Consortium on Law and Values in Health, Environment & the Life Sciences

2016-17 Student Proposal Cover Page

Applicant Information				
Applicant name(s):	Neely C. Miller		Email:	mill1425@umn.edu
Project title:	Maternal Pre-Pregnancy Body Mass Index (BMI) and Infant Neurodevelopment			
Department:	Maternal & Child Health	College:	School of Public Health	
Degree program:	Masters of Public Health	Faculty advisor name & email:	Ellen W. Demerath, Ph.D. ewd@umn.edu	☐ NA
Dept. Head:	Dianne Neumark-Sztainer PhD, MPH, RD	Dept. Head's email:	neuma011@umn.edu	
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How did you hear about this funding opportunity?
☒ Consortium e-mail ☐ Graduate & Professional Student Update ☐ The Brief ☐ Advisor ☐ Dept. email/newsletter ☐ Consortium website ☐ Other

Funding

Total amount of funding requested: **$7000.00**

Executive summary (maximum 200 words)

This project represents an interdisciplinary collaboration that combines electrophysiological measures of brain activity with analysis of inflammatory markers and infant microbiome characteristics to examine whether greater pre-pregnancy maternal weight is associated with perturbations in offspring brain function. We plan to enroll eighty pregnant women (40 in a high BMI and 40 in a normal BMI group) in a longitudinal study to investigate a model in which the association between maternal BMI and infant neurodevelopment during the first year of life is mediated by exposure to in-utero maternal cytokines and correlated with distinct infant gut microbial community features.

Approvals

Check all appropriate approvals required for your proposal. It is not necessary to have all approvals at the time of proposal submission; however, approvals must be obtained prior to receipt of funding. If you have applied for approval but have not yet received it, indicate that below.

IRB ☒ Yes ☐ No ☐ NA ☐ Application pending

Other ☐ Yes ☐ No ☒ NA ☐ Application pending Specify:

Checklist—for reviewer use

☒ The proposal is 1000 words or less excluding budget, biographies, references and citations.

☒ The proposal includes a work plan with a specific timeline using months or quarters to identify work to be done and completion dates.

☒ The proposal includes a 1-2 paragraph biography of the applicant and all co-investigators.

☒ The budget form is complete including the funds sought for this project, other pending applications for this project, and the amount/source of matching or other funds.

☒ The applicant's faculty advisor is copied on the application email. Professional students w/o advisors check NA.

☒ All necessary approvals are pending or received.

Background and Importance

This study will test the hypothesis that pre-gravid maternal obesity is associated with altered patterns of infant brain function. Specifically, we will quantify hippocampal function (recognition memory) and determine the extent to which this correlates with infant exposure to inflammation and altered gut microbial communities in infants born to obese as compared to non-obese mothers. Research supports the idea that chronic inflammation may have deleterious effects on fetal hippocampal development [1,2,3]. Obesity is associated with a chronic inflammatory state[4]; the increased levels of pro-inflammatory cytokines[5] are capable of crossing the placenta and fetal blood brain barrier[6]. Thus, inflammation is one potential mechanism whereby maternal obesity may cause disruption to hippocampal development. Postnatally, exposure to inflammation persists; breast milk contains both pro- and anti-inflammatory cytokines that pass into infant circulation and affect infant growth[7]. The effects of prenatal and post-natal (breast-milk) exposure to pro-inflammatory cytokines on brain development are not yet known.

Maternal obesity may also affect the infant microbiome postnatally. Breastfeeding promotes the colonization of the infant microbiome, and the maternal microbiome is an important contributor to infant health. It has been hypothesized that "dysbiotic" gut microbial communities present during early life induce altered development of many human systems, including the brain[8]. In healthy breast-fed infants, *Bifidobacteria* are the predominant bacterial taxa in the gut, and play a role in inflammatory and immune system regulation[9]. Lower concentrations of *Bifidobacteria* and higher concentrations of *Staphylococcus* have been found in the microbiota of infants of overweight mothers[9], demonstrating that maternal weight is an important determinant of the infant microbiome and raising the possibility that obesity-related effects on the infant microbiota could have broader impact on brain development. The hippocampus is a target of microbiome-mediated effects on brain function in adult mice; however, the relevance of these results for human infants remains unknown.

Preliminary retrospective analysis of data from our laboratory has shown an association between pre-pregnancy maternal weight and infant recognition memory that persists through the first six months of life. My masters' project expands upon this work to investigate the extent to which this phenotype is associated with inflammation and infant gut microbes. The specific hypotheses to be tested are 1) Maternal obesity is associated with poorer hippocampal-based electrophysiology outcomes; 2) Maternal obesity is associated with an increased state of post-natal inflammation (as indexed by levels of pro-inflammatory cytokines in breast milk); 3) Maternal BMI, breast milk inflammatory markers and infant brain function are correlated with distinct infant gut microbial community features.

