Homing in on precise diagnoses:
The power of combining multi-layered genomic and histopathological analysis in pancreatic neuroendocrine cancer

Tamsin Robb[1], Mee-Ling Yeong[2], Nicole Kramer[2], Cherie Blenkiron[1], Kate Parker[1], Peter Tsai[1], Sandra Fitzgerald[1], Paula Shields[1], Michael Findlay[1], Ben Lawrence[1], Cristin Print[1]

[1]University of Auckland, New Zealand [2]Auckland District Health Board, New Zealand

The NETwork Project

- Multilayered genomic and histopathological analysis of 60 patients with pancreatic neuroendocrine tumours (pNETs) led to refined diagnosis in four patients (7%).
- Targeted deep DNA sequencing and RNA expression data was employed alongside pathological examination to search for molecular drivers in pNETs.
- Combining evidence from DNA, RNA and histopathology led to tumour diagnoses reconsidered as pancreatic solid pseudopapillary neoplasms (SPNs) in two cases.
- Histological review had diagnosed these tumours as pNETs by morphological and immunohistochemical criteria but also noted uncertainty due to some variable SPN-like morphological features.
- SPNs share some cytological features with pNETs but are genomically distinct, and have a discrete clinical course, staging and follow-up programme.

Methods

- 69 sporadic well-differentiated pNETs from 60 individuals along with matched normal tissues underwent deep hybridization capture DNA sequencing of 637 genes and RNA expression analysis using Affymetrix microarrays.
- Cases selected had a clinical and pathological diagnosis of well-differentiated pNET, expressed at least one of the three neuroendocrine immunohistochemical protein markers (chromogranin A, synaptophysin or CD56) and were surgically resectable at initial diagnosis.
- Careful clinical annotation was conducted for each case, then cases de-identified prior to linking with genomic findings. Clinically relevant findings were returned to the patient's physician if deemed appropriate by an incidental findings committee, for patients who consented to this.

	Clinical Characteristics			Diagnostic Differentiation		
	AGGRESSIVENESS	5 YEAR SURVIVAL	TREATMENT	IMMUNO-HISTOCHEMICAL	MORPHOLOGICAL	
pNET	Variable malignant potential	75%[1]	Surgery, chemotherapy using somatostatin analogues, mTOR inhibitor	Synaptophysin, chromogranin A, CD56, pan-cytokeratin, E-cadherin	Nests, salt and pepper chromatin, can also feature cystic spaces and necrosis	
SPN	Low-grade malignancy (local invasion / metastasis in 20%)	95%[2]	Surgery, no chemotherapy options	Progesterone receptor, vimentin, CD10, nuclear β-catenin	Cystic spaces, necrosis, pseudopapillary structures (variable)	

