

Trainee Expansion Program

## AWARDEES SUMMER 2019



We are pleased to present the most recent TEP Bridge Fund and TEP Travel Fund awardees. Each talented individual received a scholarship of up to USD 100,000 or up to USD 10,000 to help advance their career.

### TEP Trainee Bridge Fund Awardee

#### Yarden Golan Maor



Genomic characterization of regulatory elements that effect human breast milk production

TBF Awardee 2019

### TEP Trainee Travel Fund Awardees

#### Kozeta Miliku



Genome-wide association studies (GWAS) of human milk fatty acids

TTF Awardee 2019

#### Ashley Nelson



Effect of human milk on the intestinal epithelium and its associated immune compartment

TTF Awardee 2019

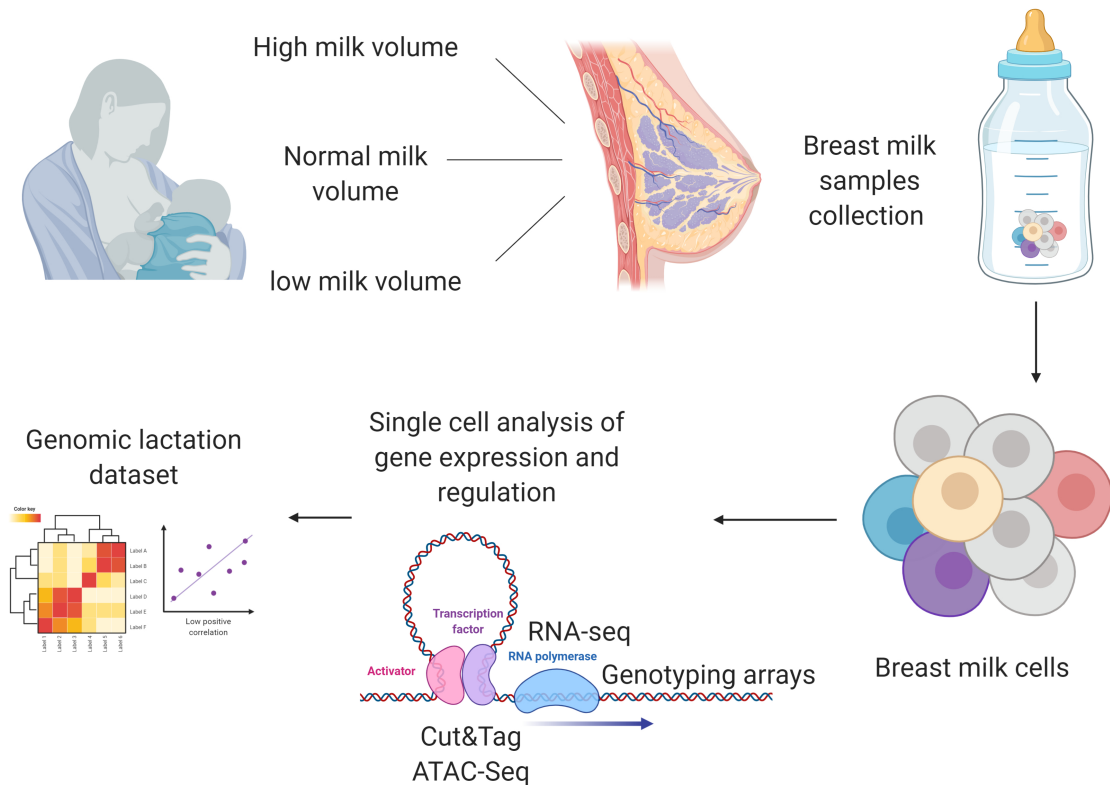
#### Tonny Jimmy Owalla



Secretion of malaria vaccine antigens in breastmilk during asymptomatic/active malaria infection

