AWARD NUMBER: W81XWH-17-1-0633

TITLE: Environmental and Nutritional Risk Factors for NF1-related tumors

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CONTRACTING ORGANIZATION: University of Minnesota, Minneapolis, MN

REPORT DATE: September 2020

TYPE OF REPORT: Annual Report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2020	Annual	15Sep2019 - 14Sep2020

4. TITLE AND SUBTITLE
Environmental and Nutritional Risk Factors for Nf1-Related Tumors

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0633

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 Materiel 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
Neurofibromatosis type 1 (NF1) is a genetic disorder. Its clinical severity is extremely variable, even among members of the same family. The source of this clinical variation is unknown, and may involve additional genetic changes, or possibly, environmental factors such as nutritional status. The *primary objectives* of this project are to 1) establish whether maternal dietary folic acid level during the peri-gestational period (in and around pregnancy) can influence the rate and severity of NF1-related tumor development in offspring in a highly controlled experimental setting using a transgenic mouse model; and 2) demonstrate feasibility of clinic-based recruitment of NF1 patients and their families for a comprehensive epidemiologic study of environmental and nutritional factors that modify risk of NF1-related tumors, with the long-term goal of conducting a large NIH-funded epidemiologic study of NF1. In the first year of this project we have worked on obtaining the necessary approvals, we have created the patient questionnaire, and optimized recruitment. We have also competed all preparations necessary to begin mouse work.

15. SUBJECT TERMS
Neurofibromatosis type I
Folic acid

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	10	19b. TELEPHONE NUMBER *(include area code)*

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Neurofibromatosis type 1 (NF1) is a genetic disorder. Its clinical severity is extremely variable, even among members of the same family. The source of this clinical variation is unknown, and may involve additional genetic changes, or possibly, environmental factors such as nutritional status. The *primary objectives* of this project are to 1) establish whether maternal dietary folic acid level during the peri-gestational period (in and around pregnancy) can influence the rate and severity of NF1-related tumor development in offspring in a highly controlled experimental setting using a transgenic mouse model; and 2) demonstrate feasibility of clinic-based recruitment of NF1 patients and their families for a comprehensive epidemiologic study of environmental and nutritional factors that modify risk of NF1-related tumors, with the long-term goal of conducting a large NIH-funded epidemiologic study of NF1.

2. **KEYWORDS:**

Neurofibromatosis type I
Folic acid

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

There are two specific aims of this project and 4 major tasks associated with these aims:
Specific Aim 1: To determine whether maternal dietary folic acid level during the perigestational period influences the incidence and severity of NF1 related tumor development in the offspring in a transgenic mouse model.
Major Task 1: Randomization and mating of female *Nf1*$^{flox/flox}$*; Pten*$^{flox/flox}$ mice
Major Task 2: Conduct aging experiment

Specific Aim 2: To conduct pilot recruitment for an epidemiologic study of NF1 cases that will examine the impact of *in utero* and early life environmental, genetic and nutritional factors that may contribute to NF1 phenotypic variability.
Major Task 3: Identify and recruit patients in an NF1 clinic
Major Task 4: Collect DNA samples, questionnaire data, and birth and medical records

What was accomplished under these goals?

Since the beginning of this award, we have obtained UMN IRB approval (March 29, 2018) and HRPO approval (April 3, 2019). We also finalized the human subjects questionnaires (child, mother, and father), and developed the online survey instrument. We began recruitment optimization for human subjects recruited from the University of Minnesota Comprehensive Neurofibromatosis Clinic in April 2018. We have supplemental funding from a local nonprofit institution to recruit 50 case-parent trios in addition to those recruited through DoD funds. While we awaited HRPO approval, we optimized recruitment using the nonprofit funds. We recruited for 12 months (April 2018 – March 2019) and enrolled 58 families in that time. Since receiving HRPO approval on April 3, 2019, we have enrolled 29 NF1 families using DoD funds, for a total of 87 families. Since some families have more than one affected child, this reflects 104 children with NF1 enrolled on the study. Due to the COVID-19 pandemic, recruitment was disrupted in March 2020. We will continue to recruit and enroll families to reach our recruitment goals.

