**AWARD NUMBER: W81XWH-17-1-0661**

**TITLE: Regulation of Cardiogenesis by GATA Transcription Factors**

**PRINCIPAL INVESTIGATOR: DR. Todd Evans, PhD**

**RECIPIENT: Joan and Sanford I. Weill Medical College of Cornell University**
**New York, NY 10065-4805**

**REPORT DATE: October 2019**

**TYPE OF REPORT: Annual**

**PREPARED FOR:** U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| OCTOBER 2019 | Annual | 30 Sep 2018 – 29 Sep 2019 |

**4. TITLE AND SUBTITLE**
Regulation of Cardiogenesis by GATA Transcription Factors

**5a. CONTRACT NUMBER**
W81XWH-17-1-0661

**5b. GRANT NUMBER**

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**
Dr. Todd Evans

E-Mail: tre2003@med.cornell.edu

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

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

Joan and Sanford I. Weill
Medical College of Cornell
University
New York, NY 10065-4805

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

In the first year of this project, we were able to evaluate loss of function mutations for gata4, gata5, and gata6, in both zebrafish and human ESC models. We were surprised to find that gata4 null mutations were tolerated in zebrafish, while the gata5 and gata6 mutations phenocopied previous analyses including a small truncated heart tube for gata6 mutants. We used western blotting to demonstrate that the gata4 mutation is null. Most strikingly, aged mutant adults showed a severe cardiomyopathy. The heart is enlarged at least two-fold in size. We are currently characterizing hearts by histology to determine if the gata4 mutants may be a model for human dilated cardiomyopathy or some other specific disorder. This is an exciting result that supports our underlying hypothesis that the mutants can be used to model human congenital cardiac disease. In addition, we found a striking defect in cardiogenesis in the human GATA6 null mutant ESCs. We correlated this with a sharp increase in expression of RALDH2, suggesting that GATA6 normally restricts retinoid signaling important for cardiac differentiation.

**15. SUBJECT TERMS**
Cardiomyopathy, hESCs, hiPSCs, directed differentiation, congenital heart disease, zebrafish

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | USAMRMC |
| Unclassified | Unclassified | Unclassified | Unclassified | 14 | 19b. TELEPHONE NUMBER *(include area code)* |

Table of Contents


| | Page |
|---|---|
| **1. Introduction** | **4** |
| **2. Keywords** | **4** |
| **3. Accomplishments** | **4-7** |
| **4. Impact** | **7-8** |
| **5. Changes/Problems** | **8-9** |
| **6. Products** | **9-11** |
| **7. Participants & Other Collaborating Organizations** | **11-14** |
| **8. Special Reporting Requirements** | **14** |
| **9. Appendices** | **14** |

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

**The overall goal** of the project is to discover mechanisms that cause congenital cardiomyopathy. As a model system, we are using animals (zebrafish) and human stem cells that carry defined mutations in the Gata4, Gata5, or Gata6 genes (Gata456), which have been associated with patients that suffer from congenital cardiomyopathy. **Our overall hypothesis** is that by manipulating Gata456 with combinatorial, temporal, and spatial specificity, we will identify important regulators and modifiers of lineage specification, tissue morphogenesis, and cell differentiation that are deregulated and thereby cause congenital cardiomyopathies related to human GATA456 gene variants. The genes and pathways we discover will provide relevant therapeutic targets to treat congenital heart disease and potentially even morbidity in adult patients that had been treated successfully by surgery. We are using zebrafish with defined mutants as an animal model and human pluripotent cells to translate to human lineages. One important goal is to find genes and pathways that interact with GATA factors that might explain why the human mutations are haploinsufficient.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Cardiomyopathy, hESCs, hiPSCs, directed differentiation, congenital heart disease, zebrafish

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

**What were the major goals of the project?**
*List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

**Aim 1. Identify the function and downstream regulatory programs for individual and combinations of Gata456 during zebrafish cardiogenesis.** We generated genetic null alleles and conditional alleles for each gene and will discover phenotypes and alterations in genetic and epigenetic programs when genes and combinations of gene functions are disrupted.

