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PRINCIPAL INVESTIGATOR: Crystal Jaing

CONTRACTING ORGANIZATION: Lawrence Livermore National Lab, Livermore,CA

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14. ABSTRACT

In this study, we will combine a rich database of patient clinical data, longitudinal collection of microbiome samples, state-of-the-art multiomic analyses, and advanced Bayesian ML methods to identify the microbial biomarkers predictive of ALS. This approach will provide a platform for identifying distinct microbial signatures linked to ALS risk and disease progression, elucidating potential solutions for treating ALS patients to slow down disease progression as well as for preventing ALS in high-risk deployed military personnel. We hypothesize that certain microbial species, or metabolites from the gut microbiome contribute to systemic re-conditioning and dysbiosis, thus leading to increased risk and more rapid progression of ALS. We will enroll 100 ALS patients and two different controls for each patient, a spouse/partner control and a control that is sex, age, and geographical location matched and will conduct comprehensive multiomics analyses, including fecal microbiome metabolomics and metagenomics. We will then utilize advanced ML approaches to identify the microbial markers most predictive of ALS risk and progression.

15. SUBJECT TERMS
None listed.

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TABLE OF CONTENTS

1. INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a devastating and fatal neurodegenerative disease. ALS etiology is still unknown, and the pathogenesis remains unclear. No effective neuroprotective therapy exists, and individual outcomes show considerable variation. The gut microbiome is an essential component of human function and could represent an integrator of overall environmental contribution to neurodegeneration. Recent microbiome studies of a limited number of ALS patients and controls showed potential differences in the gut microbiome of ALS patients. In this study, we will combine a rich database of patient clinical data, longitudinal collection of microbiome samples, state-of-the-art multiomic analyses, and advanced Bayesian machine learning (ML) methods to identify the microbial biomarkers predictive of ALS. Our multi-institutional, interdisciplinary team with clinical, epidemiological, experimental, and computational expertise is well positioned to carry out this highly innovative study. This approach will provide a platform for identifying distinct microbial signatures linked to ALS risk and disease progression, elucidating potential solutions for treating ALS patients to slow down disease progression as well as for preventing ALS in high-risk deployed military personnel. We hypothesize that certain microbial species, or metabolites from the gut microbiome contribute to systemic re-conditioning and dysbiosis, thus leading to increased risk and more rapid progression of ALS. We will enroll 100 ALS patients and two different controls for each patient, a spouse/partner control and a control that is sex, age, and geographical location matched and will conduct comprehensive multiomics analyses, including fecal microbiome metabolomics and metagenomics. We will then utilize advanced ML approaches to identify the microbial markers most predictive of ALS risk and progression.

2. KEY WORDS

ALS, microbiome, fecal sample, metagenomics, metabolomics, machine learning, outcome prediction

3. ACCOMPLISHMENTS

- Major goals of the project and what was accomplished during this performing year
  - The Specific Aims from the proposal are:
    - Specific Aim 1: Establish a Rich Database of ALS Patient Epidemiological Data and Microbiome Data to Identify Potential Biomarkers for ALS
    - Sub-Aim 1.1: Collect gut microbiome (fecal) samples and lifestyle questionnaire from ALS patients and healthy controls from UCSF ALS center and satellite clinics.
    - Sub-Aim 1.2: Characterize metagenomics and metabolomics of ALS patient biospecimens to determine microbial taxonomy and metabolite differences between ALS patients and healthy controls
    - Specific Aim 2: Integrate Patient Metadata, Metabolomics, and Metagenomics Data using Statistical and Computational Models to Understand Biomarkers Predictors of ALS Disease Onset and Progression.
  - For this report, we focus on Specific Aim 1, Sub-Aim 1.1. Progress has not been made in Aim 2.
  - Progress on Sub-Aim 1.1:

    Goal: IRB, DUA and MTA approval.

    Institutional Review Board (IRB) will be submitted and fully executed. Data Use Agreement (DUA) and Material Transfer Agreement (MTA) are already fully executed.

    Accomplishment:

- The patient consent form was approved on 7/19/2023. The IRB was approved on 8/8/2023, and it was renewed on 3/4/2024.

Goal: *OHRO review/approval.*

The approved Institutional Review Board (IRB) protocol will be submitted to OHRM for review and approval.

Accomplishment:

- OHRM submission was completed on 5/19/23.

Goal: Recruit ALS patients and controls.

