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# Establishment of three human intestinal enteroid lines to study human norovirus infection


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## Background

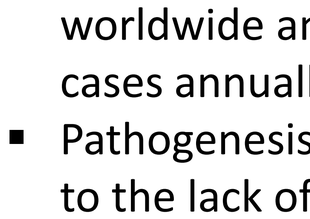
- Norovirus is the leading cause of acute gastroenteritis worldwide and is accounted for nearly 1/5 of all-cause cases annually
- Pathogenesis of norovirus remains poorly understood due to the lack of a robust tissue culture system
- Breakthrough in 2016, norovirus has finally been cultivated in an adult stem-cell derived three-dimensional human intestinal enteroid (HIE) system

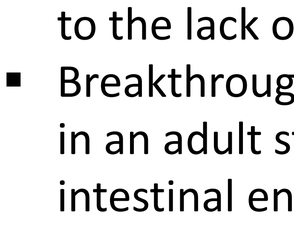
## Aim

- To establish HIE lines to study human norovirus infection

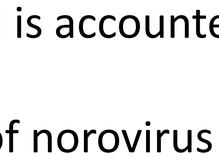
## Methods

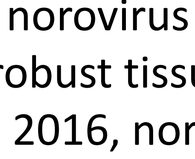
### Establishment of HIE



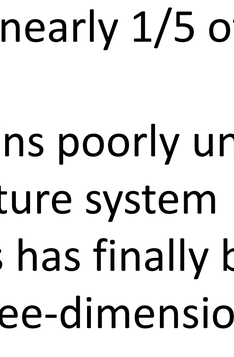



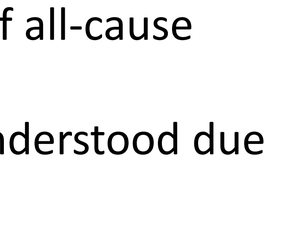
Biopsies obtained from D1 via gastroscopy in endoscopy centre (PWH)



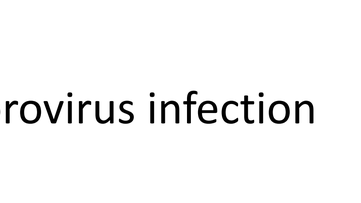


Biopsies were minced in CMGF- with scissors and wash with CCS

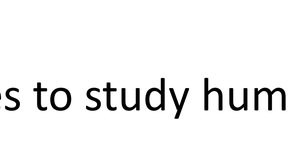


Isolate intestinal epithelial crypts by shaking at 250 rpm in orbital shaker at 4°C with 0.5 M EDTA



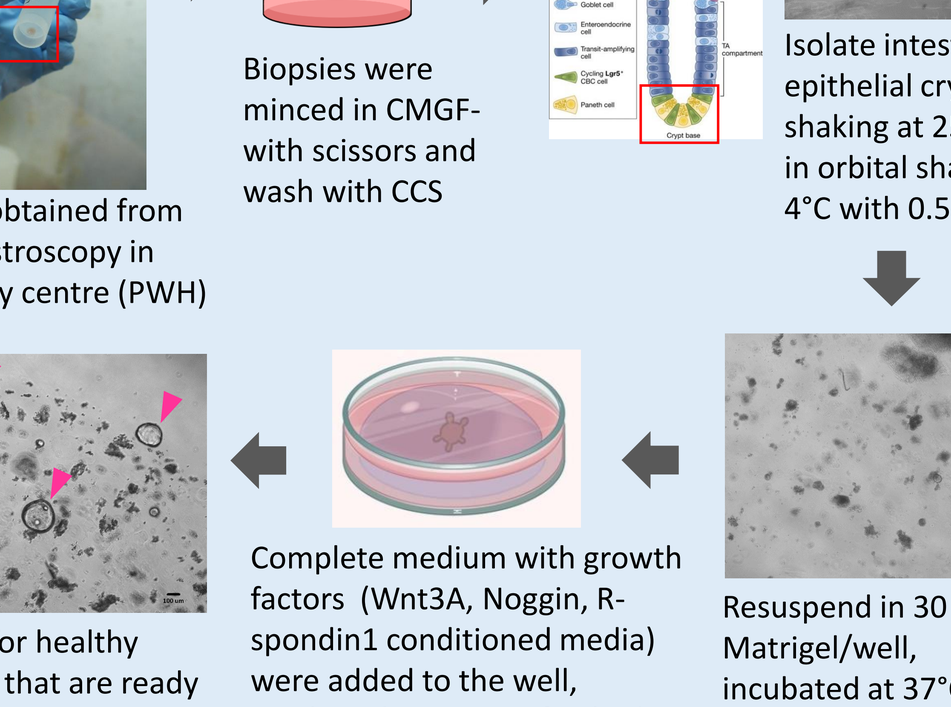
Resuspend in 30 µl of Matrigel/well, incubated at 37°C for 5-10 mins until solidified



Complete medium with growth factors (Wnt3A, Noggin, R-spondin1 conditioned media) were added to the well, incubated at 37°C, refreshed every second day

Observe for healthy enteroids that are ready for passage (Day 6/7)

### Secretor status determination




## Results

- Three patient-derived HIE lines were established from duodenal biopsies **(Figure 1)**
- Two of them were typed as secretor, and one was typed as weak secretor, of which the two secretor lines were belonged to blood group A and AB respectively **(Figure 2)**
- Enteroid lines were successfully passaged for at least 15 times with approximately 50-100 enteroids per well and could grow up to 300 µm in size robustly
- Cryopreserved stocks of HIE were made available before P6 for all lines and their viability remained high after thawing.