TTF Awardee 2019

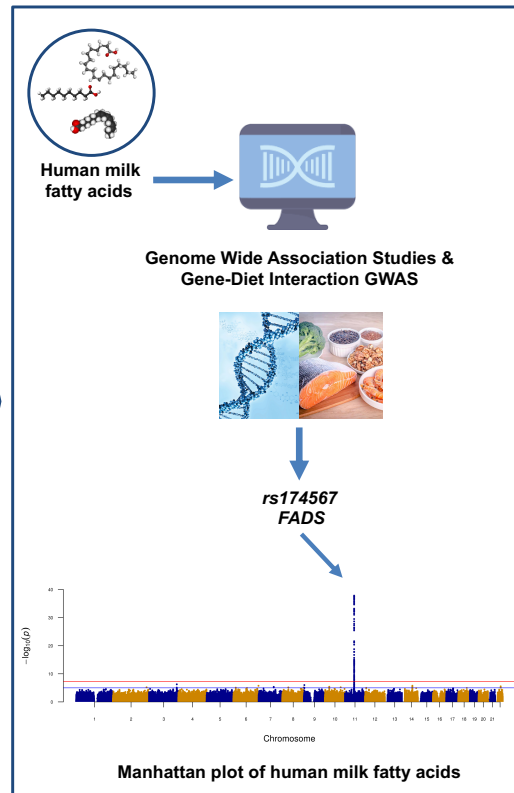
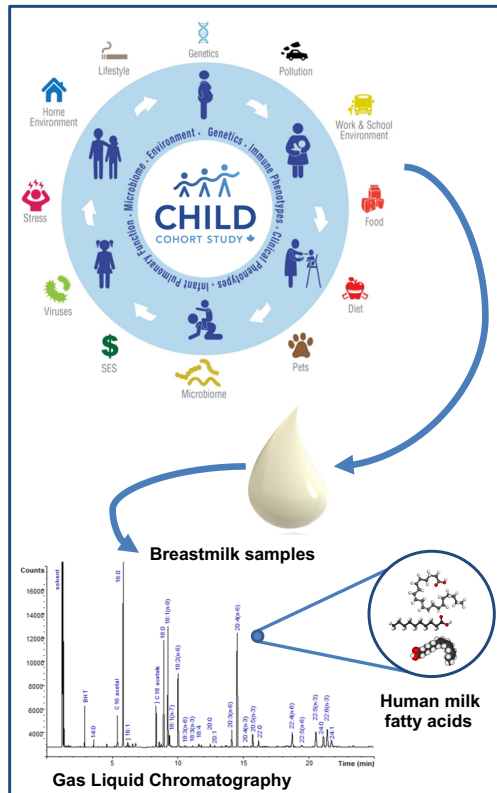
## YARDEN GOLAN MAOR



Breastmilk is the optimal dietary source for infants, as it supplies all the nutritional requirements for the first six months of life. It contains macro- and micronutrients as well as numerous bioactive compounds and several different cell types, including epithelial, myoepithelial, stem cells and leukocytes. Genetic variation in the mom may have various effects on breastmilk components and supply and subsequently affect child health and even lead to disease. However, not much is known about the genetic factors affecting breastmilk composition and production. This study will characterize the breastmilk cell transcriptome (single-cell RNA-seq), regulome (ATAC-seq and Cut&Tag), and variome (genotyping arrays) and link them to create a novel and important genomic lactation dataset. Samples will be collected under both physiological (e.g., colostrum, mature milk and involution) and pathological (e.g., low milk production) conditions, to study the mechanisms underlying different lactation pathologies and to characterize how genetic variation influences lactation outcomes and infant growth. Collectively, the results of this study will significantly impact mother and infant health worldwide and expand our toolbox to improve breastfeeding and help mothers dealing with breastfeeding issues and related diseases.

**Yarden Golan Maor**, PhD, is a molecular nutrition scientist. After completing her Bachelor of Nutrition Science with Honour she started to study for a PhD degree under the supervision of Prof. Assaraf at the Technion (Israel Institute of Technology, Haifa, Israel). Her PhD research focused on studying the transport mechanism of zinc into human breast milk and how mutations in the Zinc transporter 2 (ZnT2) may lead to Transient neonatal zinc deficiency (TNZD) in exclusively breastfed infants. During her PhD Yarden also developed a genomic test for early diagnosis of TNZD using cells that are present in the mother's breastmilk. Yarden recently started her post-doc research under the mentorship of Prof. Nadav Ahituv from the University of California San Francisco where she will continue to discover the effect of genetic variations on human breastmilk production and components.

