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)

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5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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14. ABSTRACT
In this project we tried 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 planned to use 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 and two, we identified ~163 regions in the fly genome that display either synthetic lethality or cardiac phenotypes in conjunction with tinman/pannier with about 80% 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. Surprisingly, we found many loci that ameliorated the tin/pnr double-heterozygous phenotypes in transheterozygous condition. The next steps are to follow up with identification of 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 will process these cells for mass-screening using our collaborator's 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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Unclassified	Unclassified	Unclassified	Unclassified	16	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

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	81
Mounting and Filming	4-9	Dr. Vogler	81
Analysis of movies	9-10	Dr. Vogler	81
Prioritization of candidate genes from deficiency hits	10-11	Dr. Vogler	81
Identification of specific interactors within deficiencies			
Ordering of fly stocks	12-24	Dr. Vogler	15
Candidate gene crosses	12-23	Dr. Vogler	15
Mounting and Filming	12-23	Dr. Vogler	10
Analysis of movies	12-23	Dr. Vogler	10
Confirmatory experiments (qPCR, RNAseq)	12-23	Dr. Vogler	
Milestone(s) Achieved: identified specific interactors	24	Dr. Vogler	10
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?**

In continuation of aim 1, we completed to probe the *Drosophila* genome for loci that are critical for heart development and function (aim 1) using a screen for deficiencies (deletions in the genome that remove a defined set of genes) to identify loci which, when placed *in trans* to a *tinman/pannier* double mutant condition, would alter the mild cardiac phenotype of *tin/pnr* alone (see previous technical report). We have crossed a total of 384 deficiency to flies that carry pnr^{VX6}, tin^{346} (loss-of-function alleles for *pannier* and *tinman*), covering all four chromosomes (12028 genes) and evaluated all deficiencies for phenotypes (Figure 1).

Among the deficiencies tested, we identified a total of 16 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. As expected, these deficiencies included those that cover the *tin* and *pnr* loci, rendering these crosses as lethal due to non-complementation.

As previously reported, 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} showed deviation of one or more heart

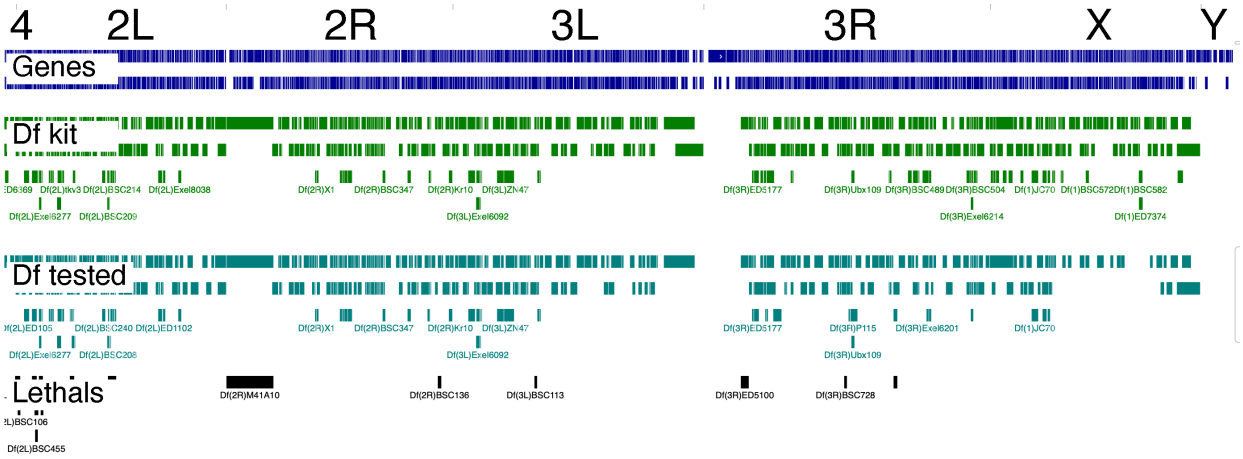

Figure 1. Map of the Drosophila genome and fly screen progress. Chromosomes and chromosome arms are shown on top. Progress on deficiency (Df) crosses is measured as Dfs tested (384) / all Dfs (BDSC Df kit). Dfs that cause synthetic lethality are shown in black.

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.

