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TITLE: Molecular Mechanisms of Transcription Factor Dosage in Heart Development and Congenital Heart Disease

PRINCIPAL INVESTIGATOR: Benoit Bruneau, Ph.D.

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14. ABSTRACT We have discovered that we can indeed understand molecular mechanisms of Congenital Heart Disease using cultured pluripotent stem cells when differentiated to cardiac cells. This requires the study of thousands of single cells rather than the whole population. These results will help us understand what type of cell is most affected upon loss of the congenital heart disease gene TBX5. Our findings in mice are also pointing a specific cell type within the developing heart that is most relevant to congenital heart disease.					
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1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Based on the identification of mutations in the transcription factor TBX5 in patients with congenital heart disease, we hypothesized that in the developing heart, TBX5 dosage is tightly regulated to confer precise gene expression patterns that control finely regulated cellular behaviors. We will test this hypothesis in mouse and human disease models by determining if specific TBX5 DNA binding events are disrupted by reduced dosage, leading to impaired regulation of discrete molecular and cellular pathways critical for normal heart development. These experiments will provide profound insights into the molecular events that lead to common congenital heart defects.

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

heart, gene expression, congenital heart disease, human

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 is to define the consequences of TBX5 haploinsufficiency in mouse and human models of CHD. The goals include clonal reduction of Tbx5 function, and to measure gene expression changes in a specific population of cells that form the interventricular septum.

Aim 2 is to identify the mechanism for TBX5 haploinsufficiency. The goals include measuring changes in chromatin state and transcription factor occupancy in a human TBX5 allelic series in iPS cells.

Aim 3 is to modulate TBX5 haploinsufficiency by a novel genetic interaction with Mef2c. The goals include imaging heart defects, and measuring gene expression changes in the mutant hearts,

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

To date we have made considerable progress on this project.

For Aim 1, the major activities included measuring, using bulk RNAseq, changes in gene expression in the compartment boundary cells. We also imaged several samples at various stage of heart development, especially in relationship to genetically labeled lineages. We varied our approach to include single cell RNAseq, which gives us an exciting view of discrete populations. For Aim 2 our major activity was to generate sufficient material to perform the required analyses; this is ongoing.

For Aim 3 the major activity was to intercross mice to expand the colony in order to obtain sufficient numbers of experimental animals.

The specific objectives to date were to determine what changes in gene expression we could detect in our mouse and human experimental models.

Significant results are several-fold. We have detected clear changes in gene expression of several mRNAs in our mouse model of CHD. Using sorting of lineage-labeled cells we have been able to obtain highly localized gene expression changes; these data are still being analyzed computationally. We are in the process of following these up with in situ hybridization to localize transcripts anatomically. Preliminary data show localized gene expression. In the case of the human TBX5 allelic series, single cell RNAseq was essential to uncover changes in gene expression in discrete cell populations, proving that we can indeed discover CHD-related gene expression changes in a human pluripotent stem cell model of disease (see figure below). We have accumulated material for the epigenomic studies outlined in Aim 2.