100 ALS patients, including veterans from UCSF clinic and four satellite clinics (Monterey, Modesto, Fresno, Santa Rosa) within 18 months of ALS diagnosis will be enrolled. Two sets of matched controls—spouse control (100) and sex/age control (100) of subjects within 60 miles of UCSF/satellite clinics—will also be enrolled.

Accomplishment:

- A total of 100 ALS patients were prescreened from March 1, 2024 to May 28, 2024.
  - 41% (29) of ineligible patients due to diagnosis greater than 18 months ago (ALS Inclusion Criteria #2)
  - 31% (22) of ineligible patients due to ALSFRS-R Swallowing score of ≤2 (ALS Exclusion Criteria #4)
  - 14% (10) of ineligible patients due to GI Problem History (ALS Exclusion Criteria #6)
  - 9% (6) of ineligible patients due to clinically obvious cognitive impairment OR has other active major neurological diseases (ALS Exclusion Criteria #2 and ALS Inclusion Criteria #4, respectively)
  - 1% (1) of ineligible patients due to recent use of antibiotics <3 months (ALS Exclusion Criteria #8)
  - We have one patient enrolled and fecal sample was collected and shipping.
  - We are actively recruiting additional patients in all sites.
- The ALS screening criteria was established previously to ensure quality and consistency of gut microbiome.
- We are re-evaluating our prescreen criteria to determine if we can relax the criteria to allow more patients to be recruited to our study.
  - Our current eligibility is 18 months from diagnosis of ALS. We are considering relaxing the eligibility to 24-26 months from diagnosis of ALS.
  - Our current eligibility for patients with ALSFRS-R≥2. We are considering relaxing the eligibility to ALSFRS score ≤2.
  - We will look at all patients we already prescreened since March 2024 using these relaxed criteria and evaluate how many more patients will be eligible. If we adopt the relaxed criteria moving forward, we will submit modified IRB in June 2024 with the relaxed enrollment criteria.
- We identified a new satellite clinic at Santa Barbara which enables us to expand our patient enrollment.

Goal: Administer a baseline questionnaire to all ALS patients and controls.

An epidemiology and lifestyle questionnaire including questions on demographics, occupation history, medication, diet, and home environment will be administered.

Accomplishment:

- Dr. Nelson designed the epidemiology and lifestyle questionnaire using standardized questions from her past large-scale epidemiologic studies of ALS and from questions she has designed for use by the CDC National ALS Registry that have been pre-tested and widely used. The questionnaire includes questions on demographic characteristics, occupational history, home pesticide use, hobbies involving toxicant exposure, cigarette smoking, alcohol consumption, caffeine consumption, and family history of ALS. In addition, questions are asked regarding factors that can influence microbiota, including medications (aspirin, proton pump inhibitors, antibiotics etc.).
- In addition, Dr. Nelson designed an approach to obtaining a subject's usual food, drink and supplement intake for 4-week period immediately prior to fecal sample. Dr. Nelson chose a validated instrument, the Dietary Health Questionnaire III (DHQIII) National Cancer Institute food frequency questionnaire [https://epi.grants.cancer.gov/dhq3/]. The DHQIII consists of 135 food and beverage items and 26 dietary supplement questions. This NCI resource enables the survey to be web-administered directly to the participants, with nutrient values ascribed to usual diet automatically calculated. Dr. Nelson constructed additional questions to include foods that have a strong influence on gut microbiota for which no standardized questions were available. She conducted a thorough literature review to inform the construction of the additional questions.

Goal: Follow up prospective ALS cohorts.

ALS patients will be followed up every 3 months. All demographic, clinical history, ALS functional rating scale data will be entered into the MOVR (neuroMuscular ObserVational Research) developed by the Muscular Dystrophy Association (MDA).

Accomplishment:

- One patient was entered in MOVR and will be followed up.

Goal: Collect fecal samples.

Each patient and control will be provided two fecal collection kits to send to UCSF. Samples will be de-identified and anonymized and sent to Lawrence Livermore National Laboratory (LLNL).

Accomplishment:

- Patients were provided collection kits and shipping labels for sample collection and shipping to LLNL.

- What opportunities for training and professional development has the project provided?

