

F. B. Will Williams

Final Report: 2018 Queenan Investigator-Initiated Research Project Award

Mentors: Drs. Benjamin Lee and Marjorie Myer

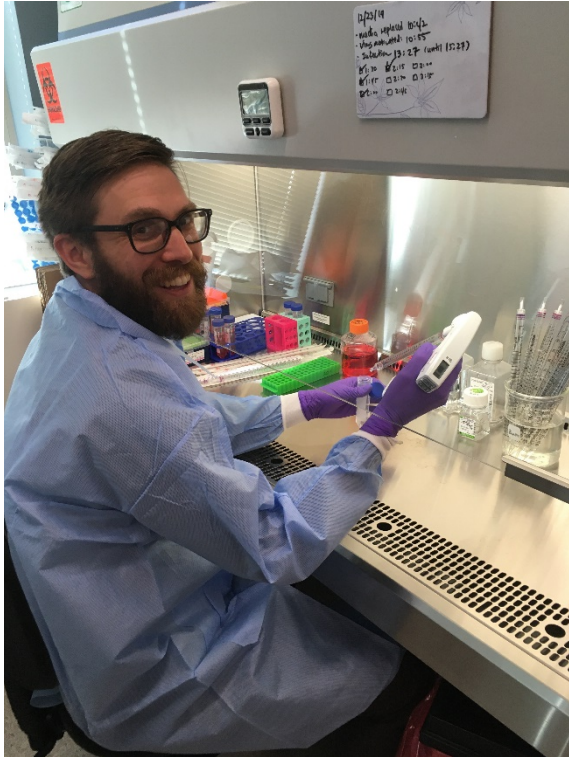
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“Maternal Histoblood Group Antigen Genotype as a Predictor for Rotavirus Vaccine Response in Breastfed Infants in Bangladesh.”

I am pleased to report that our investigations into the contributions of genetically determined breast milk components to infant rotavirus vaccine response have concluded successfully. As described in prior correspondence, our primary outcomes returned with intriguing results, and have subsequently prompted new lines of inquiry.

The Queenan Award allowed me to accomplish essential aspects of my project investigating the role maternal breast milk plays in infant rotavirus vaccine response. Our hypothesis was that maternal secretor (*FUT2*) genotype contributed to infant response to an oral rotavirus vaccine. To begin the project, I was able to characterize the maternal secretor (*FUT2*) genotype of our vaccinated cohort. Our collaborators in Dhaka concurrently completed secretor and Lewis (*FUT3*) phenotypic analysis of breast milk samples. Our preliminary analysis revealed an association between maternal secretor status and infant vaccine seroconversion, consistent with our hypothesis; to our surprise, the association runs in the opposite direction of our prediction. Both phenotypic and genotypic analysis of maternal secretor status shows that infants of nonsecretor mothers had higher rates of seroconversion. In regression modeling, maternal status outweighs that of the infant in infant vaccine response. Analysis of maternal Lewis phenotype showed no effect on infant seroconversion. These findings were of interest to the oral rotavirus vaccine research community. I had the fortune of presenting findings at both national and international meetings, including an oral presentation at the 10th International Conference on Vaccines for Enteric Diseases. The feedback generated has helped hone my thinking regarding the data and contributed to the presentation and discussion of our findings in an article titled “Maternal Secretor Status Affects Oral Rotavirus Vaccine Response in Breastfed Infants in Bangladesh” in the March 11 issue of the *Journal of Infectious Diseases*.

Our results have led us to reconsider the mechanisms by which maternal secretor antigen in breast milk may influence infant vaccine response. New hypotheses include the antigen preventing infectivity by serving as a decoy receptor for the vaccine virus or the maternal antigen modulating the infant gut microbiome, altering efficiency of vaccine virus infection. Collaborators are currently working on characterizing microbiome data from our vaccinated cohort, which will allow us to describe the relationship between maternal secretor status and infant microbiota. Questions have also been opened regarding maternal secretor status on naturally occurring rotavirus infection, leading to our current project of genotyping 155 mothers of non-vaccinated infants to correlate their secretor status with infant diarrheal outcomes. Additionally, I have delved into results from infant genome-wide association studies to correlate infant secretor phenotypes and genotypes. Importantly, our findings have continued to unearth potential mechanisms for disparities in rotavirus vaccine response in Bangladesh.