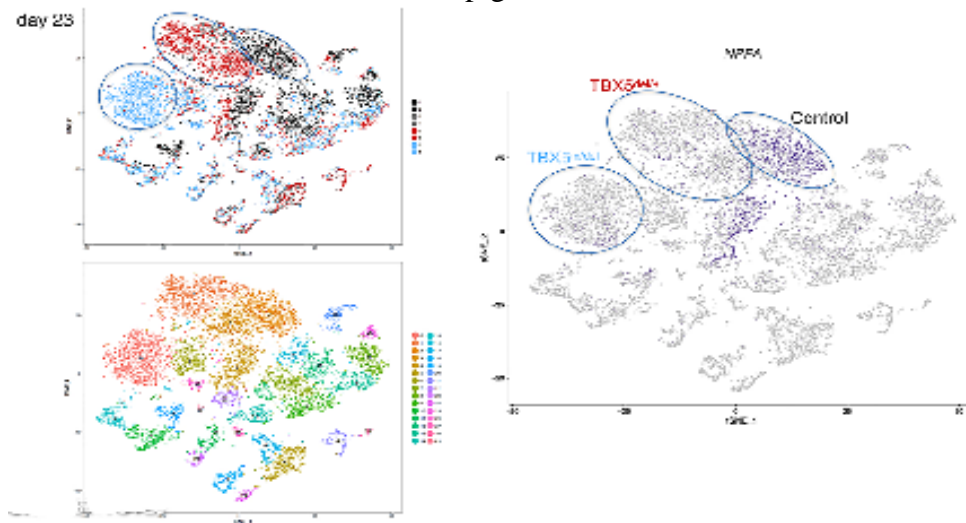


Figure: Single cells RNAseq of iPSC-derived cardiomyocytes across TBX5 allelic series. tSNE plots are shown, color-coded for genotype, top left (black: control, red: TBX5+/-, blue: TBX5-/-), by computationally defined cluster, bottom left, and to the right an example gene that has altered activity (purple) in the TBX5 mutant cells.

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.

In the next reporting period we plan to perform and complete the experiments for Aim 2, which include ChIP-seq for histone modifications and transcription factors in the TBX5 allelic series. We are also using ATAC-seq to examine chromatin accessibility. We will complete the analysis of morphological and gene expression changes in the Tbx5 mouse model; this is well underway and going ahead of schedule. We will also embark on the experiments planned for Aim 3, which have not been prioritized to date.

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).

So far, we have discovered that we can indeed understand molecular mechanisms of Congenital Heart Disease using cultured pluripotent stem cells when differentiated to cardiac cells. This requires the study of thousands of single cells rather than the whole population. These results will help us understand what type of cell is most affected upon loss of the congenital heart disease gene TBX5. Our findings in mice are also pointing a specific cell type within the developing heart that is most relevant to congenital heart disease.

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.

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.

No problems or delays were encountered.

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.

There were no changes that had impact on expenditures.

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

N/A

Significant changes in the use or care of vertebrate animals

There were no changes in use or care of vertebrate animals.

Significant changes in use of biohazards and/or select agents

There were no changes in use of biohazards.

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.*

Conference presentations:

American Heart Association, Anaheim, November 2017
Single Ventricle Summit, Palo Alto April 2018
Heart Failure Update Conference, Toronto May 2018

- **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.

N/A

- **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”.

Name: Benoit Bruneau

Project Role: PD/PI

Nearest person month worked: 2.4

Contribution to project: directed research

Funding Support: N/A

Name: Aaron Blotnick

Project Role: Research Associate I

Nearest person month worked: 10.6

Contribution to Project: cultured and differentiated stem cells

Funding Support: N/A

Name: Michael Lai

Project Role: Staff Research Scientist I

Nearest person month worked: 2.4

Contribution to project: performed electrophysiological analysis of stem cell-derived cardiomyocytes.

Funding Support: N/A

Name: Abigail Nagle

Project Role: Research Associate I

Nearest person month worked: 2.7

Contribution to project: Ms. Nagle performed general lab managing duties and participated in various aspects of the project.

Funding Support: N/A

Name: Sarah Wood

Project Role: Research Associate I

Nearest person month worked: 4.3

Contribution to project: managed mouse colony, genotyped and isolated embryos

Funding Support: N/A

Name: Elphege-Pierre Nora

Project Role: Scientist

Nearest person month worked: 2.5

Contribution to project: genomic analyses of pluripotent cell-derived cardiac precursors and cardiomyocytes.

Funding Support: N/A

Name: Irfan Kathiriya

Project Role: Postdoctoral Fellow

Nearest person month worked: 1.5

Contribution to project: Dr. Kathiriya performed most of the experimental work on this project, and directed associated personnel.

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?

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.

Closed NIH/NHLBI P01 HL089707 (Srivastava, Bruneau, Black)

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.*

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.*