**Aim 2. Identify the function and downstream regulatory programs for individual and combinations of GATA456 druing human cardiogenesis from hESCs.** Likewise, we will define cellular phenotypes and molecular alterations caused by loss or gain of function for each single and combination of genes during directed differentiation to cardiac fates from hESCs.

**Aim 3. Discover interacting pathways of GATA456 relevant to human congenital heart disease.** Screens will be carried out in hESCs carrying defined null and patient-specific alleles during in vitro cardiogenesis for gene and pathway discovery and disease modeling “in a dish”.

**What was accomplished under these goals?**
*For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant*

*results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

1) Major activities.
In the previous year we successfully characterized embryonic cardiogenesis in fish larvae carrying mutations in each gene and gene combination. We have since developed algorithms to quantitatively measure impact on progenitors and chamber tissues, leading to a focus on gata6 as a key regulator of ventricular development. We generated additional larger deletion alleles of gata4 since we want to be certain that we are evaluating null alleles that do not stimulate potential compensation by gata6. With respect to Aim2, we focused on discovery of mechanisms leading to major defects in cardiogenesis in the hESCs lacking GATA6, or heterozygous for GATA6. We also evaluated cardiogenesis from hiPSCs carrying a patient-specific GATA6 mutation.

2) Specific objectives.
We want to determine if mutations cause similar or distinct cardiomyopathies. A major objective was to determine if combinations of mutations were additive or synergistic. At this time we are still only evaluating differentiation efficiency in the human system, but will eventually start evaluating cellular function and physiology. We have characterized in more detail the phenotype of adult fish with gata4 mutations, including cardiac function. We sought to determine if the iPSCs behaved more like heterozygous or null ESCs in the differentiation. A clear objective is to find the key downstream target genes that mediate function of GATA456.

3) Key outcomes.
We have completed the primary objectives for Aim1, to characterize cardiac defects in fish lacking individual or combinations of gata456. With respect to Aim2, we found a very different result using hESCs, in that loss of GATA6 is sufficient to completely block cardiogenesis, while the heterozygous cells are moderately impaired. This has led us to focus on GATA6 in terms of mechanisms. Both the heterozygous hESCs, and the patient-specific cells demonstrate moderate phenotypes, so that we can prepare to develop the screen proposed for Aim3. The major results generated over the past funding period include:

i) We discovered that adults carrying a null gata4 mutation showed a severe cardiomyopathy. The heart is enlarged at least 2x in size. Histology suggested minor fibrosis with the major phenotype being a severely enlarged atrial chamber. This is an exciting result that supports our underlying hypothesis that the mutants can be used to model human congenital cardiac disease.

ii) We completed analysis of larvae derived from each individual mutation, plus combinations of gata45, gata46, gata56, and gata456. As expected, gata56 larvae completely lack a heart, and fail to develop any nkx2.5+ progenitors. Interestingly, the same phenotype is found in gata5-null, gata6-het larvae, but not for the gata6-null, gata5-het. This demonstrates a dosage effect, but a more severe requirement for gata5 in the fish. Gata5 mutants show cardia bifida as expected, and the phenotype is no different in gata45 mutants. Gata6 mutants show a small ventricle and enlarge atrium, suggesting a role in chamber identity. At least some of the heart tube defect is due to a failure in second heart field development.

iii) We found a striking defect in cardiogenesis in the human null GATA6 mutant ESCs. GATA4 is also impacted in these cells, so this may represent an equivalent of the double gata56 mutant

zebrafish (GATA5 is not affected n the GATA6 mutant cells). The major surprising finding was that this is associated with a very early defect in precardiac mesoderm. RNA-seq experiments showed the complete failure in expression of LGR5, a co-receptor for Wnt signaling, indicating that GATA6 may play a key role in Wnt signaling during mesoderm development, which was not previously suspected.

iv) We found that both the GATA6-het ESCs as well as the patient-specific GATA6 het iPSCs show a moderate defect in generation of cardiomyocytes during directed differentiation. This provides a foundation for studying haploinsufficiency using human cells. We have also found a defect in the GATA4-null mutant ESCs.

