FID-ISPAD Research Grant Progress Report

Year awarded: 2021

Recipient: Dr Ki Wook Kim

Project title: Characterising the population of gut viruses contributing to the development and acceleration of childhood type 1 diabetes

Project hypotheses and research questions:
Our overarching hypotheses are:

i) The frequency of viral infections of the gut is significantly higher in children who develop IA/T1D versus IA-negative controls
ii) The population of gut viruses infecting cases differs from controls

Specific research questions include:

i) How does the gut virome differ between children with IA/T1D compared to IA-negative controls?
ii) What are the most prevalent gut virus genotypes in children with IA/T1D?

Project aims:

1. Characterise the longitudinal gut virome (population of all gut viruses) prior to, at and shortly following the clinical onset of T1D using virome capture sequencing
2. Identify viruses more prevalent and/or differentially abundant in children with IA/T1D compared to controls at the genotype level

Research Progress:
This is a case-control study involving 158 cases (children with onset of IA/T1D) and 141 controls (sibling or unrelated children) from two Australian study populations: T1D and the Gut Study (TIGS) and New-Onset Westmead (NOW) cohorts. In TIGS, cases were defined as children with persistent IA or recently diagnosed T1D (from 4 weeks post diagnosis). Persistent IA was determined serologically as positivity for >1 autoantibody (IAA, GAD, IA2 or ZnT8) in two consecutive study visits. In NOW, cases were defined as children with new onset of T1D (recruited at diagnosis). Controls recruited in both cohorts were sibling or unrelated children, with no significant difference in age or sex compared to cases (5; 7).

Despite significant delays faced (Please see '*Bottlenecks'* on page 3) that have majorly impacted on the progress of this project, we have completed virome capture sequencing and bioinformatic analysis of sequence data (taxonomic classification of viral sequences) for ~50% (103/211) of all samples from the NOW cohort. Preliminary heatmap of what viruses were detected in these samples, stratified by case status (with or without type 1 diabetes) are presented (Please see '*Preliminary results'* on page 2). Most of the remaining samples have been extracted (total nucleic acid), cDNA converted and are currently in the final stages of library preparation for Next Generation Sequencing (NGS).

We have sourced all required kits, reagents and disposables needed to process all stool samples (n=180) collected from 88 children (47 cases and 41 controls) in TIGS. These samples are currently biobanked in another State (Adelaide). Dr Kim is coordinating with co-investigators Couper and Barry based in Adelaide, to organise retrieval and shipment of these samples. We anticipate receipt and commencement of sample processing in the next month.

Preliminary results:

Virome capture sequencing analysis of the first 103/211 stool samples collected from 63 cases with new-onset T1D and 40 controls detected 35 different genera of viruses. Heatmap of viruses detected represented at the genus level is presented (**Figure 1**). Overall, positivity to any virus was 72% in cases vs 85% in controls. Viruses most frequently detected (positive in >= 20 individuals) listed in the order of decreasing prevalence: enteroviruses, gyroviruses (likely dietary in origin), picobirnaviruses, mastadenoviruses, parechoviruses and rotaviruses. Although it is too early to conclude at this stage with another two-thirds of samples remaining to be sequenced, current results do not support the hypothesis that infections by enteroviruses and other gut viruses are more prevalent individuals with new-onset T1D compared to controls. Species level comparisons (data not shown) will be important to tease out case-specific associations, particularly for broad genus groups such as enteroviruses. A more detailed analysis after completion of all sequencing is needed to draw meaningful conclusions. Adjusting for potential confounders will be important.

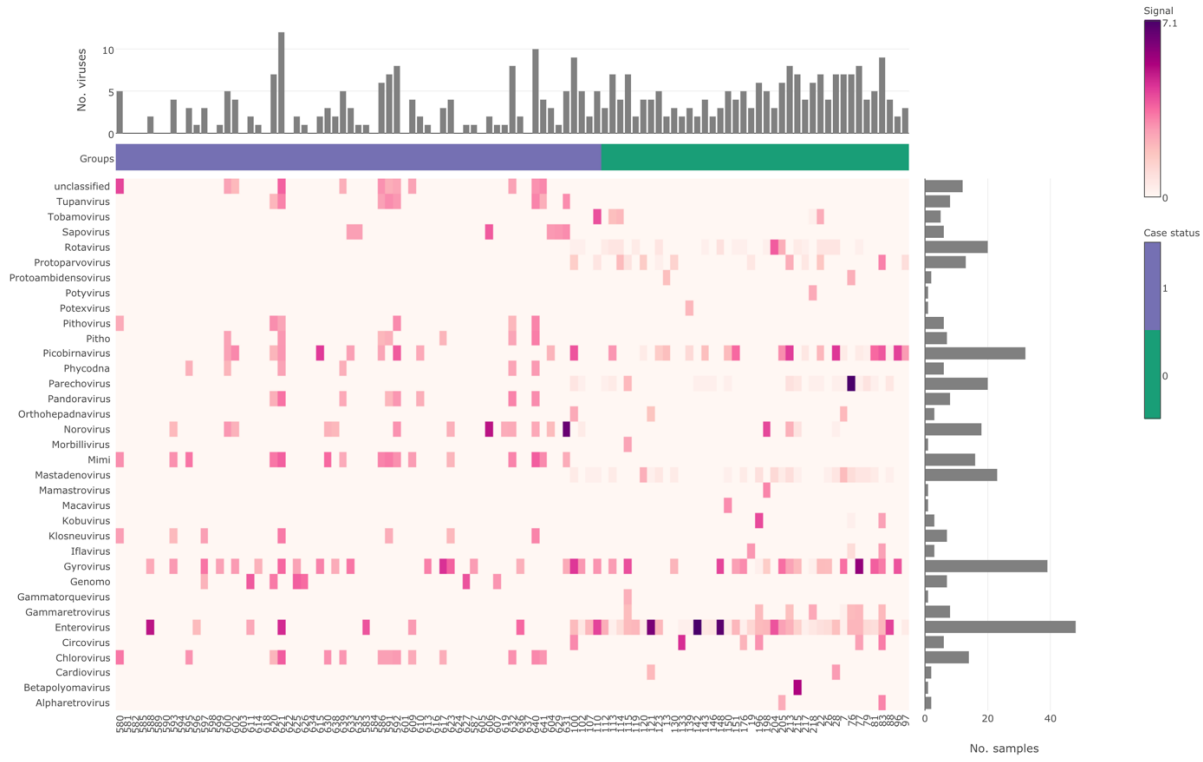

Figure 1. Viruses detected in the gut virome of 63 cases with T1D and 40 controls within the NOW cohort. Heatmap of log10(viral read counts) plotted using iheatmapr package in R (v4.0.1). Each column represents a unique individual and each row represents log10(viral read counts) for the respective virus, at the genus level. Positivity threshold set at minimum 100 viral reads.

Bottlenecks:
The following bottlenecks and challenges have contributed to ~6 months of delays in project progress (please note, these bottlenecks have resulted in similar delays across all research programs overall, not only this project):

- Delayed receipt and access of grant funds (grant was officially awarded in November 2021 but funds were received on 10th February 2022)
- COVID-related delays: including supply chain issues (frequent backorders and 2-3 months of delays in receiving next generation sequencing reagents and other essential consumables for this project), restrictions placed on all non-COVID-related research by our host institution (pause placed on all non-essential work) during the surge of the Omicron outbreak (Jan-March 2022) and increased sick leaves by research staff working on this project. Even up until very recently in Sydney (Australia), 7-day self-isolation period was enforced statewide and within the institution (Prince of Wales Hospital, where the research is based).
- Research support staff recruitment issues: a newly recruited research assistant hired to support Dr Kim in completing all experiments resigned after 1 month in the role for a different position. The replacement research assistant recruited had major performance issues during probationary period and have since resigned (8th September). We are currently preparing to advertise the role once again.

Timeline for project completion:

Table 1. Updated timeline of research milestones for 2022/2023.

Time	Milestone
Oct-Dec 2022	Complete virome capture sequencing of remaining 50% of NOW stool samples Complete nucleic acid extraction and cDNA synthesis for all TIGS samples (n=180)
Jan-Mar 2023	Complete NGS library preparation, virome capture enrichment and sequencing of all TIGS samples
Apr-Jun 2023	Complete all bioinformatic (taxonomic classification of viral sequences, differential abundance and viral genome coverage analyses) Complete all statistical analyses (identify case or control specific associations, adjusting for potential confounders)
Jun-Aug 2023	Data interpretation and reporting (peer-reviewed publication and presentation at 2023 ISPAD meeting)