester, MN
  - **Partner's contribution to the project**

- **Other.** Mayo Clinic provided de-identified iPSCs from fibroblasts of donor tissues of two patient/parent trios (7H and 158H).

8. **SPECIAL REPORTING REQUIREMENTS**
   Nothing to Report

9. **APPENDICES:** None