Following data processing of high-speed recordings of all tested deficiencies we analyzed the data for statistical differences of each deficiency in any of the 32 cardiac parameters. To this aim we performed pairwise Wilcoxon tests of each deficiency against all control crosses. This approach ensured robust testing due to the

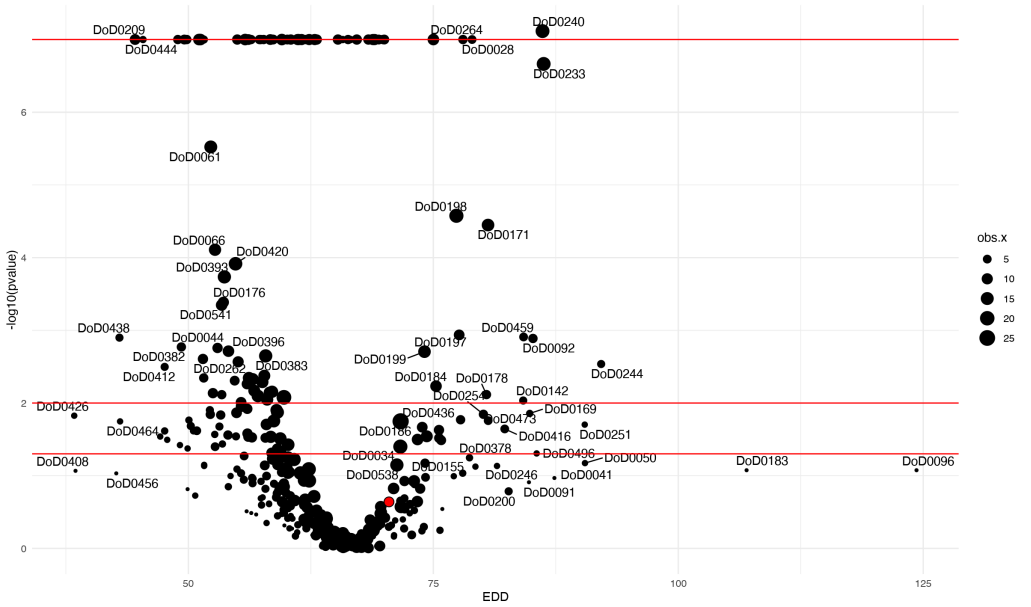

Figure 2. Volcano plot of all end-diastolic diameters (EDD) of all deficiencies tested. This screen identified genomic regions that altered heart size during relaxation (EDD) from baseline in *pnr*/*tin* double mutants. Interestingly, there are as many loci that further decrease heart size (synergism), as there are loci that increase heart size ('rescue'). Red lines indicate significance (p=0.05, p=0.01, in log-scale). Diameters are in micrometers.

conservative nature of the Wilcoxon ranked test compared to Student's t-test, as well as by pooling all controls the baseline measurements are more accurately represented. All raw p-values were adjusted for multiple-testing.

297 deficiencies crossed to pnr^{VX6}, tin^{346} had viable and robust progeny (i.e., with enough heart recordings). Several crosses had sufficient progenies (> 10 flies), but defective hearts that could not be measured automatically due to structural defects. Among the 297 crosses, we identified 28 combinations with changes in EDD (Fig. 2), 19 in ESD, 5 in FS, 30 in SI, 25 in tt10r (90% of SI length), 63 in HP, 32 in MAD_HP, and 39 with changed cardiac output (CO). In total, 163 deficiencies showed a deviation from one or more of the baseline parameters (42%). For end-diastolic diameters, we find many deficiencies that caused an increase in diameter compared to *tin*, *pnr* double-heterozygotes that are constricted compared to wild type hearts indicating that loci exist that can reverse the cardiac defect found in *tin*, *pnr* double-heterozygotes and could serve as potential targets to ameliorate cardiac injury. Going forward we will focus on identifying those loci and pathways that can

