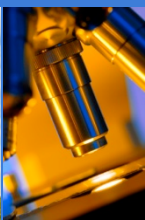# Detection of enteroviruses in tissue samples of cadaveric organ donors with type 1 diabetes

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nPOD
Network for Pancreatic Organ
Donors with Diabetes

Development of EV screening methods

- EV protein by immunohistochemistry (IHC)
  - 8 antibodies optimized
  - formalin-fixed and frozen sections

- EV genome by *in situ* hybridization (ISH)
  - formalin-fixed sections

Validation of the assays

- EV-infected cells
- Intestinal and pancreas tissue from EV-infected mice
- Pancreas from a patient who died of EV infection
- Large panel of human tissues from non-diabetic organ donors

Evaluation of immunohistochemistry and *in situ* hybridization methods for the detection of enteroviruses using infected cell culture samples

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Formalin sections

Table 2
Detection of enteroviruses in infected formalin-fixed paraffin-embedded cells using different antibodies and *in situ* hybridization.

Antibody	Formalin-fixed paraffin-embedded cells infected with different enterovirus serotypes														
	CBV1	CBV2	CBV3	CBV4	CBV5	CBV6	CAV9	CAV16	echo3	echo6	echo9	echo11	echo30	E71	PV3
Enterovirus 5-D8/1	+	++	+++	+++	+++	++	+	+	+	+++	+	+	+++	−	−
Pan-Enterovirus Blend	+++	++	++	++	++	++	++	++[a]	+	++	++	++	++	++	+++
Coxsackievirus B Blend	+	−	−	−	−	−	−	+[a]	−	−	−	−	−	+	−
In-house CBV3	++	++	+++	++	++	++	+	−	++	+++	++	++	+++	−	−
In-house CAV16	−	−	−	−	−	−	−	+++	−	−	−	−	−	+	−
Echovirus Blend	+	++	++	++	++	++	−	−	−	++	+	+	+++	−	++
In-house echovirus 11	−	−	−	−	+	+	−	−	+	−	+	++	−	−	−
Poliovirus Blend		+													+++
ISH	+++	++	+++	++	+	++	+	+	+	++	+	+	++	++	+

Frozen sections

Table 1
Detection of enteroviruses in infected frozen cells using different antibodies.

Antibody	Frozen cells infected with different enterovirus serotypes														
	CBV1	CBV2	CBV3	CBV4	CBV5	CBV6	CAV9	CAV16	echo3	echo6	echo9	echo11	echo30	E71	PV3
Enterovirus 5-D8/1	+++	+++	+++	+++	+++	+	−	−	−	+++	+	−	+++	−	−
Pan-Enterovirus Blend	+++	++	+++	++	++	++	++	++	+	++	++	−	+++	++	−
Coxsackievirus B Blend	+++	+++	+++	+++	+++	+++	−	−	−	−	−	−	−	−	−
In-house CBV3	+++	+++	+++	+++	+++	+	+++	+	+	+	+	−	+	+	−
Echovirus Blend	+++	+++	−	++	++	−	−	−	−	++	++	+++	++	−	−
In-house echovirus 11	−	−	−	−	−	−	−	−	−	−	−	+++	+	−	−
Poliovirus Blend	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+++

Results from nPOD

- EV screening panel:
  - EV protein (IHC, anti-VP1 clone 5-D8/1)
  - EV genome (ISH, pan-EV probe)
- Formalin-fixed paraffin-embedded sections:
  - 18 T1D patients: 21 pancreas, 15 spleen, 11 duodenum, 2 PLN
  - 3 auto-ab+ donors: 3 pancreas, 3 spleen, 2 duodenum, 1 PLN
  - 23 controls: 24 pancreas, 17 spleen, 1 duodenum

Proportion of donors positive for EV genome (ISH)

	T1D	Aab+	Controls
Pancreas	30 % (N=20)	33 % (N=3)	4 % (N=24)
Spleen	53 % (N=15)	33 % (N=3)	18 % (N=17)
Duodenum	40 % (N=10)	50 % (N=2)	0 % (N=1)
PLN	100 % (N=2)	100 % (N=1)	na
Total	43 %	44%	10 %

Proportion of donors positive for EV protein (IHC)

	T1D	Aab+	Controls
Pancreas	33 % (N=21)	0 % (N=3)	5 % (N=22)
Spleen	20 % (N=15)	50 % (N=2)	6 % (N=16)
Duodenum	30 % (N=10)	50 % (N=2)	0 % (N=1)
PLN	50 % (N=2)	100 % (N=1)	na
Total	29 %	38 %	5 %

