

AWARD NUMBER: W81XWH-18-1-0316

TITLE: Investigation of metabolism in hypertrophic cardiomyopathy

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**CONTRACTING ORGANIZATION: The Regents of the University of California,
San Francisco**

San Francisco, CA 94103

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14. ABSTRACT In Year 1, we compiled metabolite profiles from heart tissue in 2 mouse models of hypertrophic cardiomyopathy (HCM) in the pre-clinical stage of disease (5 week old mice). Using GC-TOF, only 1 metabolite was significantly lower in MyHC mutants compared to littermate controls at baseline. In contrast, we found differences in levels of 13 metabolites in TnT-mutants when compared to littermate-controls. Taken together, our results suggest allele-specific differences in metabolic remodeling at the preclinical stage of HCM in mouse models.						
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- 1. INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

We tested the hypothesis that individual HCM mutations confers a unique perturbation of the cardiac metabolic profile at baseline, following inotrope stimulation and exercise, by proposing metabolomics studies in heart tissue and plasma in two mouse models of HCM (R92W-TnT, R403Q-MyHC). In Year 1 of funding, we discovered mutation-specific differences in cardiac metabolite levels at baseline and following dobutamine stimulation.

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

hypertrophic cardiomyopathy, cardiac tissue metabolomics, plasma metabolomics, metabolites, mutations, mouse models, inotrope stimulation, exercise, swimming

- 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.

The main goal of the project is to compile metabolite profiles from heart tissue and plasma in two mouse models of HCM and littermate controls at 1) pre-hypertrophic stage of disease (5 weeks of age), at rest and following dobutamine (inotrope stimulation), 2) following exercise for 6 weeks (12 weeks of age).

We had proposed breeding mice in months 1-6 for studies – this milestone is complete.

We had proposed harvesting hearts and plasma for dobutamine ECHO study in Month 3, and performance of metabolomics assays in months 4-5 – this milestone is complete. We are now working on manuscript preparation for the dobutamine study.

We had proposed performing the exercise study between months 6-12- the exercise study is ongoing.

We had planned harvesting hearts between months 8-14 – we plan to harvest the exercise-hearts in month 14. We had proposed metabolomics analysis in months 15-16, and manuscript submission in months 17-18 – we anticipate achieving these milestones at the completion of the funding period.

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.

What was accomplished under these goals?

Heart Metabolomics: We performed metabolomics studies in mutant hearts and littermate controls in the pre-hypertrophic stage of disease (5 weeks of age), in collaboration with Dr. Oliver Fiehn's laboratory at UC Davis. We found mutation-specific differences in metabolites. We observed differences in 13 metabolites, including Krebs cycle metabolites, alpha ketoglutarate and succinate in TnT mutant mice, but not MyHC mutant mice (who demonstrated differences in 3-hydroxypalmitic acid-

Figures 1 and 2.

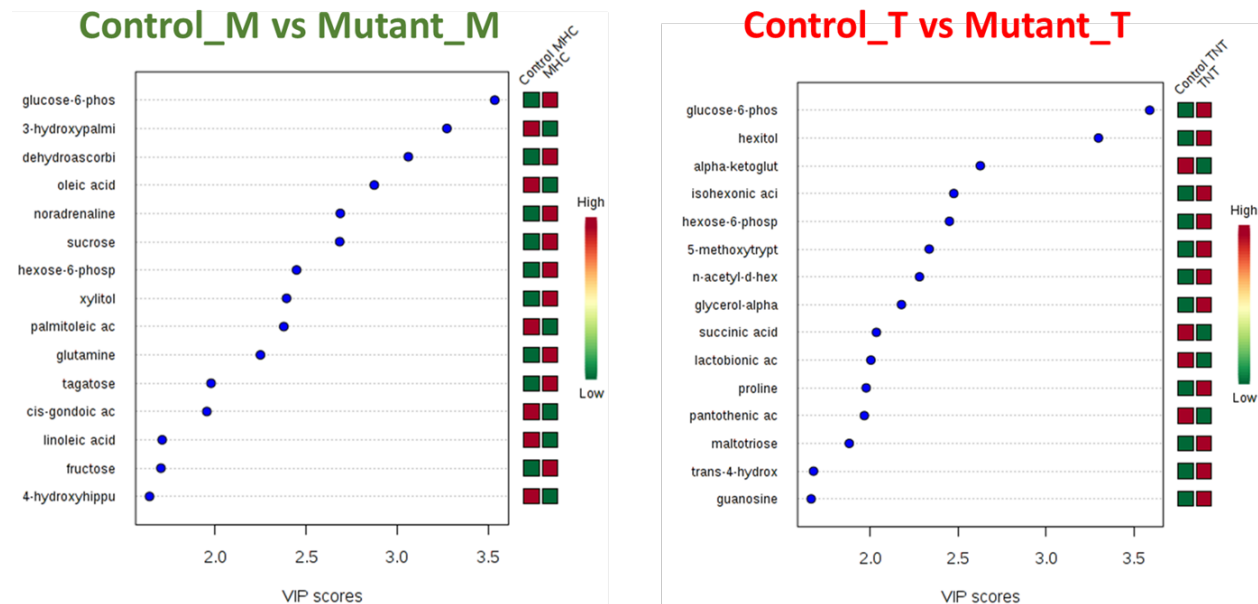


Figure 1. Partial Least Square Discriminant Analysis illustrates mutation-specific differences in cardiac metabolites. VIP score: variable importance in projection values. Control_M: MyHC littermate controls, Mutant_M: MyHC mutants; Control_T: TnT littermate controls, Mutant_T: TnT mutants

