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TITLE: Understanding Cardiovascular Disease in Mental Health/Stress Disorder

PRINCIPAL INVESTIGATOR: Prasanna Krishnamurthy

CONTRACTING ORGANIZATION: University of Alabama, Birmingham, AL

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Fort Detrick, Maryland 21702-5012

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Mental health disorder increases risk for cardiovascular disease (CVD). However, the mechanisms are poorly understood.
Purpose: Our goal is to investigate the cause-effect relationship between comorbid depression and cardiac pathology and dysfunction.
Scope: To test the hypothesis that depression alters neurotrophins and receptor interactions leading to cardiac pathophysiological changes and dysfunction.
In this reporting period (May 1, 2023 to April 30, 2024), we have obtained UAB-IACUC and UAB-Environmental Health and Safety approvals and subsequently obtained OHARO approval. We have procured breeder mouse, $p75^{NTR}$ global knockout mice from Jackson laboratory and $p75^{NTR}$ flox/flox mice from Indiana University. We are in the process of breeding and expanding the mouse colonies to obtain a critical number of mice to test our hypotheses. Due to technical challenges in obtaining homozygous knockout mice (described in section 5 of this report), we are continuing to breed the mice and we have drawn several contingency plans. Next, we performed initial set of in vitro experiments in cell culture system to determine the effect of loss of $P75^{NTR}$ on cell death. The siRNA transfections (to delete p75NTR) did not reveal efficient knockdown. Therefore, alternatively, we are preparing plasmid for CRISPR-Cas9 method of gene deletion and testing the effect p75NTR loss on cell death and proliferation. These steps will ensure preparation for experiments proposed in the next reporting period.

15. SUBJECT TERMS

P75NTR- p75 Neurotrophin Growth Factor Receptor
siRNA- small interfering RNA

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
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1. INTRODUCTION:

Mental health disorders such as major depressive disorder (MDD), psychosis, PTSD etc., are serious public health problems that has been shown to increase the risk for cardiovascular disease (CVD). However, the molecular mechanisms are poorly understood. The purpose of this study is to investigate the cause-effect relationship between comorbid depression and cardiac pathology and dysfunction.
Scope of the study: To test the hypothesis that depression alters neurotrophins and receptor interactions leading to cardiac pathophysiological changes and dysfunction. Three aims will be tested- 1) To determine the role of p75NTR signaling in cardiac homeostasis during depression; 2) To assess the effect of p75NTR on depression-associated microvascular dysfunction in the myocardium, and 3) To investigate the functional relevance of p75NTR in progression of myocardial pathophysiological changes in comorbid depression.

2. KEYWORDS:

CVD: Cardiovascular disease
P75NTR: p75 Neurotrophin Growth Factor Receptor
BDNF: Brain Derived Neurotrophic Factor
KO: Knockout
CRS: chronic restraint stress

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Determination of the role of proneurotrophin and p75 neurotrophin growth factor receptor ($p75^{NTR}$) interaction on cardiac homeostasis during depression	**Timeline** (Months)	**Status**
Major Task 1 ***Key hypothesis to be tested:*** *myocardial $p75^{NTR}$ activation during depression mediates cardiac pathophysiological alterations.*		
Subtask 1 – Obtain Institutional animal use and biosafety approvals • *We will obtain Institutional Animal Care and Use Committee (IACUC) approval for breeding and experimental use of mouse*. The process involves preparation and submission of the protocol, initial review and submission of modified protocol, Committee review, address queries and submission of modified protocol and final approval. • *Obtain regulatory and biosafety approval from UAB division of Environmental Health and Safety (EHS).* The process involves preparation and submission of Project Registration to EHS, addressing queries and resubmission of modified registration and Final approval. EHS	1-3 months	100% Completed (IACUC Date completed: 03-14-23) (EHS Date completed: 03-15-23)