Correlation between EV positivity in the pancreas and spleen

Either viral genome or protein

Spleen	Pancreas	
	positive	negative
positive	8	8
negative	2	17

P=0,012

EV VP1 in the pancreas of case #6052
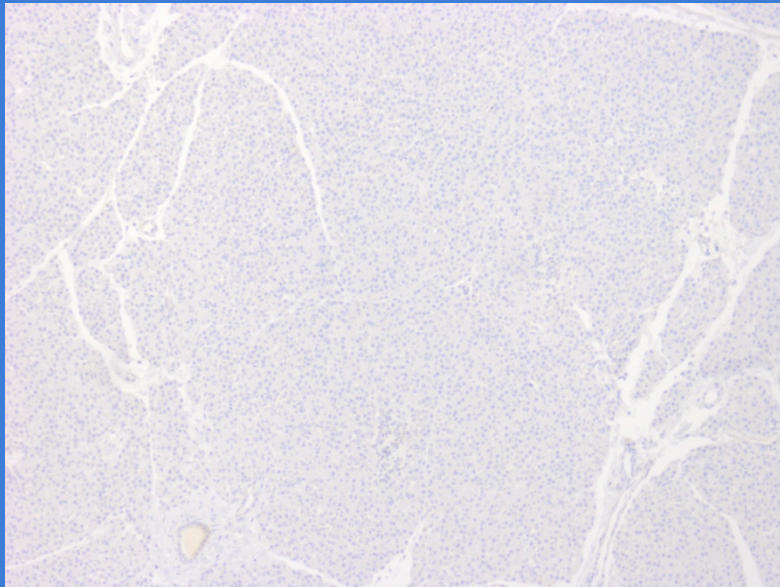
Head
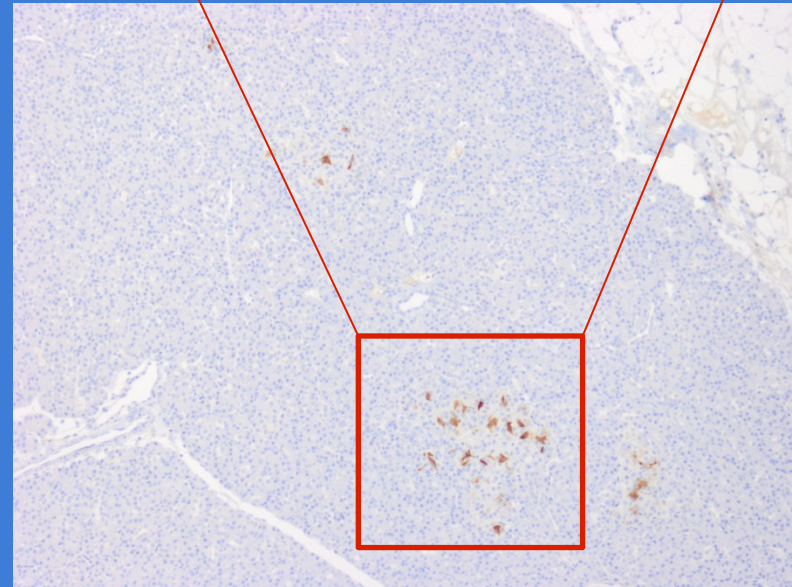
Tail

Double staining with EV and hormone antibodies
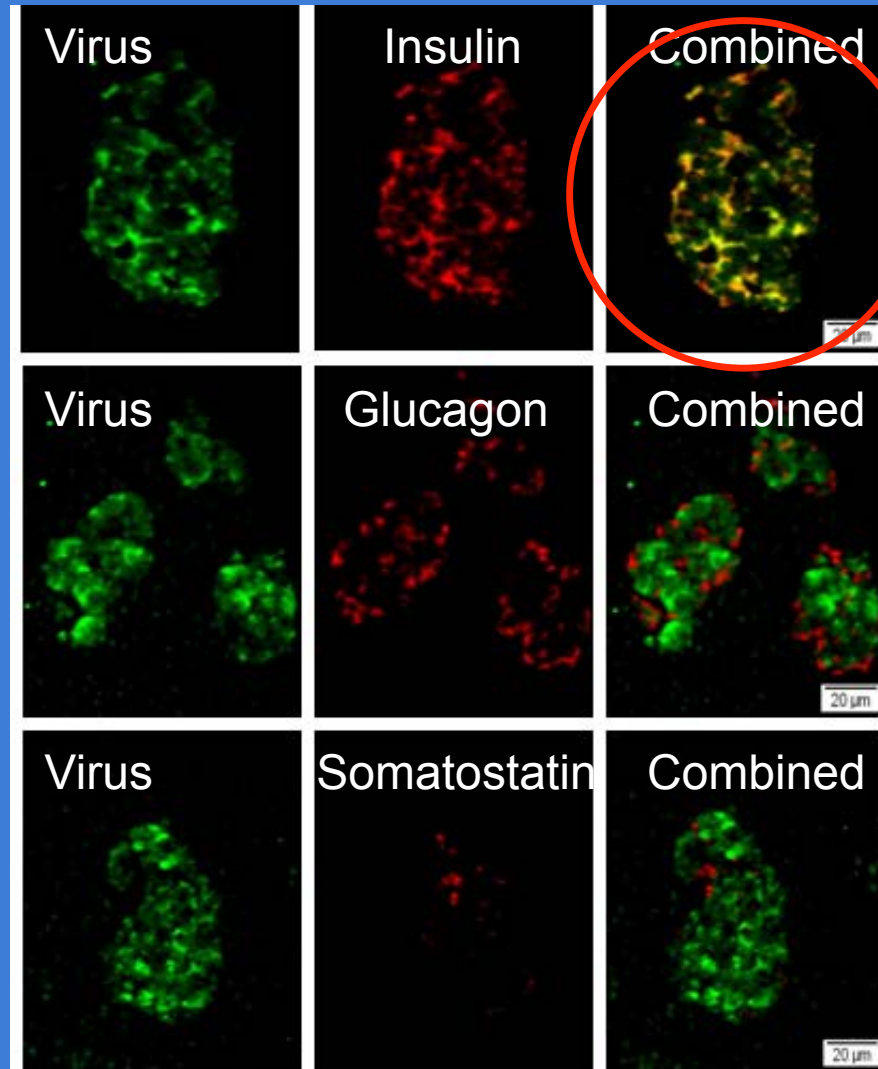
Conclusions

- EV protein and genome detected in multiple organs of T1D patients more frequently than in controls

- EV signal showed positive correlation between different organs

- EV positivity can vary between different parts of each pancreas

- Further studies:
  - analyses of multiple tissues from all nPOD donors by these assays as a primary screening of the EV status
  - analyses of different parts of the pancreas
  - selected cases analysed by PCR + sequencing

nPOD team in Tampere

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…and the nPOD study group