Methods

Study Population: We will enroll 40 obese (pre-pregnancy BMI>30) and 40 normal weight (pre-pregnancy BMI<25) mothers along with their 1 month old infants from Dr. Demerath's "Maternal Obesity, Breast Milk Composition, and Infant Growth" (IRB# 1404M50203) study. All of the infants in the parent study are full-term, without known growth or neurological conditions, free of exposure to maternal diabetes and hypertension, and without prenatal or post-natal drug exposure. All infants in the parent study are exclusively breast-fed to 1 month of age, and at least partially breast-milk fed to 3 months of age. Infants will have neurodevelopmental testing at the Center for Neurobehavioral Development at 1 and 6 months of age.

Measures of Memory: Electrophysiological data will be obtained at 1 and 6 months of age to assess hippocampal function. Infants will be fitted with a Geodesic Sensor Net that can used to collect event-related potential (ERP) data from multiple sites on the scalp.

- **1 month:** Infants will be tested using an established mother/stranger voice recognition memory ERP [10].
- **6 month:** Infants will be tested using a mother/stranger face visual recognition memory ERP[11].

Characterization of Infant Microbiome: We will collect feces from diapers at the 1-month and 6-month study visits. Bacterial 16S rDNA sequences will be generated from the samples, taxonomically classified, and community diversity and dominant taxa will be determined.

Breast Milk Inflammatory Markers: Cytokine levels will be assessed on breast milk samples collected at one and three months after birth.

To assess potential confounders, mothers will complete questionnaires regarding infant feeding and sleep, as well as reproductive history, weight history, age, diet, exercise, maternal and infant exposure to antibiotics, mental health, stress and socio-demographic information.

Statistical analysis plan:

Hypothesis 1: Maternal obesity is associated with poorer hippocampal-based electrophysiology outcomes

Analysis: We will use linear regression to test whether maternal pre-pregnancy BMI correlates with electrophysiologic outcome variables, controlling for potential confounders (noted above).

Hypothesis 2: Maternal obesity is associated with an increased state of post-natal inflammation

Analysis: We will calculate the ratio of pro-inflammatory vs. anti-inflammatory cytokines in milk samples and examine associations of maternal BMI with inflammation.

Hypothesis 3: Maternal BMI, breast milk inflammatory markers, and infant brain function are correlated with distinct infant gut microbial community features

Analysis: We will use linear regression to test whether infant gut bacterial community features (diversity, dominant taxa) correlate with maternal BMI, ERP metrics, and breast milk inflammatory markers, controlling for potential clinical confounders (noted above) with multivariable regression.

We predict that infants in the high maternal BMI group will demonstrate altered ERP responses to stimuli and that these alterations are associated with increased inflammatory marker concentrations in breast milk and altered bacterial community features as compared to infants in the normal maternal BMI group.

Contribution to Interdisciplinary Work

This study will be among the first to examine how variation in gut microbiomes is associated with early brain development in humans. The Center for Neurobehavioral Development is one of only a few centers across the world that is able to measure hippocampal function in pre-verbal infants, uniquely positioning our team to investigate the role of maternal gut microbes and inflammation during gestation on offspring neurodevelopment. Given the prevalence of obesity among women of reproductive age, obesity's effect on fetal development is of grave public health concern. Our proposal will link infant neurodevelopment to maternal weight, and help pinpoint the mechanisms by which maternal inflammation can affect the developing brain.

Project Timeline

Enrollment for this study is ongoing; data collection will continue through mid-2018.

	January 1- March 31, 2017	**April 1- June 30, 2017**	**July 1- September 30,**	**October 1- December 30,**	**January 1- March 31, 2018**	**April 1-June 30, 2018**
Enrollment	x	x	x	x		
ERP data collection	x	x	x	x	x	x
ERP data analysis	x	x	x	x	x	x
Milk sample analysis				x	x	x
Fecal sample analysis			x	x	x	x
Consortium final report						x

Biographies

Neely Miller, B.S. I am a student in the Maternal and Child Health Program in the School of Public Health at the University of Minnesota. My research expertise is in assessing brain function in children using event-related potentials. Previous and ongoing work at the University of Minnesota has focused on neurocognitive outcomes in at-risk children: infants born prematurely, children exposed to naturally occurring minerals or alcohol while in utero, infants of diabetic mothers, and infants at risk for developing autism spectrum disorder.

My research focuses on the effects of maternal nutrition and inflammation on fetal/infant neurodevelopment; exploring the role of gut microbes in neurodevelopment is a natural extension of this research.

Ellen Demerath, Ph.D. (co-I; advisor) is an epidemiologist who studies developmental origins of human chronic disease. Dr. Demerath has extensive experience in designing longitudinal clinical studies; she recently completed a 5-year study of the genetics of infant growth and later obesity among participants in the Fels Longitudinal Study.

Cheryl Gale, M.D. (co-I) is a pediatric neonatologist and a fungal molecular and cellular biologist. Her laboratory elucidated pathogenic fungal-host interaction mechanisms in the premature infant intestine as well as led the collaborative research team that developed new genomic strategies to characterize fungal microbiomes in the infant intestine.

