AWARD NUMBER: HT9425-23-1-0016

TITLE: Management of Mitochondrial Disorders Through Increased Intestinal Fermentation and Reduced Metabolic Burden

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REPORT DATE: January 2024

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
January 2024	Annual	01Jan2023-31Dec2023

4. TITLE AND SUBTITLE

Management of Mitochondrial Disorders Through Increased Intestinal Fermentation and Reduced Metabolic Burden

5a. CONTRACT NUMBER
HT9425-23-1-0016

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Development 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

This proposal explores the role of the microbiome and management of mitochondrial disease. Aim 1 proposes to obtain high quality sequencing data from a mouse model of mitochondria disease at different stages of disease, as well as from animals treated with acarbose, a drug known to alter the composition of the intestinal microbiome. Aim 2 explores therapeutic approaches based on alterations of the intestinal microbiome's composition or activity, either through microbiome transplantation or treatment with fermentable carbon sources or fermentation products.
We have gathered most of the samples required for Aim 1 and attempted microbiome tranfers in breeder animals as suggested in the original proposal. We have discovered that germ-free animals are longer lived than animals in specific pathogen free facilities. However, microbiome transfers were inconclusive. We are attempting a different strategy for the transfer experiments.

15. SUBJECT TERMS
Mitochondrial Disease, Microbiome, Acarbose

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
Unclassified	Unclassified	Unclassified	Unclassified	7	19b. TELEPHONE NUMBER *(include area code)*

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1. Introduction

This proposal investigates the role of the intestinal microbiome in mitochondrial disease. In the first aim it sets out to determine the changes in the intestinal microbiome associated with acute mitochondrial disease and successful treatment thereof. The second aim explores therapeutic strategies based on microbiome transfers and supplementation of fermentable and fermented carbon sources.

2. Keywords

Mitochondrial disease, Acarbose, Microbiome, fermentation, short chain fatty acids.

3. Accomplishments

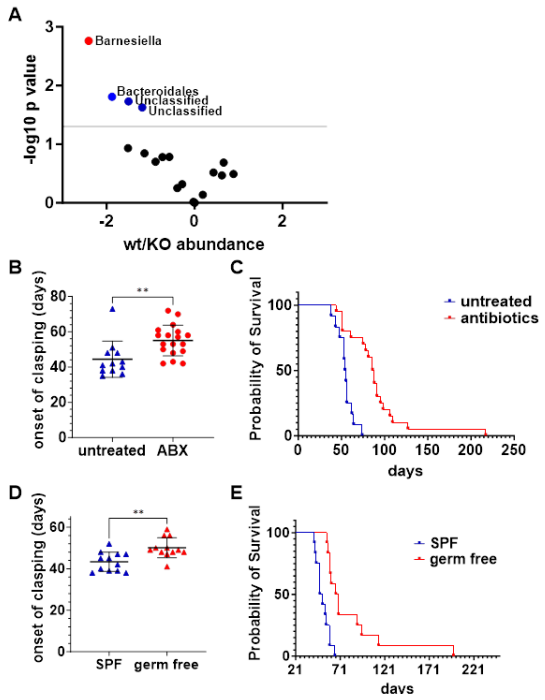

Figure 1: A. Volcano plot of the relative abundance of microbial genera in *Ndufs4*$^{-/-}$ mice compared to wild type. Gray line: linear regression -$\log_{10}$ p-value= 1.3. Blue dots are significantly significant genera, red dot is significant past false discovery rate. **B.** Onset of neurological symptoms and **C.** survival on *Ndufs4*$^{-/-}$ untreated (blue) or treated with a cocktail of broad-spectrum antibiotics red). **: p<0.01 t test. Log rank p<0.01. **D.** Onset of neurological symptoms and **E.** survival on *Ndufs4*$^{-/-}$ in specific pathogen free (SPF) condition (blue) or germ free (red with a cocktail of broad-spectrum antibiotics. **: p<0.01 t test. Log rank p<0.01.

Major Goals

Major goals for this project include 1) obtaining and analyzing the microbiome composition of *Ndufs4*$^{-/-}$ mice compared to wt, 2) quantitation of short chain fatty acids in *Ndufs4*$^{-/-}$ mice, 3) Determine the effects of recolonization of germ-free *Ndfus4*$^{-/-}$ on survival and disease progression, and 4) Dietary interventions with fermentable or fermented carbon sources and their effects on disease progression and survival.

Accomplishments

Goals 1 and 2. We have collected about 75% of the samples required for the metagenomics and metabolomics analysis. We experienced a loss of reproductivity in our colony and had to replace most of our breeders. We are back at capacity and making progress towards this goal at an accelerated pace. 16S sequencing was performed and analyzed on cohoused wild type and knockout mice between the time of submission and the time of the award and revealed some differences in the abundance of specific general between the two genotypes (Figure 1A). Considering that cohousing is a confounding factor in these experiments, we can only expect a greater magnitude of differences from the current project.

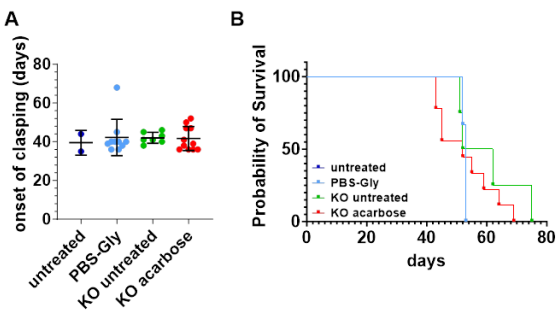

Figure 2: Recolonization of breeders does not delay symptoms of disease in *Ndufs4*$^{-/-}$ offspring. A. Onset of neurological symptoms and **B.** survival of germ-free *Ndufs4*$^{-/-}$ born from untreated (blue) or receiving PBS-Glycerol (PBS-Gly, light blue), cecum transplants from untreated *Ndufs4*$^{-/-}$ mice (KO untreated, green), or acarbose treated *Ndufs4*$^{-/-}$ (KO acarbose, red) mice.

Goal 3. We obtained germ free *Ndufs4*$^{-/-}$ and established a germ-free colony. Heterozygous breeders were recolonized as described in the proposal through oral gavage, and their offspring monitored for onset of disease and survival. We determined that simple ablation of the microbiome, either via germ-free rederivation or via antibiotic treatment, is sufficient to delay onset of disease and increase survival in *Ndfus4*$^{-/-}$ mice (Figure 1B, C, D, E). However, no differences in the onset of clasping could be detected in the offspring of recolonized breeders (Figure 2A, B). We have saved fecal pellets from recolonized breeders and their offspring to determine whether transfer of microbiota and its maintenance was successful. However, we are also attempting to recolonize *Ndufs4*$^{-/-}$ mice directly through exposure to freshly exposed feces as an alternative strategy. Regardless, the positive results with mice lacking their endogenous microbiota suggest that the microbiome has indeed a role in disease etiology and progression and that its manipulation is an avenue for therapeutic interventions.

Goal 4. Because of the reduced output from our breeders, we prioritized allocation of animals to goals 1 and 2. Regarding goal 4, we confirmed our preliminary results with tributyrin and demonstrated that its effects do not add up to those of rapamycin (see https://doi.org/10.1038/s42255-023-00815-w). We are supplementing this goal with cell culture based experiments.

Opportunities for training and professional development.

Nothing to report.

Results dissemination.

A portion of these results was included in a Nature Metabolism article, available at https://doi.org/10.1038/s42255-023-00815-w. Other results have been included in invited seminars at the University of Washington, Drexel University, SUNY Binghamton, and Case Western Reserve University.

4. Impact

The finding that ablation of the endogenous microbiome is sufficient to delay disease in *Ndufs4*$^{-/-}$ has major implications for the field of mitochondrial disease and organismal health in general. It supports the hypothesis that mitochondrial function has a direct impact on the composition of the microbiome and can alter it in pathogenic ways.

No impact of technology transfer and beyond science and technology to report.

5. Changes/Problems

As mentioned in point 2, we are attempting a different strategy to study the effects of recolonization. This is not a major change in the goals of the project, but rather a small adjustment in the strategy. We are seeking IACUC approval for these new animal experiments.

6. Products

Nothing to report.

7. Participants & Other Collaborating Organizations

Name:	*Alessandro Bitto*
Project Role:	*PI/PD*
Researcher Identifier (e.g. ORCID ID):	0000-0002-6658-1931
Nearest person month worked:	*0.24*
Contribution to Project:	oversee the project and provide expertise in the rearing and handling on *Ndfus4*$^{-/-}$ mice
Funding Support:	K01 DK128128-01A1, R56AG082916-01, U01AG077920-04

Name:	*Kahte Culevski*
Project Role:	*RSE1*
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	*9.7*
Contribution to Project:	Handling, rearing, genotyping, scoring of mouse experiments.
Funding Support:	

R56AG082916-01 was awarded in July 2023.

No other organization to report.

8. Special Reporting Requirements

No special reporting requirements.

9. Appendices

No appendices.