project registration is required for IACUC approval.		
Subtask 2: Submit IACUC-approved documents to the Office of Human and Animal Research Oversight (OHARO) for review and approval.	2-3 months	100% Completed (Approved date: 03-16-2023)
Subtask 3: Breeding and expansion of global $p75^{NTR}$ knockout (KO) mice colony. • We will procure four $p75^{NTR}$ KO mice (from Jackson laboratory; Strain # 002213), which is homozygous for $p75^{NTR}$ (also called NGFR, Nerve Growth Factor Receptor). We will breed and expand the colony until we achieve a minimum of 20 mice (10-males and 10-females) in each experimental group (depression vs control; described in Subtask 6). *Note: Jackson Lab delivery of live mice takes 12-weeks from ordering. Also, breeding will continue to produce sufficient mouse for experimental use in Major Task 3.*	4-12 months	50% Completed Live $p75^{NTR}$ KO mice are derived from cryopreserved embryos. The procurement from Jackson laboratory has been completed. (Mouse order placed: 06/22/23 Mouse delivery date: 09/11/23). Since delivery of the breeder mice, we have placed KO mice for breeding to obtain enough mice for experimentation. After several rounds of breeding, we faced difficulty with obtaining homozygous KO mice. The challenges and alternative strategies are described under "accomplishments section" below. We anticipate that this subtask might be delayed by another 2-3 months.
Subtask 4 (Alternative approach): Generation of cardiac-specific $p75^{NTR}$ KO mice. • We will procure four $p75^{NTR}$ flox mice (from Jackson laboratory; Strain #031162), which possesses loxP sites that will allow for tissue-specific deletion of gene (cardiac tissue in our case). Cardiac-specific $p75^{NTR}$ knockout (KO) mice will be generated by crossing $p75^{NTR}$ flox mice with alpha myosin heavy chain (α-MyHC-cre) mice (already available in the lab). We will breed and expand the colony until we achieve a minimum of 20 mice (10-males and 10-females) in each experimental group (depression vs control; described in Subtask 6). *Note: Although global $p75^{NTR}$ KO mice are fully viable and have normal life span, some studies have reported impaired sympathetic innervation density, which may/may not affect their breeding pattern. As mentioned in our proposal, if this is the case, we will alternatively generate cardiac-specific $p75^{NTR}$ KO mice.*	4-12 months	50% Completed <u>We took two approaches: Because the commercially available mice are on a mixed genetic background, we also procured p75NTR-flox/flox mice from the original inventory, who possesses these mice on C57BL/6 background.</u> **1. Procurement of NGFR-flox ($p75^{NTR}$ flox/flox) from Indiana University** • Material Transfer agreement (MTA) approved: June, 2023 • Took 4 months (September 2023) to receive the mice from Indiana University (due to weather and sipping logistics). We are currently expanding the breeder mouse colony. **2. Procurement of NGFR-flox ($p75^{NTR}$ flox/flox) from Jackson Laboratory** • We also procured NGFR-flox mouse on a mixed background (C57BL/6/129S) from Jackson Labs. We are currently

		backcrossing them for atleast 10 generations to get a pure line on C57BL/6 background for future studies. a. Order placed: 10/09/23, cryo-recovery b. Mice received: 01/08/24 (6-weeks old). We anticipate that this subtask might be delayed by another 2-3 months.
Subtask 5: In vitro evaluation of $p75^{NTR}$ role in fibroblasts and cardiomyocyte function • Using either Viral or Plasmid vectors, $p75^{NTR}$ will be either overexpressed (gain-of-function) or inhibited (loss-of-function) in mouse ventricular myocytes and primary human cardiac fibroblasts (Lonza). The cell response to recombinant proBDNF (Sigma Aldrich, MO) will be tested. Cell death, cell size, fibroblast cell proliferation and expression of hypertrophic markers and fibrosis-related markers will be evaluated by qRT-PCR and western blotting.	4-12 months	25% Completed We first performed loss-of-function studies using RNA interference against NGFR gene ($p75^{NTR}$). We are first testing transfection efficiency in two different easy to grow cell lines- mouse cardiac endothelial cell line (MCEC) and RAW 264.7 cells (mouse macrophage cell lines). However, we did not observe a significant knockdown of NGFR using this approach. Therefore, as an alternative strategy, we plan to knockout $p75^{NTR}$ in the cells using CRISPR-Cas9 system and additional siRNA/viral vectors. We have already designed plasmids for CRISPR-Cas9 experiments. We are in the process of propagating and extracting desired quantity of plasmids for treating cardiomyocytes and fibroblasts. Due to challenges in mice breeding and expansion of colonies (in subtask 3 and 4) and due to poor transfection efficiency of siRNA in subtask 5, we anticipate that this subtask might be delayed by another 2-3 months due to the above challenges.
Subtask 6: To test the hypothesize that $p75^{NTR}$ deficiency (KO) in mice prevents cardiac dysfunction and adverse structural remodeling in mice subjected to major depressive disorder (MDD). • Wild-type (WT) mouse and global $p75^{NTR}$ KO mouse will be subjected to chronic restraint stress (CRS)-induced depression. Another set of mice that will not be subjected to depression will serve as control group. A total of 20 mice (10	13-16 months	Experiments have not been started yet.

male+10 female) will be used for each group. • To confirm depression-like behavioral changes, we will assess behavioral changes using forced swim test, open field test, sucrose preference test, and elevated plus maze test, first at baseline, and at 16 days after completion of CRS protocol. We will measure Brain-Derived Neurotrophic Factor (BDNF) in the hippocampus of the brain. • We will perform echocardiography to measure left ventricular (LV) cardiac function, at baseline and at 7 days and 16 days after CRS protocol. The captured data will be used to analyze heart function parameters such as percent fractional shortening (%FS), Ejection fraction (EF%) and structural remodeling such as LV chamber size and LV wall thickness. • The mice will be euthanized at 16 days after CRS, and heart tissue will be collected for RNA-sequencing, histology, gene and protein expression analyses (specified in Subtask 7). • *Statistical analyses:* The data from behavioral studies and echocardiography, will be analyzed by unpaired, two-tailed students t-test (heteroskedastic correction for unequal variance). Two-way ANOVA with appropriate post-hoc testing will be used to compare more than two groups and time series experiments. P values of <0.05 will be considered statistically significant.		
*Please note that the SOW beyond 16 weeks is excluded in this report. Report for SOW beyond 13 weeks will be included in the next annual report.		

What was accomplished under these goals?

Major activity: To determine if loss of $p75^{NTR}$ affects depression-induced cardiac pathology. Our first objective is to generate $p75^{NTR}$ knockout mouse.