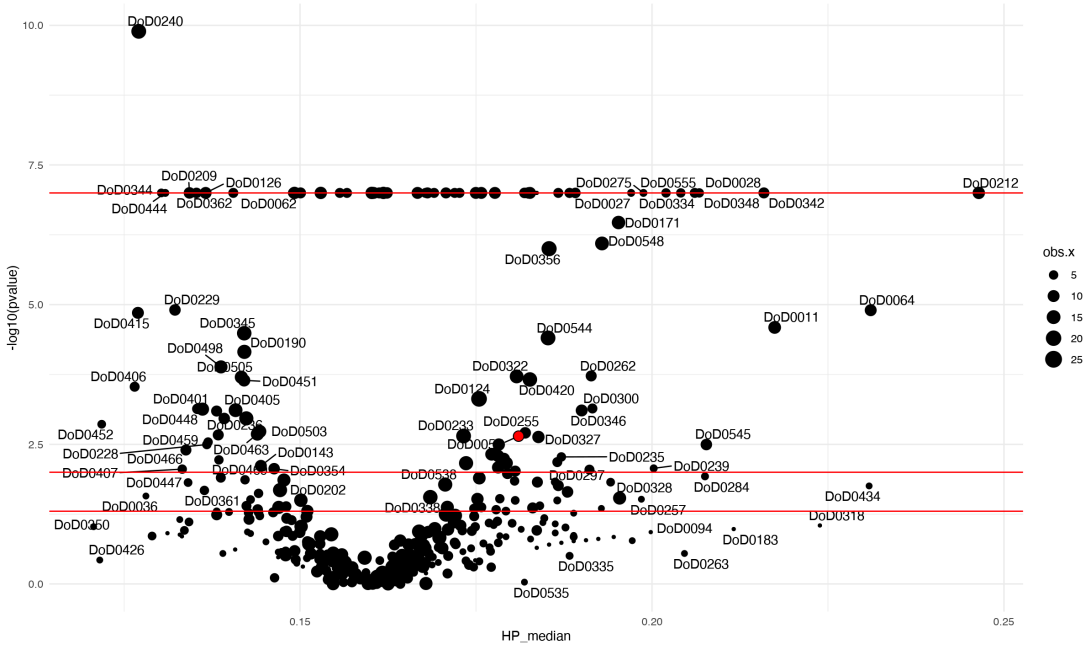

Figure 3. Volcano plot of deficiencies with reduced or prolonged heart periods (HP_median).

rescue the *tin*, *pnr* deficiency. The most prominent phenotype with 63/163 deficiencies among all tested parameters was median heart-period (HP_median, Fig. 3), indicating that heart rate is most sensitive to genetic backgrounds compared to structural parameters (e.g., EDD, Fig. 2). Overall, this screen resulted in a large set of loci (n=163) that are likely genetically linked to the cardiac TFs *tin*/*pnr* in adult *Drosophila* hearts. Follow-up studies include testing each deficiency by itself to identify synergistic interactors versus genetic interactors, and prioritization of genetically interacting deficiencies to identify the interacting genes within each deficiency.

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 still in the process of generating the cells necessary for the subsequent experiments (cardiac progenitors and cardiomyocytes).

During the extension period we focused on two aspects: (1) to further characterize the extent to which deficiencies could modify the baseline phenotype (Fig. 4) and (2) to take a first step towards identifying a modifier gene within a candidate deficiency.