We received University of Minnesota IACUC approval on July 30, 2018. We submitted documents for ACURO approval on August 16, 2018 and received approval on October 25, 2018. We began breeding mice for use in the project in November 2018. As of November 12, 2019 we have generated all $Nf1^{flox/flox}$; $Pten^{flox/flox}$ female mice and 16 of the necessary male mice. We are currently generating additional *Dhh*-Cre; $Nf1^{flox/flox}$ male mice. These females and males will be the parent generation of our experimental animals. We randomized the first set of 20 female mice to the experimental diets in the last week of May 2019 and they started experimental diets at that time. Randomization occurred according to the unbalanced sample size design: 10 mice to the low folic acid group, 7 to the control group, and 3 to the high folic acid group. Due to problems generating the male mice at that time, no males were available for mating during the fertile period of these females and they were sacrificed. We generated additional females in parallel with generation of males and we currently have more than the required 60 females required for mating and therefore will be prepared to generate offspring more quickly than expected once males are ready for mating.

On December 8, 2019, Bryant Keller, the graduate student responsible for mouse breeding, feeding, genotyping, and weaning, passed away suddenly and unexpectedly. Unsurprisingly, this has presented a significant challenge for the mouse work within this study. We are currently working to reconstitute the work done to date and gather all the files necessary to catalog the mice and resume work on the project. This has involved working with Bryant's family at a very sensitive time to gain access to his laptop which was in his apartment at the time of his death. We are also reviewing the paper files he kept in his lab desk.

Some staff members in Dr. Largaespada's laboratory stepped in to assist with the project until we were able to hire a full time staff member to resume the work. We were able to access Bryant's electronic files and in February of 2020 we determined that all mice previously bred for this project were unfortunately too old to continue breeding and that the best course of action would be to generate new breeding pairs. As of May 2020, laboratory staff is able to resume animal research at 50% time. We resumed breeding and have 16 females on the experimental diet as of October 2020. We have additionally have 4 males available to mate with females once they have finishing the one-month experimental diet run-in. A chart showing the females currently enrolled is below. We have several more females that we expect to enroll on experimental feed within the coming month.

We have recruited a senior level technician, hired as a Research 5, Mr. Justin Tibbits, to replace Bryant's efforts. Mr. Tibbitts has extensive experience in mouse models of cancer and mouse husbandry, having spent the last 8 years working with Dr. Lisa Coussens at the Oregon Health Sciences University. Mr. Tibbits started on June 8, 2020 and has quickly become familiar with the project's status and goals.

Mouse Number:	DOB:	Folate Feed Level:	Date placed on feed:
F569	7/30/20	0.3 mg/kg	10/5
F570	7/30/20	2.0 mg/kg	10/5
F571	7/30/20	8.0 mg/kg	10/5
F572	7/30/20	0.3 mg/kg	10/5
F600	7/30/20	0.3 mg/kg	10/5
F574	8/8/20	0.3 mg/kg	10/5
F575	8/8/20	0.3 mg/kg	10/5
F576	8/8/20	2.0 mg/kg	10/5
F577	8/8/20	2.0 mg/kg	10/5
F591	9/11/20	8.0 mg/kg	10/5/20
F592	9/11/20	0.3 mg/kg	10/5/20
F593	9/11/20	2.0 mg/kg	10/5/20
F594	9/11/20	0.3 mg/kg	10/5/20
F595	9/11/20	0.3 mg/kg	10/5/20
F601	9/19/20	2.0 mg/kg	10/19/20
F603	9/20/20	2.0 mg/kg	10/19/20

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

In April 2019 I attended the American Association for Cancer Research annual conference in Chicago, IL. While there I met with colleagues, including Dr. Kimberly Johnson who is a Co-Investigator on this project, and attended relevant sessions on NF1 biology and treatments.

How were the results disseminated to communities of interest?

In October 2020 I gave a presentation at the Minnesota Neurofibromatosis Symposium to present this ongoing work to patients and family members.

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

During the next year we plan to continue recruitment from the UMN Comprehensive Neurofibromatosis Clinic. We also plan to continue breeding and aging for the mouse study.

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?

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

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

The COVID-19 pandemic has delayed our work since March 2020 when all laboratory research was paused. As of May 2020, laboratory staff was able to resume work at 50% time. We now have active breeding and mating ongoing for this project and we do not anticipate further delays.

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

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

Not applicable

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.

  Nothing to report

  Books or other non-periodical, one-time publications.

  Nothing to report

  Other publications, conference papers and presentations.

  Nothing to report

- **Website(s) or other Internet site(s)**

  Nothing to report

- **Technologies or techniques**

- Nothing to report

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**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Erin Marcotte, PhD
Project Role: Principal Investigator
Nearest person month worked: 1
Contribution to project: No change

Name: Justin Tibbits
Project Role: Researcher
Nearest person month worked: 2
Contribution to project: Managed mouse breeding, feeding, and performed genotyping

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?

Washington University in St Louis
St Louis, MO
Dr. Kimberly Johnson serves as Co-Investigator on this project.

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.