Our accomplishments:

1. **Subtask 1.** Obtained IACUC approval on 03/14/2023
2. **Subtask 1.** Obtained *UAB Environmental Health and Safety (EHS) approval on 3/15/2023*
3. **Subtask 2.** Submitted IACUC-approved documents to the Office of Human and Animal Research Oversight (OHARO) for review and approval was obtained on 03-16-2023.
4. **Subtask 3.** Live $p75^{NTR}$ KO mice were procured from Jackson laboratory (on 9/11/2023). Since delivery of the breeder mice, we have placed KO mice for breeding to obtain enough mice for experimentation. Timeline of event, breeding pattern and challenges are described below:

i). Procurement of NGFR (p75^{NTR}, B6.129S4-Ngfrtm1Jae/J) global knockout mouse from Jackson Laboratory (Strain #: 002213).

a. Order placed: 06/22/23, cryorecovery
b. Mice received: 09/11/23 (5 weeks old).
c. Mice received from Jackson lab: 9 male homozygous mice (genotyping was performed in the lab; see **Figure 1**). The mice had to attain 12 weeks before breeding.

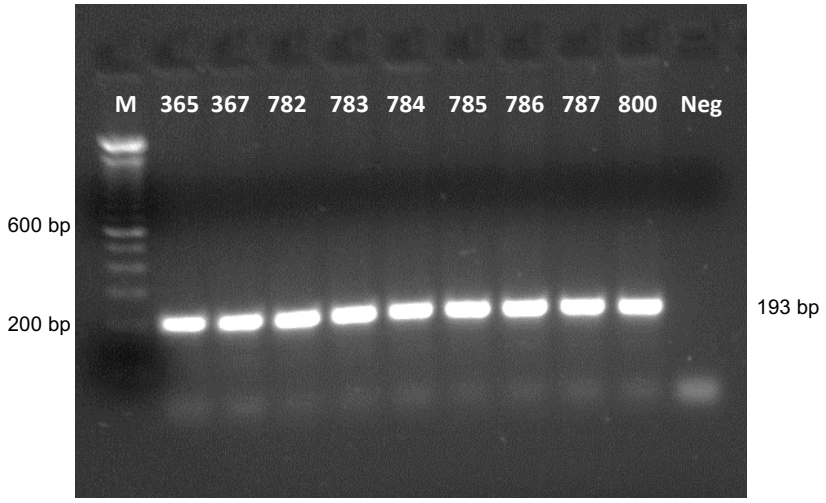

Figure 1. Genotyping data for 9 homozygous (B6.129S4-Ngfrtm1Jae/J) males received from Jackson Lab.

Genotyping data observation (Amplicon size):

Wild type mice:	386 bp (one band)
Heterozygotes KO mice:	193 bp and 386 bp (two bands)
Mutant mice (homozygous KO):	193 bp (one band)

d. Because all the mice that we received from Jackson Lab were male homozygous, to obtain homozygous female mice, we first set up breeding with Wild-type (C57BL/6) female mice to get F1 heterozygote mice. Below is the breeding pattern and the expected F1 generation of mice.

C57BL/6 (WT) ♀ p75^{NTR} KO ♂

p75$^{NTR+/+}$ × p75$^{NTR-/-}$

\- -

+	+ -	+ -
+	+ -	+ -

F1 p75$^{NTR+/-}$

e. All mice in F1 generation were expected to be heterozygotes ($p75^{NTR+/-}$). Please see data in **Figure 2**.

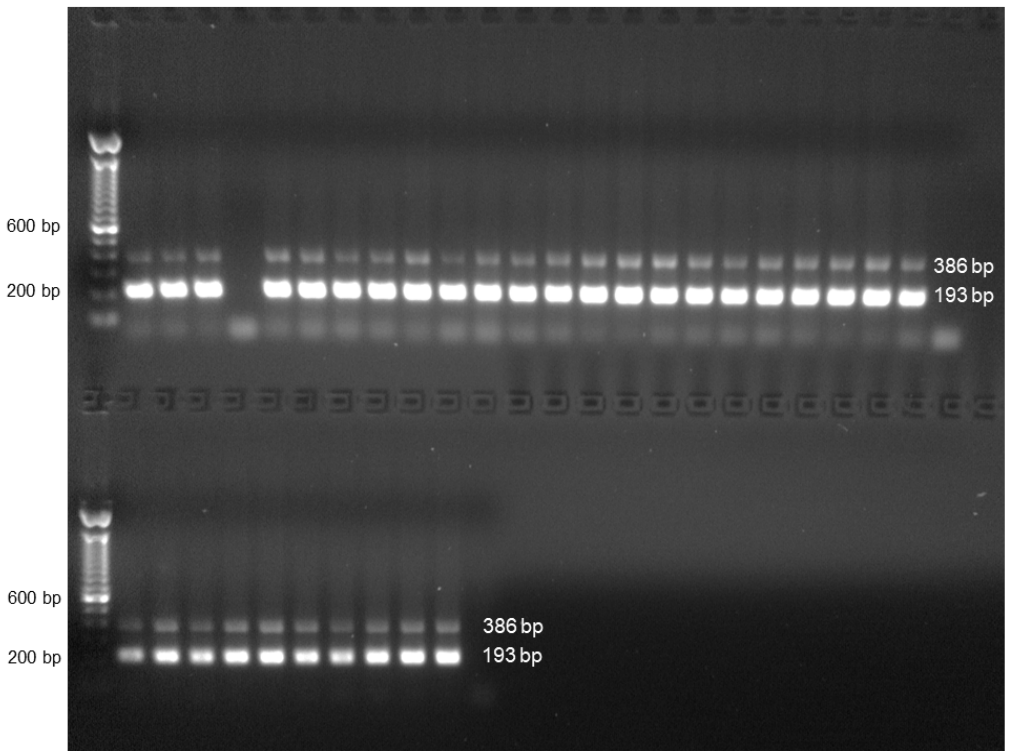

Figure 2. Genotyping data for mouse offspring from F1 generation.