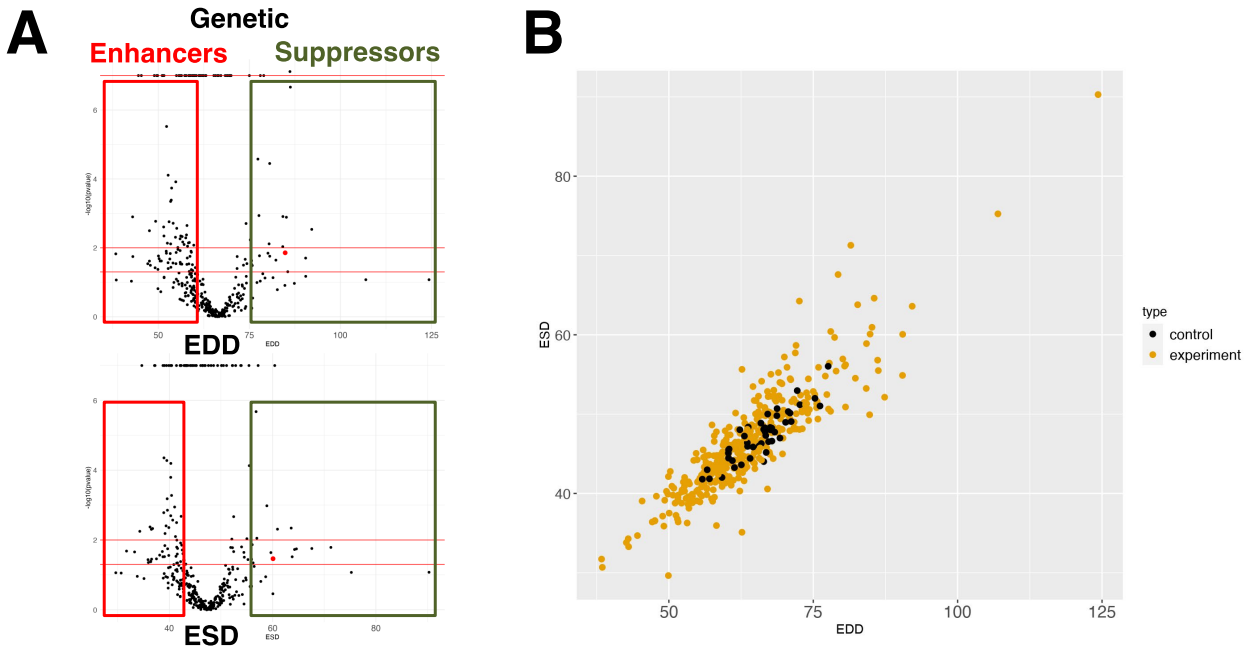

Figure 4. Deficiencies that alter *tin/pnr* baseline phenotypes. (A) End-diastolic and end-systolic diameters (EDD, ESD, resp.) can be significantly altered by genomic deletions in the background of *tin/pnr* heterozygotes. Similar to heart period (Fig.3), the constricted heart diameters of the sensitizer line can be further decreased ('enhanced', red) or increased ('suppressed', green) in the presences of a deficiency. **(B)** Scatter-plot showing the phenotypic range of controls and their enhancement/suppression by deficiency lines.

Candidate deficiency *Df(2L)ED334* is a 341kb-spanning deletion line containing 64 gene loci. Among these, the G-protein coupled receptor (GPCR) *Trissin receptor* (*TrissinR)* stands out as it has previously been reported to induce elevated Ca++ levels in vitro via its binding peptide Trissin (Ida et al., 2011). TrissinR is widely expressed in the adult fly heart (our contribution, see Fly Cell Atlas), and we have therefore obtained RNAi and Gal4 lines that would allow to target cardiac TrissinR, as well as the upstream neuronal circuit (peptidergic neurons expressing Trissin peptide). A first RNAi line (obtained from BDSC, Bloomington Drosophila Stock Center) did not show a phenotype, and we are currently obtaining two additional, likely more potent RNAi lines from VDRC (Vienna Drosophila Resource Center) to corroborate a link between *TrissinR* and heart period.

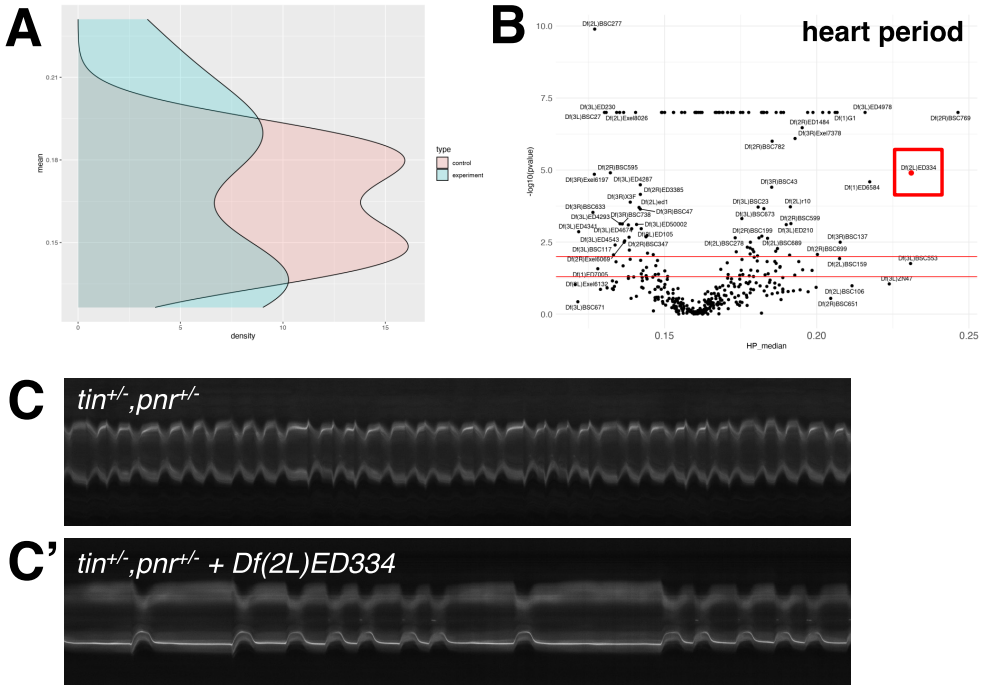

Figure 5. Deficiency *ED334* prolongs heart period (HP). (A) Density-plot showing a biomodal distribution of mean heart period for ED334 in tin/pnr background compared to controls. **(B)** Candidate screen deficiency (original genotype, non-blinded; compare to Fig.3) showing the overall spread of HP phenotypes. **(C)** M-modes of beating fly hearts in sensitized control background (*tin/pnr*) vs. combination with candidate deficiency.