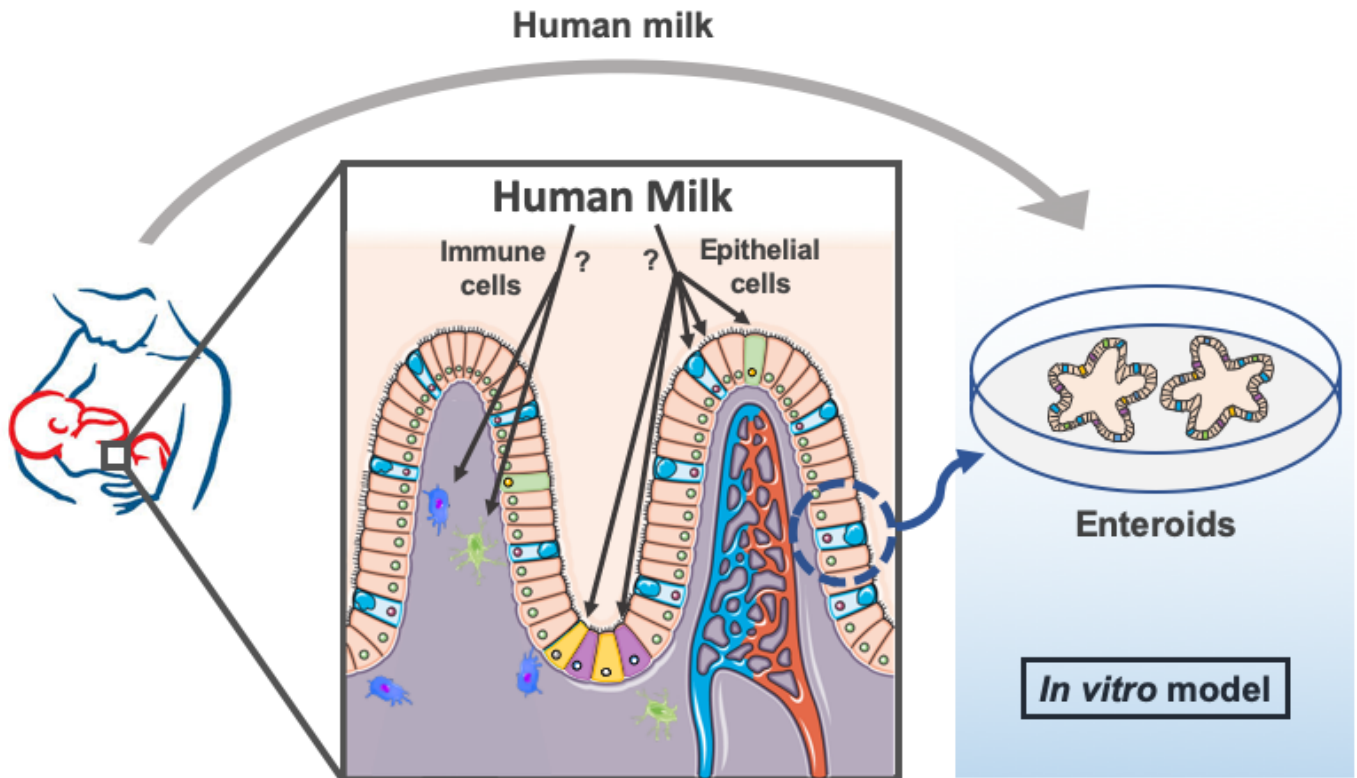
## KOZETA MILIKU



Fatty acids are a vital component of human milk. They influence infant growth, neurodevelopment and immune function, and they provide ~50% of milk's energy content. In the CHILD Cohort Study Dr. Miliku and authors have recently shown that mothers' diets (fish oil supplements and dietary fish intake) are associated with omega-3 milk fatty acids levels and *FADS* gene variants determine the concentrations of milk omega-6 fatty acids. Yet, not much is known on other genetic determinants of human milk fatty acids levels, and the combined interactions of gene and diet in human milk fatty acids are not widely explored.

**Kozeta Miliku**, MD, PhD, is a post-doctoral fellow at Children's Hospital Research Institute of Manitoba, University of Manitoba in Winnipeg, Canada. Her research interest are the fields of human milk and lactation and the developmental origins of health and disease. Dr. Miliku's current work in the Azad lab is focused in determining the role of human milk composition on infant health outcomes and identifying the mechanisms. She will visit Dr. Duan's lab at Queen's University to advance her genetic/genomic research skills and study the genetic determinants of human milk fatty acids.

## ASHLEY NELSON





Human milk (HM) is known to promote infant health and many of its effects occur in the gastrointestinal tract. However, our current understanding of the specific effects of HM on the gut epithelium and its associated immune compartment is limited. This project aims to expand our knowledge of the effects of HM on different cell subsets of the intestinal epithelium through use of human intestinal enteroids. Enteroids, aka “mini-guts”, are generated from primary biopsies of the human intestinal epithelium and form structures in vitro that maintain their cellularity and closely resemble the intact gut epithelium. Thus, enteroids are an optimal tool to elucidate the specific effects of HM on the human intestinal epithelium. Under the mentorship of Dr. Sasirekha Ramani at Baylor College of Medicine, Ashley will learn the nuances of enteroid culture, enabling her to establish this cutting edge model in her research on the mechanisms whereby HM promotes health.