In addition to the academic production the Queenan award has supported, my research skills have expanded substantially through the duration of the project. Having come into the lab with virtually no prior experience, I worked with laboratory technicians to hone basic skills in pipetting, solution preparation, and sterile technique before moving into DNA extraction. Dealing with scant samples of peripheral blood mononuclear cells, I worked with Dr. Lee to generate Standard Operating Protocols to optimize and validate extraction procedures. Next, I delved into Polymerase Chain Reaction technique to amplify our target sequence from extracted DNA, followed by electrophoresis to confirm successful reactions. I worked with the Vermont Integrative Genomic Resource to run Sanger sequencing on our samples before importing the raw data into GeneiousPrime sequence data analysis software to generate calls on documented

polymorphisms associated with nonsecretor status. The temptation of rabbit holes was plentiful, with opportunities for deep dives into the NIH's dbSNP database and the literature on polymorphisms associated with the nonsecretor phenotype. I was particularly intrigued that the majority of nonsecretors in our sample had a null polymorphism that seems to be unique to Bangladesh (at least in published reports), while the classic nonsecretor polymorphism was only the second most common in our group.

The Queenan award was integral to providing these opportunities, with funds used for kits and reagents, Sanger sequencing costs, travel, and publication fees.

The entire process afforded plentiful opportunities for missteps and mistakes. Troubleshooting was a regular part of my life. The rigorous checks and balances required of a wet lab, from meticulous documentation in lab notebooks to deep cleans for potential contamination, saved countless hours invested. Outside logistics additionally contributed unforeseen obstacles, most importantly the move of the entire lab from one floor of the Microbiology & Molecular Genomics building to another due to a planned renovation. Thankfully, most of the lab work had concluded prior to the COVID-19 pandemic, which could have brought the project to a grinding halt.

Equally important to my growth as a physician scientist were the opportunities for development outside the confines of the laboratory. With the help of the Queenan Award, I was welcomed into Vermont's Translational Global Infectious Disease Research (TGIR) Center. With weekly lectures and a mission to support young researchers, I was exposed to a diverse array of thinkers who provided mentorship and exposure to fascinating ways of understanding the realm of infectious disease. In particular, the mentorship from physician-researchers Beth Kirkpatrick and Benjamin Lee was critical to my development. Biostatistician Dorothy Dickson provided

outstanding direction in data management and analysis, while modelers Laurent Hébert-Dufresne and John Hanley offered insights into the use of modeling and machine learning in the infectious disease. I was even able to experiment in the realm of data presentation, giving my first attempt at the reimaged Poster 2.0 at *TropMed 2019*.

Abstract #233
Maternal Secretor Status Affects Oral Rotavirus Vaccine Response in Breastfed Infants in Bangladesh
 PRESENTER: Frank Williams


METHODS
 Substudy of PROVIDE, a Rotarix™ vaccine efficacy trial in Dhaka, Bangladesh
 • Cohort: 246 maternal-infant dyads randomized to vaccination arm
 • Samples:
 - Infant saliva
 - Maternal breast milk
 - Infant serum
 • Predictor variables:
 - Maternal secretor status
 - Infant secretor status
 • Primary outcome: Infant rotavirus-specific IgA seroconversion

RESULTS


Maternal Status	Infant Status	RV-specific IgA Seropositivity (%)	95% CI
Secretor	Secretor	24%	(18, 31)
Secretor	Non-secretor	12%	(7, 18)
Non-secretor	Secretor	18%	(12, 24)
Non-secretor	Non-secretor	10%	(5, 15)

BACKGROUND
 • Secretor status controls expression of 2-fucosylated oligosaccharides on mucosal surfaces and in secretions
 • Infant secretor status has been associated with symptomatic rotavirus infection and oral rotavirus vaccine (RV) response
 • The effect of maternal secretor status on RV response remains undescribed

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Maternal secretor status affects Rotarix™ seroconversion rates, with infants of non-secretor mothers more likely to seroconvert.



Take a picture to download the full abstract

Professionally, the grant has opened doors for future directions for an aspiring physician scientist. In 2019, I was invited to present my findings at the TGIR Center’s Annual Meeting, receiving direct feedback from the Center’s External Advisory Board as a potential future candidate for funding. The project has led me to investigate potential avenues for a research-focused career as a clinician scientist, attending the 2019 NICHD workshop for physician scientists, as well as going through the application process for the Reproductive Scientist Development Program through an immunology lab in Boston. Through the team at the TGIR Center, I have connected with Imaging the World, a non-governmental organization based in Uganda that is implementing antenatal ultrasound for women in rural clinics across eastern Africa looking for guidance on outcomes-based research. I have also been able to develop a growing network in the Maternal-Fetal Medicine Global Health community, joining the SMFM Global Health Committee this year.

I look forward to continuing to build the skills I have developed through the Queenan Fellowship as I transition to the role of junior faculty committed to reducing pregnancy and early childhood health outcome disparities through global health research. None of these would have been possible outside of your support. I am grateful for the generous funding from the Queenan Fellowship for Global Health from the Foundation for SMFM.