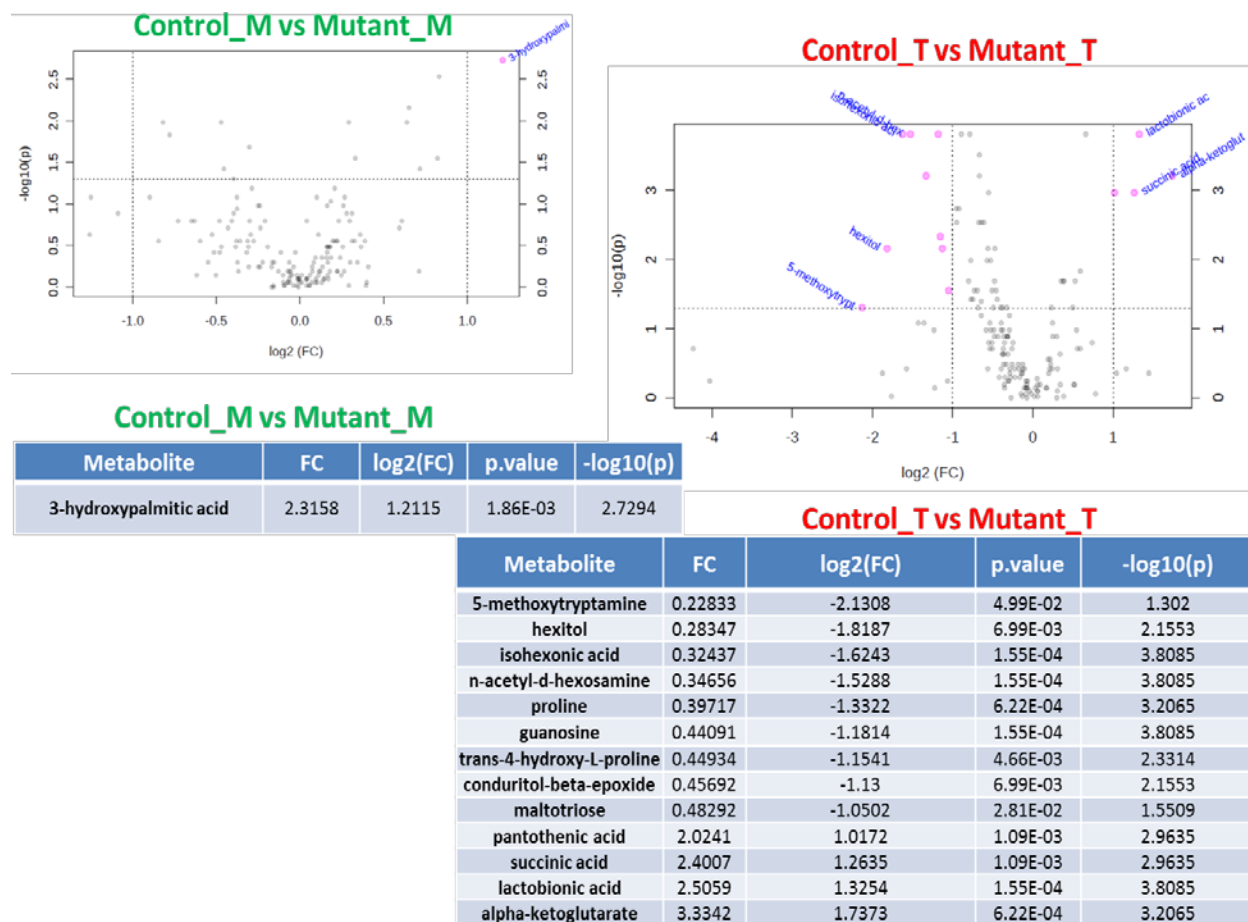


Figure 2. Significant primary metabolites (GC-TOF analysis) in littermate control versus mutant MyHC or TnT mice at the pre-hypertrophic stage of disease (5 weeks of age) are shown on volcano plots with fold change threshold at 2 on the x-axis and t-test threshold at 0.05 on the y-axis. Pink dots represents metabolites that are above the threshold. The tables list the significant metabolites in the 2 mouse models in the pre-hypertrophic stage of disease. Mutant_M: MyHC mutants; Control_M: MyHC littermate controls. Control_T: TnT littermate controls, Mutant_T: TnT mutants

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

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

We plan to sacrifice mice after the completion of swim-exercise, perform tissue/plasma metabolomics analysis, analyze data and write a manuscript, according to the milestones proposed in the grant application.

We are currently writing up the metabolomics data from 5 week old mice and anticipate submitting the manuscript in the next 2 months.

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).

Nothing to Report

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:*

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.

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.

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).*

Nothing to Report

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”.

Maria Roselle Abraham

Principal Investigator

MAHABRA

1 month

Contribution to project: Dr. Abraham helped establish the protocols for intraperitoneal dobutamine administration and exercise in HCM mouse models. She was involved in interpretation of the metabolomics data from the dobutamine project, and is working on writing up the results as a manuscript.

Junaid Afzal

Associate Researcher

2 months

Contribution to project: Dr. Afzal has performed mouse echocardiography with/without dobutamine and ECHO-data analysis in the 2 HCM mouse models. He is currently involved in swim-training of mice

Yamin Liu

Postdoctoral Researcher

7 months

Contribution to project: Dr. Lu helped maintain the mouse colony, performed mouse genotyping, intra-peritoneal dobutamine administration, euthanized mice, and processed heart tissue for metabolomics. Dr. Lu assisted with mouse echocardiography and is working with Dr. Afzal on swim-training of mice.

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.

Nothing to Report

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.*

University of California Davis (UC Davis) performed the metabolite analysis
Collaboration

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.*