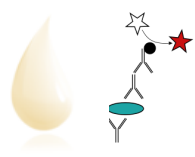
**Ashley Nelson** is a second year PhD candidate in the Immunology program at the University of Rochester School of Medicine and Dentistry in New York. She received her BSc with high honors in Biotechnology and Molecular Bioscience at the Rochester Institute of Technology. Ashley is interested in studying the effects of human milk on gut epithelial function, with a focus on epithelial cell subsets as well as immune cell populations. She is also the president of a graduate student organization at the University of Rochester called Graduate Students Raising Families which helps support and advocate for student parents.

# TONNY JIMMY OWALLA

88 breastmilk samples of asymptomatic *Plasmodium falciparum*- infected breastfeeding mothers in Uganda





High sensitivity ELISA for detection of *Plasmodium falciparum* antigen pLDH and pHRP2 in breastmilk


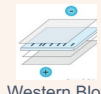




*Plasmodium falciparum* HRP2 & pLDH antigens are shed in 15% of breastmilk samples of *Plasmodium falciparum* infected mothers (Van den Elsen, Verhasselt and Egwang, in press)

**Is Circumsporozoite protein (CSP) antigen, an immune component of the leading malaria vaccine (RTS,S), present in breastmilk ?**




- Develop highly sensitive and specific techniques for detection of *P. falciparum* CSP vaccine antigen in breastmilk
- Determine if CSP is found free or bound to Antibody
- Analyse levels of CSP in 88 breastmilk samples






ELISA      Western Blot      Mass Spectrometry

**Public health significance of malaria CSP antigen shedding in breastmilk in Ugandan infants**



- Influence on immune reactivity and susceptibility to *Plasmodium falciparum* infection?
- Influence on malaria vaccine efficacy?



According to the World Health Organization, more than 200 million cases of malaria occur yearly worldwide with the majority in infants aged under 5 years. This highlights the need for a successful strategy of prevention of malaria infection especially in early life. Breastfeeding is the most efficient way to prevent child morbidity and mortality related to respiratory and gastro-intestinal infectious disease. In contrast, there is conflicting evidence on malaria prevention by breastfeeding. We propose the original hypothesis that the presence of malaria antigen in breastmilk may stimulate antimalarial immune defences and reduce malaria risk in breastfed infants. Our preliminary data confirms the shedding of *Plasmodium falciparum* histidine-rich protein 2 (pHRP-2) and lactate dehydrogenase (pLDH) in breastmilk of 15% of breastfeeding mothers with asymptomatic-malaria in Uganda. The goal of this project is to investigate whether (i) the malaria vaccine antigen CSP is secreted in breastmilk since this may influence specific antimalarial immune responses and malaria risk in vaccinated infants; (ii) CSP is free or complexed with maternal antibodies in breastmilk. These questions are significant because CSP is the major component of the new leading RTS,S vaccine. Therefore, we expect that shedding of CSP antigens in breastmilk may be particularly efficient for infant immunisation and prevention of malaria in breastfed

infants. To address these questions, I will set up ELISA, Western blotting and Mass Spectrometry analysis, for detection of CSP in breastmilk samples from Ugandan mothers under the supervision of Professor Valerie Verhasselt during my visit to the University of Western Australia.

**Tonny Jimmy Owalla** is a Research Associate at Med Biotech Laboratories, Kampala-Uganda with a strong background in malaria. He holds a BSc in Biomedical Laboratory Technology from Makerere University, Kampala-Uganda and expects to attain the MSc in Molecular Biology and Biotechnology from the same university in January 2020. During his undergraduate research, Tonny studied natural immune responses to blood stage malaria vaccine candidates in pregnant women and children in Uganda under Professor Thomas Egwang. This cultivated in him a special interest in maternal and infant immunology. He is currently interested in understanding if malaria vaccine antigens are secreted in breastmilk during asymptomatic/active malaria infection. He anticipates that this Trainee Travel Fund will enable him to acquire important skills relevant to his future research on the role of breastfeeding in antimalarial immunity. Tonny will be jointly mentored by Professors Verhasselt and Egwang.