v) While perhaps less directly related to GATA factor function, we made use of our iPSC platform to model human congenital cardiac disease, focused on Noonan Syndrome (NS), in collaboration with the Kontraridis laboratory. We used patient-derived *RAF1*$^{S257L/+}$ and CRISPR-Cas9-generated isogenic control iPSC-derived cardiomyocytes (iCMs) to model NS *RAF1*-associated HCM and to further delineate the molecular mechanisms underlying the disease. We found that mutant iCMs phenocopy the pathology seen in patient NS hearts by exhibiting hypertrophy and structural defects. Through pharmacological and genetic targeting, we identified two perturbed concomitant pathways that, together, mediate HCM in *RAF1* mutant iCMs; hyper-activation of MEK1/2, but not ERK1/2, causes myofibrillar disarray, whereas the enlarged cardiomyocyte phenotype is a direct consequence of increased ERK5 signaling, a pathway not previously known to be involved in NS. RNA-sequencing revealed genes with abnormal expression in *RAF1* mutant iCMs and identified subsets of genes dysregulated by aberrant MEK1/2 or ERK5 pathways that could contribute to the NS-associated HCM. Taken together, the study identified the molecular mechanisms by which NS *RAF1* mutations cause HCM and revealed downstream effectors that could serve as therapeutic targets for treatment of NS, and perhaps other, more common, congenital HCM disorders as well.

**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."*

*Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

Nothing to Report.

**How were the results disseminated to communities of interest?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

| |
|---|
| Nothing to Report. |

**What do you plan to do during the next reporting period to accomplish the goals?**
*If this is the final report, state "Nothing to Report."*

*Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

| |
|---|
| The following experiments are ongoing:<br>i) We continue to screen for generation of conditional zebrafish alleles. While we believe we generated alleles in F0 putative founders, we have yet to isolate germline-transmitting strains. We have several stragies using different donor DNAs.<br>ii) We are in the process of using Crispr/Cas9 to revert the mutant patient-specific GATA6 allele back to the "wildtype" sequence, so that we can compare phenotypes with an otherwise isogenic line.<br>iii) We are characterizing Wnt signaling in the GATA6 null lines and will test the ability to rescue development when Wnt signaling is normalized.<br>iv) We will profile by RNA-seq and ATAC-seq to compare the GATA4-null, GATA6-het, and GATA6-null ESC lines. GATA6 ChIP-seq is also planned.<br>v) We will carry out functional analysis for the cardiac cells that are generated in the GATA4-null, GATA6-het, and patient-specific GATA6 mutant PSCs.<br>vi) We plan to initiate the Crispr/Cas9 genetic screen in the background of either or both of the GATA6-het ESCs and patient-specific GATA6-het iPSC lines. |

4. **IMPACT:** Describe 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?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

| |
|---|
| We have discovered that in human embryonic development the GATA6 gene has a very early role that was not previously appreciated. Our current studies to explore gene regulatory networks controlled by GATA6 will shed new light on early steps of human development. Our studies on the later function of GATA6 should help explain why patients with mutations in this gene can be born with heart defects. |

**What was the impact on other disciplines?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

| |
|---|
| Nothing to Report. |

**What was the impact on technology transfer?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

| |
|---|
| Nothing to Report. |

**What was the impact on society beyond science and technology?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

| |
|---|
| Nothing to Report. |

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

**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.*

| |
|---|
| One of our goals was to generate conditional alleles in zebrafish. Using Crispr/Cas9 we have evidence that we made these alleles but thus far we have not been able to generate germline transmitting conditional mutant alleles in the fish model. We have several alternative strategies |

and will continue to pursue these in the coming year. This has not impacted our ability to make progress in many other areas.

**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.*

Nothing to Report.

**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.*

Nothing to Report

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
*Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

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:** 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**
  Report only the major publication(s) resulting from the work under this award.

  **Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title;*

*journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Jaffré, F., Miller, C.L., Schanzer, A., Evans, T., Roberts, A.E., Hahn, A., Kontaridis, M.I. (2019). Inducible Pluripotent Stem Cell-Derived Cardiomyocytes Reveal Aberrant Extracellular Regulated Kinase 5 and Mitogen-Activated Protein Kinase Kinase 1/2 Signaling Concomitantly Promote Hypertrophic Cardiomyopathy in RAF1-Associated Noonan Syndrome. ***Circulation.*** 140: 207-224. PMCID: PMC6709678. YES.