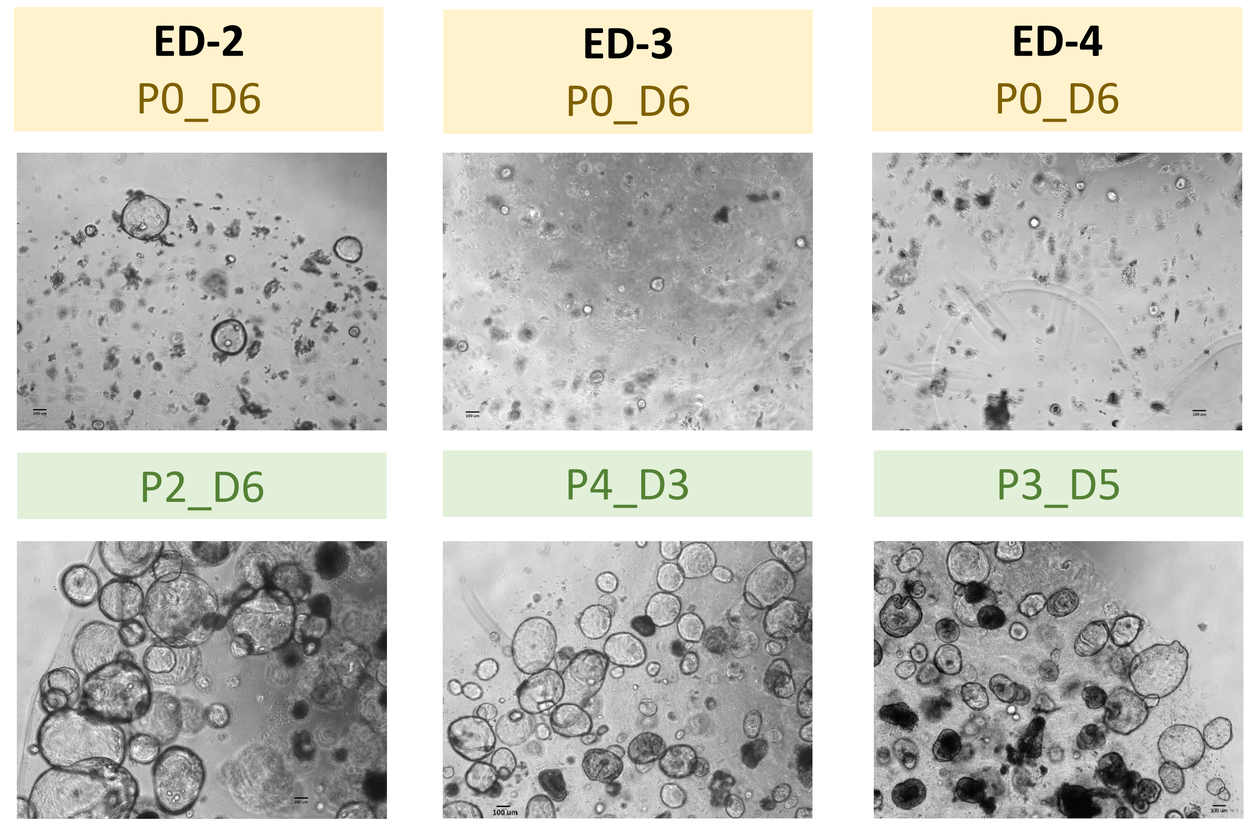



**Figure 1**. HIE images of ED-2, ED-3 and ED-4 at different passage

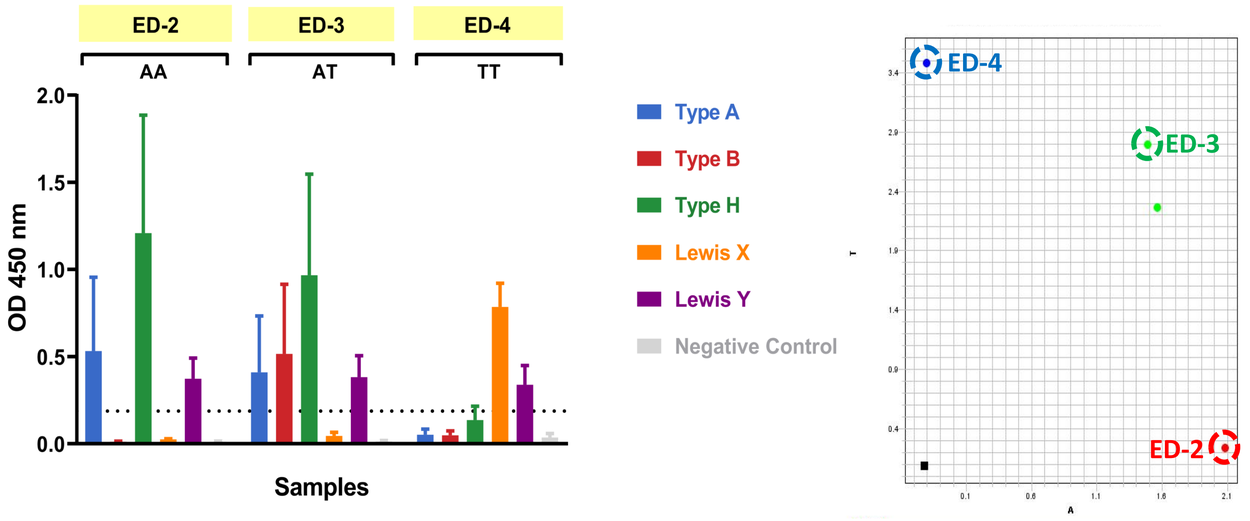



**Figure 2**. Secretor status phenotyping and genotyping of HIE donors

## Conclusion

We have successfully established three HIE lines of different histo-blood groups. The susceptibility of the two secretor enteroid lines to infection by norovirus GII.4 Sydney strains will be evaluated in the future.

## Acknowledgements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