f. All the heterozygote adult mice from F1 generation above were again set up for breeding. Below is the breeding pattern. The expected number of offsprings is wildtype (25%; +/+), heterozygous (50%; +/-) and homozygous (25%; -/-). However, unfortunately, we only found one male homozygous knockout mouse (out of 40 pups) in our F2 generation (1st set of breeding; please see **Figure 3**).

F1 $p75^{NTR+/-}$ × $p75^{NTR+/-}$

	+	-
+	++	+ -
-	+ -	- -

F2 $p75^{NTR+/+}$, $p75^{NTR+/-}$, $p75^{NTR-/-}$

(WT) (Het) (KO)

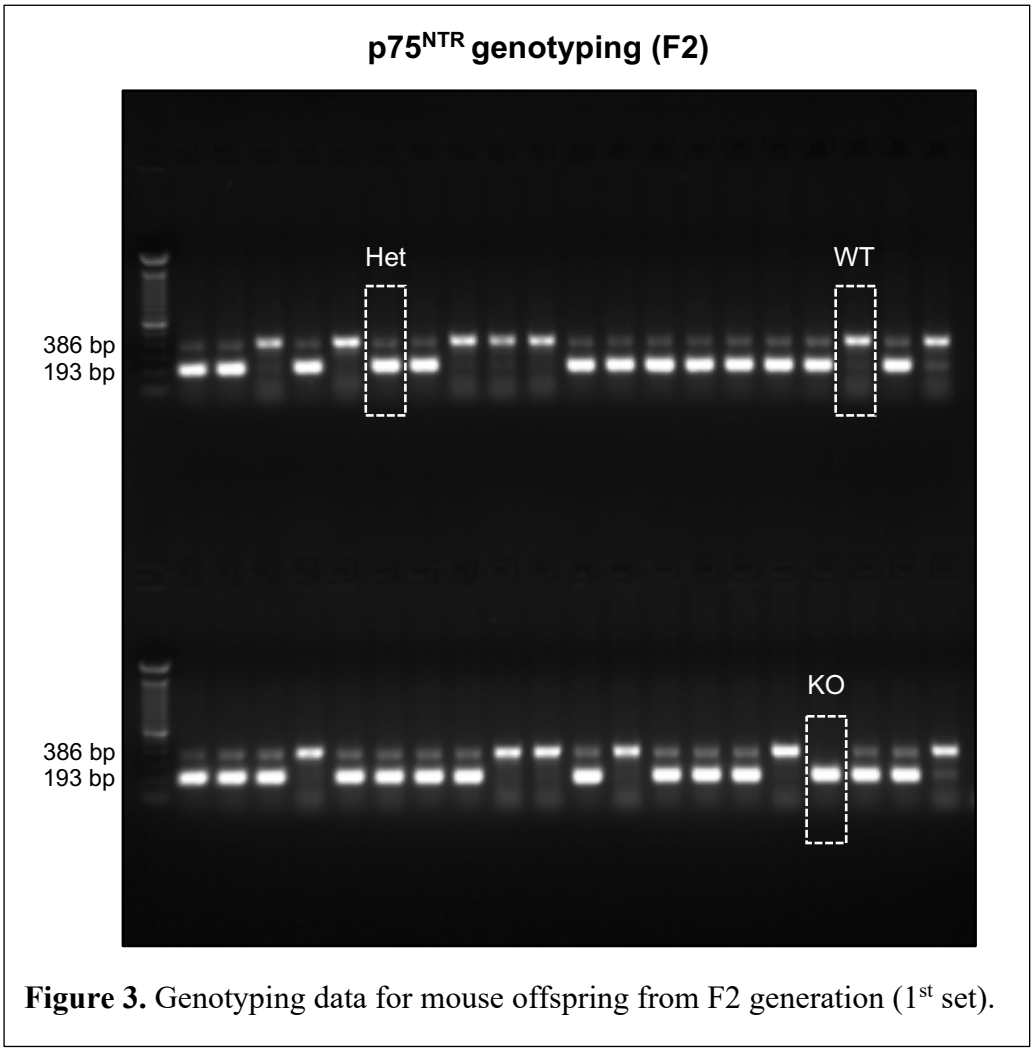

Figure 3. Genotyping data for mouse offspring from F2 generation (1st set).

g. We set up another set of breeding F2 generation. Again, in the second set of breeding also, we only found one male homozygous knockout mouse (please see **Figure 4**). Up until this point, we only obtained 2 homozygous mice, unfortunately, both are male again.

