[1]Academic Endocrine Unit, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom; [2]Department of General and Visceral Surgery, Evangelisches Krankenhaus Wien, Hans-Sachs-Gasse 10-12, 1180 Vienna, Austria; [3]Cell and Molecular Oncology, Department of Oncology, Medical Sciences Division, University of Oxford, Oxford, United Kingdom

Disclosure: K.A. English: None. **A. Selberherr:** None. **O.A. Shariq:** None. **E. O'Neill:** None. **K.E. Lines:** None. **R.V. Thakker:** None.

Background: DNA methylation and hydroxymethylation (5hmC) are epigenetic alterations that can silence or maintain gene transcription, respectively. Pancreatic neuroendocrine tumours (PNETs), which can be hormone-secreting (e.g. insulinomas) or non-functioning (NF-PNETs), have a low mutational burden and are reported to be associated with DNA hypermethylation, but the role of 5hmC in PNETs is unknown. Aim: To determine the patterns of DNA 5hmC in NF-PNETs, insulinomas, and normal pancreatic islets. **Methods:** NF-PNETs (n=31, mean age 54 ± 13 years (y), 14 males (M), tumour grade (G) G1=15, G2=13, G3=3) and insulinomas (n=21, mean age 50 ± 21y, 9M, G1=17 and G2=4) were collected from patients who did not have a genetic syndrome associated with PNETs together with 14 normal pancreata (mean age 63 ± 12y, 11M). Samples were obtained following ethical approval from the Universities of Oxford and Vienna. DNA 5hmC was assessed by immunohistochemistry (IHC) and MethylationEPIC array (that interrogates 5hmC CpG sites). Multivariate analysis was used to determine variables associated with 5hmC. Transcriptome analysis was undertaken by RNA-Seq. **Results:** NF-PNETs had reduced 5hmC when compared to insulinomas ($p<0.01$) and to normal adjacent islets ($p<0.001$). Thus, 5hmC was ~3 fold lower in NF-PNETs compared to normal adjacent islets (n=12; $p<0.001$), whilst 5hmC between insulinomas and normal adjacent islets (n=15) was not significantly different. Multivariate analysis confirmed this finding, with NF-PNETs being associated with lower 5hmC when controlled for patient age, sex and tumour grade ($p<0.01$). In normal pancreatic tissue 5hmC was ~2 fold higher in normal islets when compared to exocrine tissue (51 islets assessed in total, range 1-9 islets/sample from 34 fields of 305,866um^2 studied; $p<0.001$). MethylationEPIC array analysis, using DNA from NF-PNETs (n=5), insulinomas (n=4) and normal pancreatic tissue (n=7), revealed NF-PNETs and insulinomas to have a significant decrease in 5hmC compared to normal pancreatic tissue ($p<0.001$). Furthermore, subtype analysis revealed that NF-PNETs had lower 5hmC compared to insulinomas (0% vs 0.003%, adjusted $p<0.08$). To determine possible mechanisms underlying these differences in tumour subtype 5hmC, RNA-Seq analysis using NF-PNETs (n=7) and insulinomas (n=6) was performed. This revealed 79 genes to be differentially expressed (adjusted $p<0.01$), of which 33 were upregulated (including Calcium/Calmodulin Dependent Protein Kinase 2 Gamma (*CAMK2G*)); and 46 were downregulated (including Glucagon Like peptide 1 receptor (*GLP1R*)) in NF-PNETs. Interestingly, *CAMK2G* and *GLP1R* are both involved in DNA 5hmC modulation via cAMP. Conclusions: DNA 5hmC, an epigenetic mechanism, is significantly lower in NF-PNETs when compared

Abstract citation ID: bvad114.2195

Tumor Biology

OR16-03

Epigenetic Studies Of Pancreatic Neuroendocrine Tumours (PNETs) Reveal Decreased DNA Hydroxymethylation In Non-functioning PNETs But Not Insulinomas

***Katherine A. English, FRACP*[1]*, Andreas Selberherr, PhD, FEBS*[2]*, Omair A. Shariq, MD*[1]*, Eric O'Neill, PhD*[3]*, Kate E. Lines, PhD*[1]*, and Rajesh V. Thakker, MD, FRS*[1]

to normal islets and insulinomas and may involve dysregulation of cAMP-mediated signalling pathways.

Presentation: Friday, June 16, 2023