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TITLE: Solving the Unsolved. Integrating Omic approaches to enable genomic diagnosis of virtually all patients with mitochondrial disease

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14. ABSTRACT
New genomic technologies are transforming diagnosis of inherited disease but perhaps half of all children with mitochondrial diseases or other inherited disorders are not currently being diagnosed. This project focuses on using new computational and ''Omic'' approaches to push clinical genomic diagnostic rates from about 50% to close to 100%. These Omic approaches include long-read DNA sequencing technologies, RNA sequencing and quantitative proteomics. The major focus is on several retrospective cohorts of patients that are among the largest well-characterized groups of patients internationally. The requirements for ethical approval of applying genomic analyses to retrospective cohorts collected over many years have taken longer than we anticipated to finalize in Australia so as to ensure HRPO compliance. Those approvals are now in place but they delayed finalizing Collaboration agreements with other centers, which are now nearing completion. We are thus anticipating having final approvals in place to be able to formally initiate the study by January 1, 2020.

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1. Introduction

Oxidative phosphorylation (OXPHOS) is a metabolic pathway located in the mitochondrial power plants in all our cells. OXPHOS is the main pathway used to convert food into the energy needed to make our brain transmit signals, our heart pump and our muscles work. Defects in this pathway are amongst the most common cause of inherited metabolic diseases, with at least 50 children born each year in Australia developing severe OXPHOS disease in their lifetime and about 800 in the USA. As all cells generate energy, patients can present with a disease that affects any or all organ systems. Diagnosis of OXPHOS disorders remains extremely complicated. Until recently, for most children diagnosis typically relied on invasive muscle or liver biopsies and many families have had to wait months or years to receive an accurate diagnosis. The development of massively parallel sequencing (MPS) technologies has improved our ability to obtain genetic diagnoses and spared many children the need for invasive biopsies. However, the diagnostic sensitivity and specificity of these new technologies have not been defined and need ongoing refinement. This project aims to define these quality parameters for retrospective and prospective cohorts and further improve our ability to achieve diagnosis of OXPHOS disease by implementing new computational, “Omic” and cell biology approaches. This information is needed to ensure the implementation of these technologies into routine diagnostic laboratories achieves optimal sensitivity and specificity in order to greatly improve the speed and accuracy of diagnosis. For our retrospective cohort we do not need to obtain new samples from patients as all the patient samples needed for the study have been previously referred to us for diagnostic investigation. Prospective patients and family members are not being actively recruited within this study but are patients who have undergone genomic sequencing in other research or clinical studies without having achieved a confirmed genetic diagnosis. If their existing consent information does not give permission for the studies within this project, or if additional samples are need from parents or relatives, they will be re-consented for this project.

2. Keywords

Mitochondrial disease, rare diseases, oxidative phosphorylation, genetic diagnosis, massively parallel sequencing, quantitative proteomics, retrospective cohorts, oligogenic analyses, functional rescue

3. Accomplishments

Major Goals of the Project

1. Identify monogenic disorders in enriched patient cohorts unsolved by Exome Sequencing by utilizing extended computational, functional, RNA-Sequencing, Whole Genome Sequencing and quantitative proteomic analyses.
2. Utilize statistical analyses of oligogenic interactions to detect multiple mutations in different genes interacting through shared pathways.

Milestones: We noted the need for obtaining local IRB approval for some of the SOW Aim 1 sub-tasks in months 1-5 of the project and these have all now been obtained. We are still in the process of obtaining final HRPO approval of the project, as described below, and thus have been unable to initiate experimental studies. We have therefore not listed specific objectives or significant results/key outcomes but anticipate rapid generation of results following a proposed revised start date of January 1st, 2020.

What was accomplished under these goals?

1) Major activities

The requirements for ethical approval of applying genomic analyses to retrospective cohorts collected over many years have taken longer than we anticipated to finalize in Australia so as to ensure HRPO

compliance. This was largely due to two issues. Firstly, the introduction of a new State-wide online Ethics approval system, which caused some months delay in implementation and in upskilling of users. The larger issue was the need to ensure compliance with a 2018 Update of the Australian *National Statement on Ethical Conduct in Human Research 2007 (Updated 2018).* This update was introduced into Australia subsequent to submission of our DoD grant application and subsequent to our previous Ethics approval providing a waiver of consent for genetic studies on retrospective cohorts of patients. Final Ethics approval was obtained from the Royal Children's Hospital Human Research Ethics Committee (IRB equivalent) on June 13, 2019. The approval does not require any modification to the protocol specified in the grant application.
Initial review of the research protocol and supportive documents by the U.S. Army Medical Research and Development Command (USAMRDC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) resulted a list of items to be addressed in order to proceed with the full review. Those items have now been submitted other than some documents related to documentation of human subject protection training (not previously required in Australia), updated CVs and finalization of two research agreements, which could not be done until we knew the protocols were compliant. We anticipate these items will be submitted by mid-November. We have been advised that we cannot initiate the study until we receive approvals from the USAMRDC, ORP and HRPO and we are thus anticipating having final approvals in place to be able to formally initiate the study by January 1, 2020.

What opportunities for training and professional development has the project provided?
Nothing to report.

How were the results disseminated to communities of interest?
Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
In the next year, we plan to have identified genomic diagnoses in up to half of our exome-unsolved cases by completing most tasks specified in Year 1 of the SOW Timeline, as follows:
Aim 1 Major Task 1 Subtasks 1 to 4: Computational, RNA, protein and mitochondrial DNA analyses in all relevant patients in the retrospective cohort and additional prospective cases if resources permit.
Aim 1 Major Task 2: RNA-sequencing analyses on 40 unsolved patients.
Aim 1 Major Task 3: Whole genome sequencing to have commenced on 20 unsolved patients.
Aim 1 Major Task 4 Subtask 1: Quantitative proteomic analysis on cell lines from 20 unsolved patients.
Aim 1 Major Task 4 Subtasks 2 to 3: iPSC lines generated from 5 patients and differentiation experiments commenced.
We expect to commence all other tasks and subtasks in the second year after the Start Date.

4. Impact

As the experimental phases of the project are yet to commence we have nothing to report.

5. Changes/Problems

Changes in approach and reasons for change
Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
We have had a delay in obtaining the required approvals as noted above but anticipate no other substantive changes other than changing the proposed start date to January 1, 2020 so have nothing further to report.

Changes that had a significant impact on expenditures
Nothing to report other than delay in start date noted above

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report.

Significant changes in use or care of human subjects
Nothing to report.

Significant changes in use or care of vertebrate animals
Not applicable.

Significant changes in use of biohazards and/or select agents
Nothing to report.

6. Products

Publications, conference papers, and presentations
No journal publications or other publications derived from DoD support. However, I have acknowledged my support from the DoD CDMRP at two international conference presentations in 2019:
- Mitochondrial Medicine (United Mitochondrial Disease Foundation), Washington DC, USA, June 2019
- 16th Asian Society of Mitochondrial Research and Medicine, Fukuoka, Japan, October 2019

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?
Given the advice that we cannot initiate the study until we receive approvals from the USAMRDC, ORP and HRPO nobody was worked actively on the project as yet. We received an initial quarterly payment, which we have maintained in our account to be used once approvals are authorized.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report.

What other organizations were involved as partners?
Nothing to report.

8. Special Reporting Requirements

Not applicable.

9. Appendices

Nothing to report.