- The project provided training and development opportunities for Dr. Carol Mascarenhas, a post-doctoral fellow at Lawrence Livermore National Laboratory and she started in Oct 2023. Dr. Mascarenhas is being trained to conduct microbiome analysis of samples and will be working on the microbiome samples collected during the duration of the project. She has participated in the project biweekly meetings and presented a poster of LLNL genomic capabilities at the LLNL-UC ALS workshop (May 2024) to foster collaborations in ALS research.
- Zane Ashkar was hired in Sept 2023 at University of California, San Francisco as a student intern and clinical research coordinator. He was onboarded with Good Clinical Practice Training; trained on MOVR and is onboarding an additional coordinator to go to clinics (training this individual). Through this project, Zane has been provided the unique opportunity to spearhead patient recruitment and trouble-shoot along the way. In his role at UCSF, many of Zane's clinical research responsibilities are in established clinical trials with a set protocol. This project has allowed him to be involved in design of recruitment methods and collaborate with investigators from other centers.

- How were the results disseminated to communities of interest?

  - Project members participated in the Inaugural UC system-wide ALS Workshop, *Reinvigorating Innovation in ALS*, held in May 16-17, 2024 in Livermore, California. The methodologies and approach of the project were discussed with workshop participants.
  - The project team plans to attend and present a poster of our work at the International ALS Meeting in Dec. 2024 and at the January 2025 CA ALS Research Summit.

- What do you plan to do during the next reporting period to accomplish the goals?

  - Specific Aim 1: Establish a Rich Database of ALS Patient Epidemiological Data and Microbiome Data to Identify Potential Biomarkers for ALS
    - Sub-Aim 1.1: Collect gut microbiome (fecal) samples and lifestyle questionnaire from ALS patients and healthy controls from UCSF ALS center and satellite clinics.
      - We are planning to recruit a total of100 patients and 200 controls. We will re-evaluate our prescreen criteria to relax the criteria to allow more patients to be recruited to our study. This includes opening up the eligibility to 24-26 months from diagnosis of ALS and patients with ALSFRS score ≤2. We will submit modified IRB in June 2024 with the relaxed enrollment criteria.
      - We will provide fecal collection kits and shipping labels to patients and controls so they can ship samples to LLNL.
    - Sub-Aim 1.2: Characterize metagenomics and metabolomics of ALS patient biospecimens to determine microbial taxonomy and metabolite differences between ALS patients and healthy controls.
      - We will conduct metagenomics analysis at LLNL to determine microbial profiles from patients and controls.
      - We will conduct metabolomics analysis at UCSF to determine microbial metabolite profiles from patients and controls.
    - Specific Aim 2: Integrate Patient Metadata, Metabolomics, and Metagenomics Data using Statistical and Computational Models to Understand Biomarkers Predictors of ALS Disease Onset and Progression.
      - We plan to integrate patient data from MOVR, metagenomics and metabolomics data and apply machine learning and statistical analysis to understand the biomarkers predictive of ALS onset and progression.

4. IMPACT

- **What was the impact on the development of the principal discipline(s) of the project?**

  - Traditionally, ALS clinical research targets patients who live in cities near large ALS multidisciplinary clinics and who are able to travel in person for research visits. Our proposal changes this traditional model and brings the clinical research to where our patients live, focusing on five California regions where we have satellite locations of ALS care—San Francisco, Santa Rosa, Monterey, Modesto, and Fresno. We have also recently added a new satellite location in Santa Barbara. We will use the MOVR (neuroMuscular ObserVational Research) database, developed by the Muscular Dystrophy Association, to track all ALS patients from the day of their diagnosis until their death, with important subtype information, including site of onset, spread over time, genetic markers, and rate of progression. Our research project will leverage our outstanding scientific collaborations and deep clinical,

experimental, and computational expertise to study regional ALS populations (and controls), including patients in rural or underserved areas. We will correlate the biomarkers we collect with clinical markers to establish predictors of risk, rate of progression, and survival.