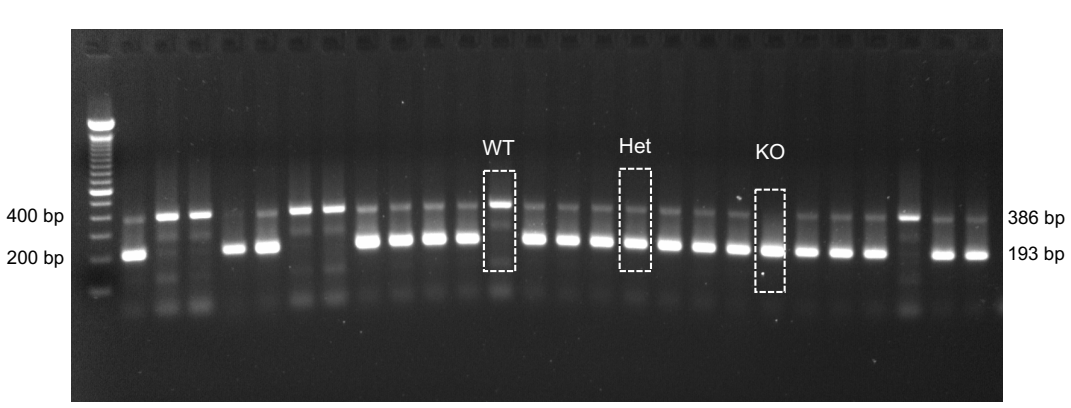

Figure 4. Genotyping data for mouse offspring from F2 generation (2nd set).

h. Setback and overcoming challenge: Jackson laboratory mentioned that probability of getting homozygous KO mice from breeding between Heterozygous × Heterozygous and Heterozygous × Homozygous mice is low. Therefore, we set up extensive breeding to obtain more homozygous mice. In the 3rd set of breeding, we successfully obtained several homozygous knockout mice.

i.In the third round of breeding (using F1 generation mice), we could successfully obtain one male and five female homozygous knockout mice (please see **Figure 5**).

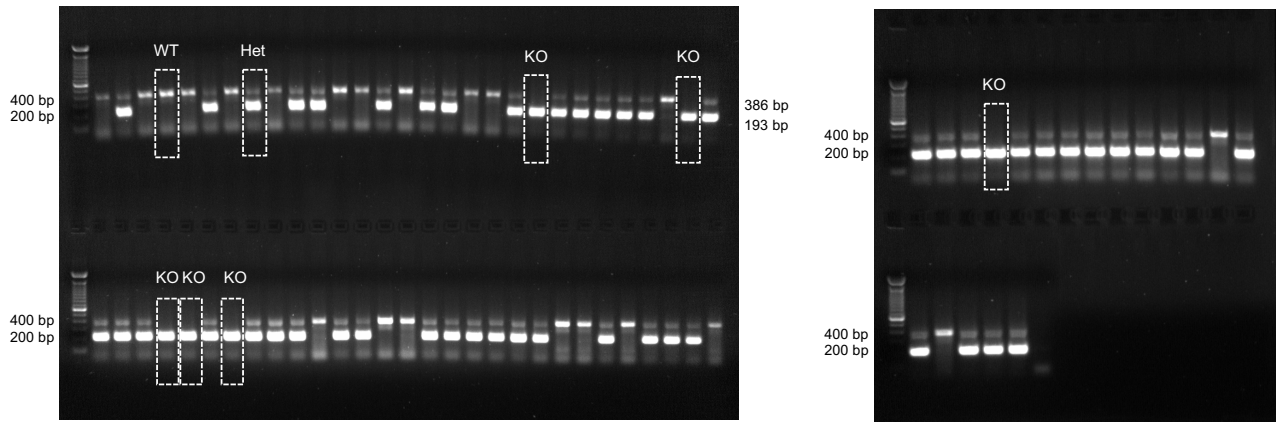

Figure 5. Genotyping data for mouse offspring from F2 generation (3rd set).

h. On-going breeding: We are performing extensive breeding between Heterozygous × Heterozygous mice, Heterozygous × Homozygous, and Homozygous × Homozygous mice to get more of homozygous KO mice to set up depression studies proposed in Subtask 6.

5. Subtask 4 (Alternative approach): Generation of cardiac-specific $p75^{NTR}$ KO mice.
Objective: $p75^{NTR}$ flox mice (which possesses loxP sites) breeding with alpha myosin heavy chain (α-MyHC-cre) mice will result in cardiac-specific $p75^{NTR}$ KO mice, which will then be tested to determine the effect on depression-induced cardiac pathophysiology (proposed in subtask 6).

Our accomplishments:

i)Procurement of NGFR-flox ($p75^{NTR}$ flox/flox) from Indiana University:

The NGFR-flox mouse line available at Jackson labs (Strain #: 031162) is on a mixed genetic background (a mix of C57BL/6 and 129S2 mouse strain). To get a pure C57BL/6, we need to backcross the mixed background line with c57BL/6 for atleast 5-10 times. Therefore, based on previous publication, we contacted the original author Dr. Brain Pierchala (Indiana University) who created this mouse line to check and determine if they have NGFR-flox on C57BL/6 background. This would save us significant amount of time in backcrossing (approximately 1.5-2yr). Dr. Pierchala has this line backcrossed at least 3 times agreed to send us the mouse line. Therefore, we initiated the material transfer agreement (MTA) for this line in June, 2023. The approval and shipment took 4 months. We received shipment of the mouse line from Indiana University in September 2023.

ii) Setback and overcoming challenge: Unfortunately, upon arrival and genotyping, we found that only one female mouse was heterozygous for NGFR-flox (please see **Figure 6A**). We again requested Dr. Pierchala to send us second shipment of NGFR-flox mice, which we received in November, 2023. We received one breeding pair (male, $NGFR^{fl/wt}$, female $NGFR^{fl/fl}$; Please see **Figure 6B**).

