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

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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
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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 (60% complete)
Major Task 2: Conduct aging experiment (0% complete)

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 (90% complete)
Major Task 4: Collect DNA samples, questionnaire data, and birth and medical records (60% complete)

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 21 NF1 families using DoD funds, for a total of 79 families. Since some families have more than one affected child, thus we have a total of 93 children with NF1 enrolled on the study. We will continue to recruit and enroll families to reach our recruitment goals.

To date, we have collected DNA for all 93 affected children within the 79 families, including 47 trios, 29 duos, and 3 case only samples. We have consents to obtain newborn blood spots for all 93 cases, and of those we have successfully retrieved 10 (only children born in Minnesota since August 1, 2014 will have a retrievable blood spot). We have obtained phenotype questionnaire data for all 93 affected cases. Additionally, we have signed medical record consent forms for all 93 cases and have retrieved 11 of those to date.

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 October 28, 2019 we started 37 female mice on the experimental diet and they were randomized according to the experimental design (18 deficient folic acid, 13 control, 6 supplemented folic acid). On November 4, 2019 we started an additional 17 females on the experimental diet (8 deficient folic acid, 6 control, 3 supplemented folic acid). Thus we currently have 54 female mice on experimental diets. The remainder will begin experimental diet during the week of November 18. We currently have 16 male mice available for mating, and given the rolling start of females on experimental diets, these will be a sufficient number for mating the first and second waves of females as they finish the month long experimental diet prior to mating. Additionally, we are generating more male mice currently. We expect to have the first litters of pups by mid- to late- December.

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?

Nothing to report

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

Some of the male mice we have bred have had health problems such as hind limb paralysis and therefore would not be appropriate breeders. We have worked to generate additional male mice to have the number we need.

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: Michelle Roesler
Project Role: Coordinator
Nearest person month worked: 1
Contribution to project: Managed creation of the REDCap database, prepared all documents for start of human subjects recruitment

Name: Bryant Keller
Project Role: Graduate Student
Nearest person month worked: 1
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?

GRANT AWARDED:
No Sponsor Award # Marcotte (PI) 7/1/18-6/30/20
Children's Cancer Research Fund
'Telomere length as a predictor of age at diagnosis of neurofibromatosis type-I associated tumors'
Major Goals: This peer-reviewed award supports a project to determine whether telomere length at birth and the telomere genetic risk score are predictive of age at tumor diagnosis among children with NF1.
Role: Principal Investigator

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:

QUAD CHARTS:

9. APPENDICES: