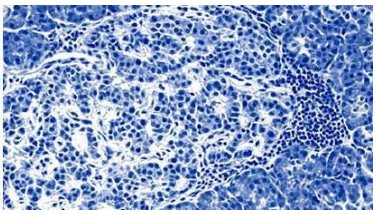
nPOD
Network for Pancreatic Organ Donors with Diabetes



Characterizing the virome of nPOD pancreatic donor islets using comprehensive capture sequencing

AUTHORS

Ki Wook Kim[1], Alberto Pugliese[2], Rick Lloyd[3], Heikki Hyöty[4], Sonia R. Isaacs[1], William D. Rawlinson[1,5], Maria E. Craig[1,5,6,7] and nPOD Viral Working Group (nPODv).

[1]School of Women's and Children's Health, University of New South Wales, Sydney, NSW, Australia
[2]Miller School of Medicine, University of Miami, Miami, FL, USA
[3]Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX, USA
[4]Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland
[5]NSW Health Pathology, Sydney, NSW, Australia
[6]Institute of Endocrinology and Diabetes, The Children's Hospital at Westmead, Sydney, NSW, Australia
[7]Discipline of Child and Adolescent Health, University of Sydney, Sydney, NSW, Australia

PURPOSE

Over a century of experimental and epidemiological data support the role of viruses in the development of type 1 diabetes. The most compelling evidence in recent years has been the detection of enterovirus RNA and protein in the Network for Pancreatic Organ Donors with Diabetes (nPOD) specimens and in pancreatic biopsy samples of new onset individuals. Viruses appear to be present in very few cells within the pancreas at ultra-low copy numbers, often below the limit of PCR detection. The purpose of this study was to determine whether comprehensive virome capture sequencing (VirCapSeq-VERT) can improve low-level enterovirus detection from nPOD specimens and identify additional viruses that may be potentially involved in disease pathogenesis.

METHODS

Pancreatic islet cells were obtained from a single donor with established type 1 diabetes. Purified islet cells were maintained in culture for 15 days untreated and in co-culture with HEK cells expressing viral proteases (2Apro and 3Cpro). Supernatants were collected on days 1, 2, 4, 6, 8, 11 and 15. Cells were harvested at day 0, 11 and 15. Total nucleic acid was extracted using the MagMax isolation kit and treated with Turbo DNase. RNA was converted to cDNA using Superscript III with random hexamers and Klenow fragment. Sequencing libraries were prepared using KAPA HyperPlus (Roche) with unique dual-index adapters. VirCapSeq-VERT enrichment of library fragments containing vertebrate-infecting viral genome sequences was performed following the SeqCap protocol (Roche). Post-capture library pool was sequenced on the Illumina NextSeq500 at UNSW Ramaciotti Center for Genomics, Sydney, Australia. Sequencing analysis (*de novo* assembly and

taxonomic classification) was performed using three published bioinformatic pipelines: VirusTAP, Genome Detective and ViromeScan.

SUMMARY OF RESULTS

Sequences corresponding to enterovirus could not be identified in any of the supernatants and cell extracts harvested from the untreated cells. In contrast, all samples collected from cells co-cultured with HEK cells expressing a viral protease construct (2Apro and 3Cpro) produced a large number of reads corresponding to the 2A and 3C protease regions of poliovirus 1. High number of reads corresponding to human papillomavirus 18 and human adenovirus 5 were detected across all timepoints of both untreated and co-cultured islets, but not at day 0. No human viruses were detected in the two negative controls (media only). Consistent with previous studies, bovine viruses (bovine diarrhea virus and bovine parvovirus 3) were readily detected across all samples including the negative controls as a common contaminant of cell culture media and fetal bovine serum. Overall, there was good concordance on the range of viruses detected and the number of viral reads between the three bioinformatic pipelines used.

CONCLUSIONS

This was a pilot and proof-of-concept study conducted using pancreatic islets harvested from a single donor. In this preliminary analysis, no clinical enteroviruses were detected. Testing of additional donor islets is needed to evaluate the performance of VirCapSeq-VERT in detecting low-level enterovirus presence, as well as to investigate the presence of other viruses that may be important in the pathogenesis of type 1 diabetes. If higher sensitivity is demonstrated, we anticipate routine application of VirCapSeq-VERT to screen pancreatic tissue or laser captured islets from nPOD organ donors with type 1 diabetes or islet autoantibodies. Determining the complete breadth and genotype of viruses that contribute to type 1 diabetes is paramount for the development of viral vaccines for primary and secondary prevention.