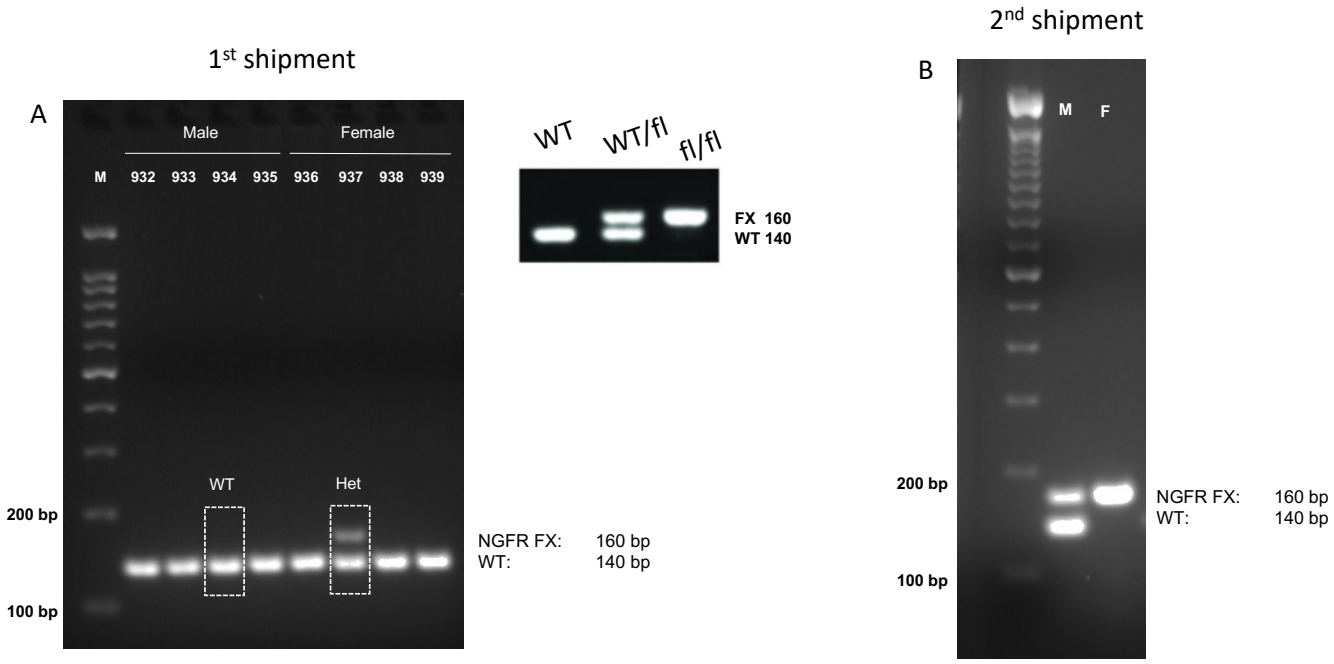

Figure 6. A. Genotyping data for NGFR-flox mouse received from Indiana University (1st shipment). **B.** Genotyping data for 2nd shipment of mice.

iii) On-going: First set of breeding was set up among heterozygotes to obtain homozygous $NGFR^{fl/fl}$ mice. The breeding pattern is shown below and the resultant mouse offsprings is shown in **Figure 7**. We are now further breeding these mice to get enough number of homozygous $NGFR^{fl/fl}$ mice, which will then be backcross with C57BL/6 mice for upto 3 generation to get pure C57BL/6 background mice. These NGFR-flox mice will be used to generate cardiac-specific and endothelial-specific NGFR knockout mouse line (proposed in subtask 6 and Aim 2).