Badieyan, Z.S. and Evans, T. (2019). Application of Chemically Modified mRNA in Cell Fate Conversion and Tissue Engineering. ***Stem Cells Trans. Med.*** 8: 833-843. PMCID: PMC6646692. YES.

**Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report.

**Other publications, conference papers and presentations**. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report.

- **Website(s) or other Internet site(s)**
  *List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.*

Nothing to Report.

- **Technologies or techniques**
  *Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.*

Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  *Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.*

| Nothing to Report. |
|---|

- **Other Products**
  *Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:*
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*
  -

| • 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".*

Note: person-months listed below have been rounded to the nearest whole number.

**Todd Evans, Principal Investigator:** "No Change"

Funding Support:

**Brandoch Cook, Assistant Professor**
Nearest person-month worked: 3

Contribution to Project: Dr. Cook is an expert in developmental signaling pathways and assisted the team in evaluating alterations caused by GATA mutations by western blotting and immuno-precipitation assays.

**Katherine Zollo, Research Technician**
Nearest person-month worked: 12
Contribution to Project: Ms. Zollo is our lead fish technician and she maintains all the zebrafish strains needed for the project. She also assists in husbandry and maintaining lab stocks and supplies.

**Fabrice Jaffre, Instructor**
Nearest person-month worked: 3
Contribution to Project: Dr. Jaffre is an expert in using hPSCs for disease modeling. He assists the team in optimizing differentiation assays using our hESC and hiPSC models. He also led the team studying the role of specific RAF1 mutations to model human congenital heart disease in vitro.

Funding Support: American Heart Association

**Ingrid Torregroza, Research Associate**
Nearest person-month worked: 5
Contribution to Project: Ms. Torregroza has functioned as laboratory manager to help coordinate research supplies and data collection. She is also working to generate and validate conditional zebrafish mutants.
Funding Support: R35HL135778, Tri-Institutional Stem Cell Initiative, NYSTEM

**Yi-Fan Lin, Postdoc**
Nearest person-month worked: 4
Contribution to Project: Dr. Lin is investigating alterations in mitochontrial metabolism in the context of defined mutations.
Funding Support: Tri-Institutional Stem Cell Initiative and NYSTEM

**Joseph Bisson, Postdoc**
Nearest person-month worked: 12
Contribution to Project: Dr. Bisson has focused effort primarily on the GATA6 mutant hESCs and has discovered alterations in the Wnt signaling pathway. He has also helped to generate and validate additional mutant cell lines.

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

*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

**For Todd Evans, PI**

**Ended since last reporting of this DOD contract:**
Research award (Evans, Todd, Co-PI) 07/01/16-06/30/19
Tri-Institutional Stem Cell Initiative
"TET-dependent epigenetic mechanisms controlling human embryonic stem cell differentiation"
Role: Co-PI

**No Cost Extension awarded since last reporting of this DOD contract:**
Research Award (Evans, Todd, PI) NCE 08/01/14-12/31/19
Tri-Institutional Stem Cell Initiative
"Molecular control of primitive versus definitive endoderm fates from stem cells".
Role: PI

**For David Christini, co-PI**

**Ended since last reporting of this DOD contract:**
SP0039539-PROJ0010850 (Lucks, Julius, PI) 07/01/18-01/31/19
Cornell University - Ithaca
"CAREER: Uncovering Quantitative Design Principles of RNA Regulators for Synthethic Biology" (support for grad student Angela Yu)
Role: subaward PI

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

*Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.*

*Provide the following information for each partnership:*
*Organization Name:*
*Location of Organization: (if foreign location list country)*
*Partner's contribution to the project (identify one or more)*

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

**8. SPECIAL REPORTING REQUIREMENTS**
**COLLABORATIVE AWARDS:** N/A

**QUAD CHARTS:** N/A

**9. APPENDICES:** N/A