- **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?**
  Nothing to report (end of grant).

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:	4.5
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:	2.1
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?**

CHANGES IN ACTIVE OTHER SUPPORT

Name of Individual: Vogler, Georg

Current Appointments: 09/2007 Research Assistant Professor

CURRENT

NEW AWARD

Title: Atrial Fibrillation Gene Networks Underlying Electrical Remodeling and Arrhythmia

Major Goals: We propose to identify AF specific gene networks that will provide more specificity and potential treatment targets. The proposed research will apply a new, integrated multi-model systems approach with unprecedented throughput (including with age) to discover conserved genetic regulators contributing to the most prevalent form of arrhythmia in humans, AF.

Specific Aims: 1. Define age-dependent genetic interactions in a KCNA5/Sh-centric gene network.
2. Identification of Transcription Factor (TF)-mediated regulation of cardiac rhythm (APD, SI, AI).
3. Identification of Gene Regulatory Networks (GRN) with validation in fly and zebrafish.

Project Number: SBP MPI 2023
Name of PD/PI: Ocorr, Karen
Source of Support: Sanford Burnham Prebys Medical Discovery Institute
Project Performance Period: 09/2023 – 08/2024
Total Award Amount (including Indirect Costs):
Time Commitment per Budget Period:

YEAR (YYYY)	Person Months (##.##)
1. 2024	0.30 calendar months

Grants Management Officer: N/A
Overlap: None

THIS AWARD - CLOSED

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, Georg
Source of Support: Department of the Army
Project Performance Period: 02/2021 – 08/2023
Total Award Amount (including Indirect Costs):
Time Commitment per Budget Period:

YEAR (YYYY)	Person Months (##.##)
1. 2022	2.40 calendar months
2. 2023	2.40 calendar months

Grants Management Officer: Rahul G. Thakar, 301-619-4500, rahul.g.thakar.ctr@mail.mil
Overlap: None

Name of Individual: Colas, Alexandre

Current Appointments: 09/2023 Associate Professor

CURRENT

NEW AWARD

Title: Development of a Novel Cell Painting Assay to Morphologically and Temporally Profile Human Oncogenic Transformation and Identify Novel Regulatory Pathways
Major Goals: To develop of a new phenotypic assay to morphologically and temporally profile human oncogenic transformation and identify novel regulatory pathways.
Project Number: P30 CA030199 (Pilot Project)
Name of PD/PI: Colas, Alexandre

Source of Support: NIH/NCI
Project Performance Period: 10/2022 – 12/2023 (NCE)
Total Award Amount (Including Indirect Costs):
Time Commitment per Budget Period:

Year	Person Months
1. 2023	0.00 calendar months

Grants Management Officer: Ethan Meek; ethan.meek@nih.gov
Overlap: None

NEW AWARD

Title: Characterization of the role of Fstl1 during cardiac reprogramming in human cardiac fibroblasts
Major Goals: To test synergistic effect of FSTL1.37 with AJSZ KD on reprogramming efficiency.
Project Number: CSRA 23-004
Name of PD/PI: Colas, Alexandre
Source of Support: Regencor, Inc.
Project Performance Period: 11/2023 – 03/2024
Total Award Amount (Including Indirect Costs):
Time Commitment per Budget Period:

Year	Person Months
1. 2024	0.40 calendar months

Grants Management Officer: Sean Edwards, sean@regencor.com
Overlap: None

NEW AWARD

Title: Characterization of the Role of Proteoglycan Sulfation as Barrier to Cardiac Reprogramming
Major Goals: Using Chst7 as an entry point, the proposed work aims at gaining insight on how PG sulfation oppose cell fate reprogramming in differentiated cells.
Specific Aims: 1. To build a transgenic cell line enabling to determine Chst7 subcellular expression and to identify Chst7-interacting proteins during CR.
2. To identify barrier core protein(s) sulfated by Chst7 during CR.
Project Number: SBP PD 2023
Name of PD/PI: Colas, Alexandre
Source of Support: Sanford Burnham Prebys Medical Discovery Institute
Project Performance Period: 09/2023 – 08/2024
Total Award Amount (Including Indirect Costs):
Time Commitment per Budget Period:

Year	Person Months
1. 2024	0.36 calendar months

Grants Management Officer: N/A
Overlap: None

THIS AWARD - CLOSED

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, Georg

Source of Support: Department of the Army

Project Performance Period: 02/2021 - 08/2023 (NCE)

Total Award Amount (including Indirect Costs):

Time Commitment per Budget Period:

Year	Person Months
1. 2022	0.60 calendar months
2. 2023	0.60 calendar months

Grants Management Officer: Rahul G. Thakar; 301-619-4500, rahul.g.thakar.ctr@mail.mil

Overlap: None

CLOSED

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

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, Alexandre

Source of Support: NIH/NHLBI

Project Performance Period: 06/2019 – 10/2023 (NCE)

Total Award Amount (including Indirect Costs):

Time Commitment per Budget Period:

Year	Person Months
1. 2020	3.00 calendar months
2. 2021	3.00 calendar months

3. 2022	3.00 calendar months
4. 2023	3.00 calendar months

Grants Management Officer: Karen Brummett, 301-594-6268, brummettk@gmail.nih.gov
Overlap: None

CLOSED

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, Alexandre
Source of Support: NIH/NHLBI
Project Performance Period: 02/2021 – 04/2023
Total Award Amount (including Indirect Costs):

Time Commitment per Budget Period:

Year	Person Months
1. 2022	0.00 calendar months
2. 2023	0.00 calendar months

Grants Management Officer: Kristen Williams, 301-827-5513, Kristen.williams@nih.gov
Overlap: None

- **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:** See Award Expiration Transition Plan attached below.

Transition Plan Questionnaire

Directions: Please answer all questions that apply for each product under development. Please fill out one document per product.
This is not an application for funding; however, answers will help us understand the outcomes and products from your award.

1. After the award closes, would you be willing to periodically provide voluntary information (via email) regarding the project status (i.e. where the research is headed)? **Yes** ☑ or **No** ☐
These responses will help CDMRP demonstrate the return on its investments and will help demonstrate that the CDMRP is a responsible and successful steward of federal research funding.

2. What **conclusion(s)** does your final data support?
-Genetic interaction screening is a viable methodology to uncover potential cardiac disease genes.
- Genetic deficiencies (reduced gene dosage) could alleviate a phenotype.

3. Will you/have you applied for/obtained follow-on-funding for this project? **If yes**, please list (a) funding organization, (b) total budget requested/obtained, and (c) title of the funded proposal. *This information will be recorded as an outcome to this award*.
Yes. We will apply for federal funding (NHLBI) and private funding (AHA) to study the potential role of Trissin-Receptor function in the heart. We are planning to identify additional candidate genes from the screen, link their expression to tinman/pannier to connect TF-circuitry with organ function.

4. What will be **the next step(s)** for this project?
Prioritized deficiencies will be characterized in detail to identified tinman/pannier mediators. Data will be integrated in ongoing single-cell sequencing project.

5. How would you classify your **lead candidate product**? d
(a) Therapeutic (Small Molecule, Biologic, Cell/Gene Therapy): Please choose, if applicable
(b) Diagnostic
(c) Device
(d) Research Tool to Address a Research Bottleneck
(e) Knowledge Product (Non-material product such as a compound library, database, something that improves clinical practice, education, etc.)
(f) Other - Please Specify:

6. How does your candidate product aid the Warfighter, Veteran, Beneficiary, and/or General Population?
Basic research of heart genetics is a necesscity to interpret potential disease genes and their disease mechanisms. This work is intended to provide such information.

7. Therapy / Product Development, Transition Strategies, and Intellectual Property

Describe the steps and relevant strategies required to move the candidate product (knowledge or tangible) to the next phase of development and/or commercialization. Please address any issues with intellectual property.

PIs are encouraged to explore the technical requirements and the current regulatory strategies involved in product development as well as to work with their organization's Technology Transfer Office (or equivalent regulatory/legal office), federal/international regulatory experts, to develop the transition plan and to explore developing relationships with industry, DoD advanced developers (e.g. USAMMDA), and/or other funding agencies to facilitate moving the product into the next phase.

N/A