Michael Georgieff, M.D. (co-I) is a pediatric neonatologist and is the Director of the Center for Neurobehavioral Development. Dr. Georgieff is one of the world's experts on the role of nutrients in brain development as well as the development of novel neuroimaging tools to quantify hippocampal function in infants and young children.

Dan Knights, Ph.D. (collaborator) is a computational biologist with extensive expertise in microbial DNA sequence data analysis including predictive modeling of microbiome temporal dynamics, inference of microbial function from sequence data, as well as identification of microbial interactions. Dr. Knights introduced machine learning to the field of microbiome-based biomarker discovery and has continued to develop novel bioinformatics methodologies for linking microbiome structure and function to clinical outcomes in humans.

Michael Sadowsky, Ph.D. (collaborator) is a microbiologist and molecular microbial ecologist who has extensive experience in microbial DNA sequencing and analysis of bacterial metagenomes and is an international leader in the field of human GI tract microbiology and microbiomes. In addition, he has a successful track record in collaborating with physicians to bring microbiome-based therapies to the clinic, as evidenced by his work in transplantation of fecal microbial communities into patients with *Clostridium difficile* colitis.

References

1**.** Niculescu MD, Lupu DS. High fat diet-induced maternal obesity alters fetal hippocampal development. Int J Dev Neurosci 2009;27(7):627-633.

2. Tozuka Y, Wada E, Wada K. Diet-induced obesity in female mice leads to peroxidized lipid accumulations and impairment of hippocampal neurogenesis during the early life of their offspring. FASEB J 2009;23(6):1920-1934.

3. Bilbo SD, Tsang V. Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. FASEB J 2010;24(6):2104-2115.

4. Ramsay JE, Ferrell WR, Crawford L, Wallace AM, Greer IA, Sattar N. Maternal obesity is associated with dysregulation of metabolic, vascular, and inflammatory pathways. J Clin Endocrinol Metab 2002;87(9):4231-

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6. Zaretsky MV, Alexander JM, Byrd W, Bawdon RE. Transfer of inflammatory cytokines across the placenta. Obstet Gynecol. 2004;103(3):546-550.

7. Schuster S, Hechler C, Gebauer C, Kiess W, Kratzsch J. Leptin in maternal serum and breast milk: Association with infants' body weight gain in a longitudinal study over 6 months of lactation. Pediatr Res. 2011;70(6):633-7.

8. Borre YE, O'Keeffe GW, Clarke G, Stanton C, Dinan TG, Cryan JF. Microbiota and neurodevelopmental windows: Implications for brain disorders. *Trends in Molecular Medicine*. 2014;20(9):509–518.

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10. deRegnier RA, Nelson CA, Thomas KM, Wewerka S, Georgieff MK. Neurophysiologic evaluation of auditory recognition memory in healthy newborn infants and infants of diabetic mothers. J Pediatr. 2000;137(6):777-784.

11. De Haan, M. & Nelson, C.A. Recognition of the mother's face by 6-month-old infants: A neurobehavioral study. Child Dev. 1997; 68: 187-210.

Consortium on Law and Values in Health, Environment the Life Sciences
Proposed Budget

Project Title: Maternal Pre-Pregnancy Body Mass Index (BMI) and Infant Neurodevelopment

	Provide justification along with costs.		**Requested funding**	**Matching/other funding** *Provide this information is you have other funding sources for this project.*	
	Category& instructions	**Justification**	**Amount**	**Amount**	**Source**
1	Your stipend *Maximum of $5,000*	*Collection/artifact detection/analysis of high-density ERP data; 2.5 hours/session * 160 sessions @ hourly rate of $22.50 (Note: total amount exceeds allowable stipend)*	$5,000		
2	Speaker honoraria (for colloquia)		$0		
3	Supplies & Services *Identify and explain use here or in the body of your proposal.*	*DNA isolation, amplicon generation, and sequencing at the U of MN Genomics Center; 80 samples @ $25/sample* *(Note: The total sample size is 160. Award from Masonic Children's Hospital Research Fund will supplement.)*	$2,000	$2,000	Masonic Children's Hospital Research Fund
4	Equipment *Identify and explain use. Allowable only if the equipment is necessary for this project. All equipment must be given to your dept. at the completion of your project.*		$0		
5	Travel *Indicate the purpose of the travel, estimated dates of travel, transportation, housing and allowable per diem costs (see travel.umn.edu).*		$0		
	Subject Reimbursement	Subjects receive a $20 gift card at each session. 80 subjects * $20 * 2 sessions = $3200. This portion of the project was funded by the J.B. Hawley Student Research Award.	$0	$3,200	J.B. Hawley Student Research Awards program
		Subtotal research expenses (2-5)	**$7,000**	**$5,200**	
	TOTAL BUDGET		$7,000	$5,200	