- **What was the impact on other disciplines?**
    - The team plans to publish results in peer-reviewed journals and present data at national ALS conferences and the Military Health System Research Symposium (MHSRS). Our information sharing will trigger interest from the scientific community in advancing therapeutic development for ALS. Data collected during this study will be made publicly available after results are successfully cleared by the PI's institutional and DoD/CDMRP document review processes.
- **What was the impact on technology transfer?**
    - Upon completion of this project, the MOVR database will be updated. The broader scientific community can request access to the MOVR database to review the clinical data collected from this study. All raw and processed data with a descriptive metadata file will be uploaded into corresponding NCBI databases or other government-identified repositories for data archiving. The integrated analysis of clinical, demographic, survey, and multiomic data and result summaries from these data will be made available via a project publication. The LLNL-developed codes will be distributed to the project collaboration team, and then to the wider research community via publicly available LLNL GitHub repositories with open-source licensing, concurrently with project publication. Results will be made available via a project publication.
- **What was the impact on society beyond science and technology?**
    - This innovative project will advance development of ALS therapeutics and lead to improvement of patient quality of care. One immediate application from our proposal, if we identify particular bacteria or their metabolites that affect disease onset or rate of disease progression, would be treating patients with targeted metabolites, antibiotics, and/or probiotics to create a healthier gut microbiome. In addition to helping current patients, unlike most other therapies under development for ALS, this low-cost therapy would be available for groups at high risk of ALS to use prophylactically and thus to potentially begin protecting our military personnel from this fatal disease before and during deployment.
    - The project will have an impact on the caregiver community to understand better practice to care for ALS patients.

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**
    - Patient enrollment was delayed due to several reasons:
        - Project execution was delayed due to PI change from Dr. Michael Morrison to Dr. Crystal Jaing at LLNL in Nov 2023.
        - Project execution was delayed due to a staffing change from Dr. Moriah Sandy to Dr. Darren Dumlao to lead the metabolomics analysis at UCSF.
        - Recruitment was delayed due to delay in procuring and shipping of fecal collection kits for metabolomics and metagenomics analysis.

- Enrollment was delayed due to the development of the questionnaire that would be used for initial pre-screening. We wanted to make sure the questionnaire was as complete as possible to fully understand the various aspects that may impact a patient's microbiome.

- **Changes that had a significant impact on expenditures**
  - Nothing to Report.
- **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**
  - *Nothing to Report*
- **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?**

Name:	Crystal Jaing
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-9933-3005
Nearest person month worked:	0.5
Contribution to Project:	Dr. Jaing leads the overall research project. She manages the collaborations with UCSF and Stanford,

	runs biweekly meetings, and monitors progress toward research aims and objectives.
Funding Support:	This award

Name:	Carol Mascarenhas
Project Role:	Post-doc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.2
Contribution to Project:	Dr. Mascarenas participates in project meetings and learning microbiome analysis.
Funding Support:	This award

Name:	Priyadip Ray
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.2
Contribution to Project:	Dr. Ray participates in project meetings and will conduct computational analysis of microbiome and clinical data.
Funding Support:	This award

Name:	Catherine Lomen-Hoerth
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.5
Contribution to Project:	Dr. Lomen-Hoerth directs the ALS clinical research at UCSF, patient enrollment, patient follow up, and MOVR data. She also meets with the LLNL team regularly to update progress on the project.
Funding Support:	UCSF

Name:	Zane Ashkar
Project Role:	Clinical coordinator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.5

Contribution to Project:	Mr. Ashkar is a student and Clinical Coordinator for the UCSF ALS clinic. He facilitates patient prescreen, phone interview and patient onboarding.
Funding Support:	This award

Name:	Hannah George
Project Role:	Clinical coordinator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.5
Contribution to Project:	Ms. George is the Clinical research coordinator for this project. She leads the IRB submission, coordinates patient screening and recruitment.
Funding Support:	This award

Name:	Darren Dumlao
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0
Contribution to Project:	Dr. Dumlao leads the metabolomics analysis of patient and control fecal samples for the project.
Funding Support:	UCSF

Name:	Amy Gryshuk
Project Role:	Strategic Alliances
Researcher Identifier (e.g. ORCID ID):	0000-0003-0416-3934
Nearest person month worked:	0.1
Contribution to Project:	Dr. Gryshuk serves as an UCSF Alliance Manager supporting the multi-institutional collaborative project.
Funding Support:	UCSF internal

Name:	Lorene Nelson
Project Role:	Co-Investigator, Epidemiologist
Researcher Identifier (e.g. ORCID ID):	0000-0002-9813-8088
Nearest person month worked:	0.6
Contribution to Project:	Epidemiology and statistical methods

Funding Support:	This award

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

    - Dr. Darren Dumlao from the UCSF Metabolomics Core has replaced Dr. Moriah Sandy to lead the metabolomics analysis of fecal samples.

- **What other organizations were involved as partners?**

    - Nothing to report.