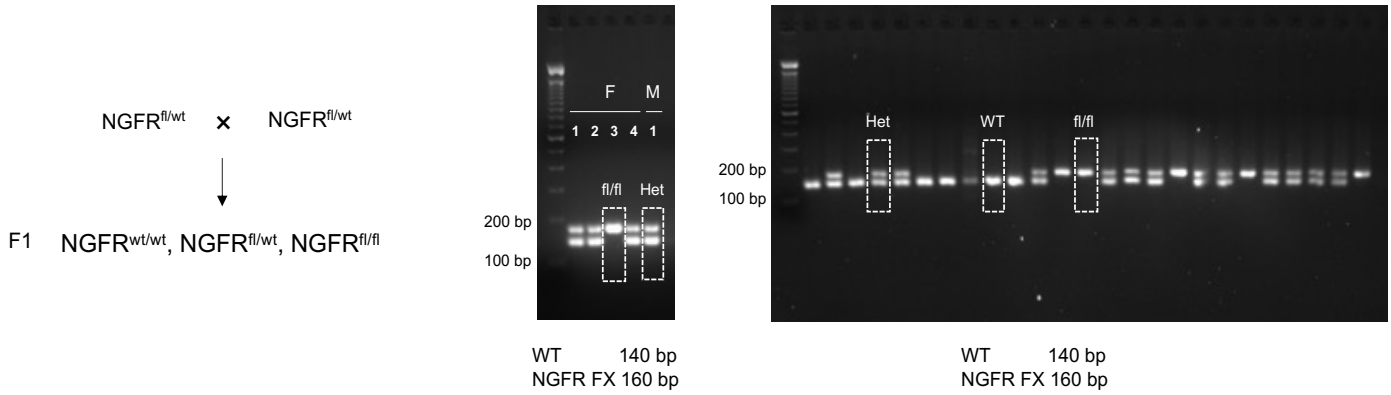

Figure 7. Genotyping data for NGFR-flox mouse after first round of breeding.

***6. Subtask 5, In vitro* studies:** To investigate the $p75^{NTR}$ role in cardiac cells during depression, we first performed loss-of-function studies using RNA interference against NGFR gene ($p75^{NTR}$). We are first testing transfection efficiency in two different easy to grow cell lines- mouse cardiac endothelial cell line (MCEC, **Figure 8A**) and RAW 264.7 cells (mouse macrophage cell lines, **Figure 8B**). However, we did not observe a significant knockdown of NGFR using this approach.

Setback and alternative plan: As an alternative strategy, we plan to knockout $p75^{NTR}$ in the cells using CRISPR-Cas9 system and using different siRNA/viral vectors. We have already designed plasmids for CRISPR-Cas9 experiments. We are in the process of propagating and extracting desired quantity of plasmids for treating cardiomyocytes and fibroblasts.

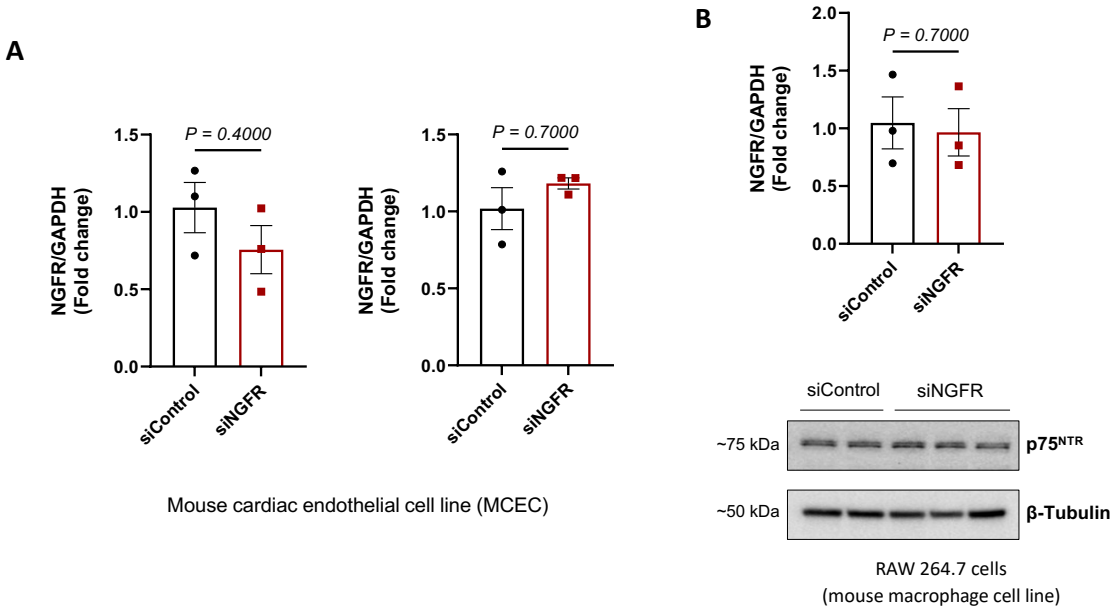

Figure 8. qRT-PCR and Western blotting showing p75NTR siRNA transfection efficiency in mouse cardiac endothelial cell line (MCEC) and RAW 264.7 cells (mouse macrophage cell lines).

Summary and conclusions: While we encountered initial setback in obtaining p75NTR homozygous knockout mice after breeding three rounds, we are now successful with obtaining female mice that are homozygous. According to Mendelian inheritance, we expect that the breeding between homozygous male and homozygous female should result in 100% homozygous offsprings. These mice will be subsequently used in Subtask 6. The subtask 3 is approximately 50% complete and due to the above challenges, we anticipate a delay of 2-3 months in completing this subtask 3 and obtaining atleast 20 mice for experiments proposed in subtask 6 (which is also anticipated to be delayed by 2-3 months).

Also, in Subtask 4, we encountered initial setback in obtaining male p75NTR-flox heterozygous knockout mice, we are now successful with obtaining p75NTR-flox/flox homozygous mice. This initial setback has delayed our subsequent breeding plan. Once we generate a greater number of homozygous mice, we will begin crossing with alpha-MyHC-Cre mice to obtain cardiac-specific knockout mice. These mice will be subsequently used in Subtask 6. This subtask 4 is approximately 50% complete and due to the above challenges, we anticipate a delay of 2-3 months in completing this subtask 4 and obtaining atleast 20 mice for experiments proposed in subtask 6 (which is also anticipated to be delayed by 2-3 months). In subtask 5, due to challenges in mice breeding and expansion of colonies (in subtask 3 and 4) and due to poor transfection efficiency of siRNA in subtask 5, overall, we anticipate delay of 2-3 months. However, by overcoming some of these initial setbacks, we anticipate to be on track in the next reporting period.

PROTOCOL (1 of 1 total):

Protocol [ACURO Assigned Number]: ACURO protocol PR220330.e001

Title: Understanding Cardiovascular Disease in Mental Health/Stress Disorder

Target required for statistical significance: 480

Target approved for statistical significance: 480

Total subjects to date: 10

SUBMITTED TO AND APPROVED BY:

IACUC-22724 approval date: 03-14-23
ACURO approval date: 03-16-2023

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

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

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

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
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
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

In subtask 3, we encountered initial setback in generating female p75NTR homozygous knockout mice after breeding three rounds. We are now successful with obtaining female mice that are homozygous. In Subtask 4, we encountered initial setback in obtaining male p75NTR-flox heterozygous knockout mice, we are now successful with obtaining p75NTR-flox/flox homozygous mice. These subtasks will be delayed by atleast 2-3 months. Now we have the required mice, we expect we will resume speed with these tasks.

In subtask 5, due to challenges in mice breeding and expansion of colonies (in subtask 3 and 4) and due to poor transfection efficiency of siRNA in subtask 5, there has been delay in completing these tasks by atleast 2-3 months. As an alternative strategy, we plan to knockout $p75^{NTR}$ in the cells using CRISPR-Cas9 system

and using different siRNA/viral vectors. We have already designed plasmids for CRISPR-Cas9 experiments. We are in the process of propagating and extracting desired quantity of plasmids for treating cardiomyocytes and fibroblasts. Dr. Praveen Dubey, who is an expert in CRISPR-Cas9 technology and molecular biology has been assigned some of these experiments. We expect to resume these experiments and complete the proposed subtasks.

Please note that these setbacks and proposed action plans do not change the scope of the study.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

In this reporting period, Dr. Praveen Dubey has dedicated 12 calendar months to this project. Recently, he has been awarded Career Development Award (CDA) from American Heart Association. Therefore, his calendar months on this grant might reduce in the next reporting period.

Dr. Sarojini Singh has been partially supported through American Heart Association Fellowship (8.0 calendar months). Now, her fellowship has ended. She will increase her calendar months on this grant in the next reporting period (up to 12.0 calendar months).

Ms. Vasanthi Rajasekaran (Technician) has dedicated 12.0 calendar months in this reporting period. However, she transitioned to another lab at UAB in the middle of May 2024.

Dr. Prasanna Krishnamurthy, PI has originally committed 4.2 calendar months to this project. Now his other supports have ended, he has increased his calendar months and will go up to 6.6 calendar months in the next reporting period.

Due to the above changes in personnel and delays in achieving subtask 3 and 4, we are planning to recruit either a Graduate student or a postdoctoral fellow, who could start immediately. However, these changes do not affect the annual or overall approved budget of the project.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

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: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **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?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name	Prasanna Krishnamurthy
Project Role	PI
Researcher Identifier e.g., ORCID ID)	0000-0002-4842-6364
Nearest person month worked	5.1 calendar months
Contribution to Project	As a Principal Investigator, he will oversee the progress of the entire project and direct the design of the studies related to accomplishing each of the Specific Aims. He meets with the investigation team weekly in achieving the aims of the project.
Funding Support	None

Name	Sarojini Singh
Project Role	Postdoctoral fellow
Researcher Identifier e.g., ORCID ID)	0000-0001-6143-277X
Nearest person month worked	4.0 calendar months
Contribution to Project	Primary roles: Experiment design, acquisition and analyses of data, record and documentation of data, written and oral presentation of the project-related finding. She was involved in procurement of heterozygous NGFR-Flox mice (C57Bl/6 background) from Dr. Brain Pierchala (Indiana University), crossing, breeding, and colony maintenance to generate NGFR-Flox homozygous mice on C57Bl/6 background.
Funding Support	American Heart Association (8.0 calendar months)

Name	Praveen Dubey
Project Role	Researcher V
Researcher Identifier e.g., ORCID ID)	0000-0002-8560-684X
Nearest person month worked	12.0 calendar months
Contribution to Project	Primary roles: Experiment design, acquisition and analyses of data, record and documentation of data, written and oral presentation of the project-related finding. He was involved in procurement of NGFR global knockout mice from Jackson Laboratory (Cryorecovery), crossing, breeding, and colony maintenance to generate NGFR homozygous global knockout mice.
Funding Support	None

Name	Vasanthi Rajasekaran
Project Role	Researcher II (Technician)
Researcher Identifier e.g., ORCID ID)	Not available
Nearest person month worked	12.0 calendar months
Contribution to Project	Assisted the investigative team in maintaining KO mice colony, breeding and genotyping.
Funding Support	None

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

The below mentioned grant is now closed:
Title of the project: Targeting paracrine mediators to enhance cardiac regeneration and repair after injury.
Funding agency: American Heart Association (Transformational Project Award #19TPA34850100)
Point of contact at the funding agency: Kendall Plemons (Kendall.Plemons@heart.org; awards@heart.org)
Role: Krishnamurthy (PI)

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

None