In tumours from two patients initially diagnosed as pNETs, DNA, RNA and histopathological evidence contributed to re-diagnosis as SPNs
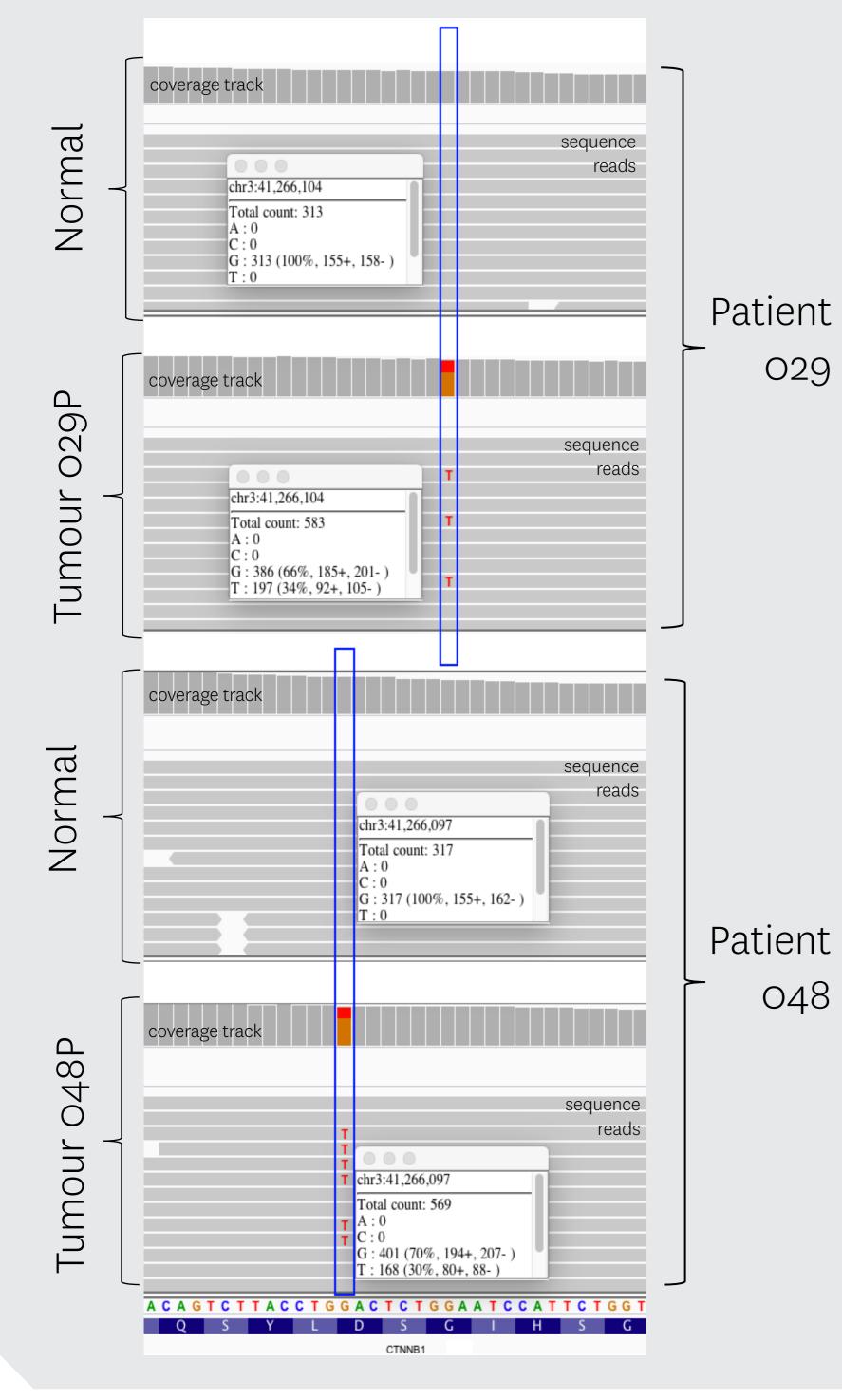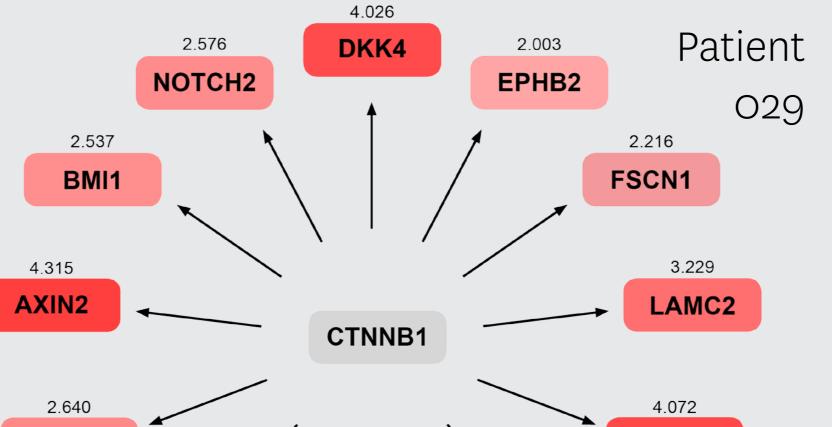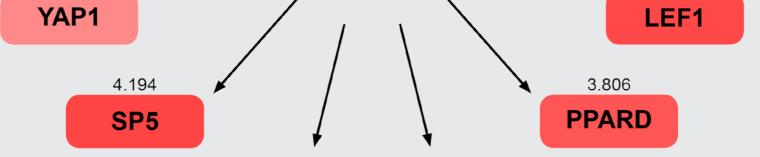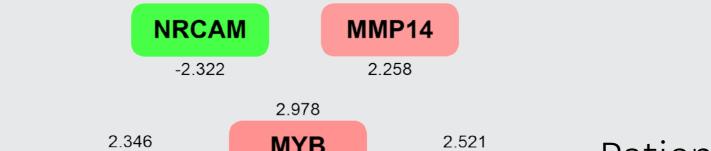
Deep targeted DNA-seq

Revealed **activating** mutations[3] in *CTNNB1* encoding B-catenin, pathognomonic for SPNs - present in 90% of all SPN cases[4].
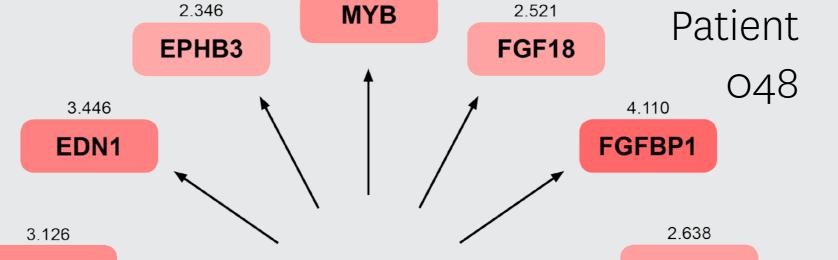

RNA expression analysis

RNAs known to be up-regulated by β-catenin[5] were highly expressed, in accord with the mutation's activating effect.

RNAs known to be up-regulated by β-catenin activity in colorectal cell lines were intersected with those RNAs with significantly high (≥1 SD above the mean of expression of the RNA in pNETs) or low expression (≥1 SD below the mean of expression of the RNA in all assayed pNETs). Colours map RNA expression from maximally low expression (green) through mean (white) to maximally high expression (red) across pNETs. Numbers indicate Z-transformed expression, i.e. number of SD above/below mean expression of each gene across the tumours.
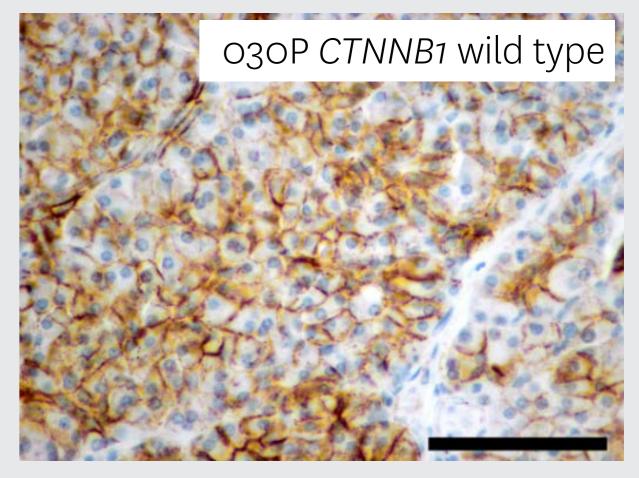
Histopathological analysis
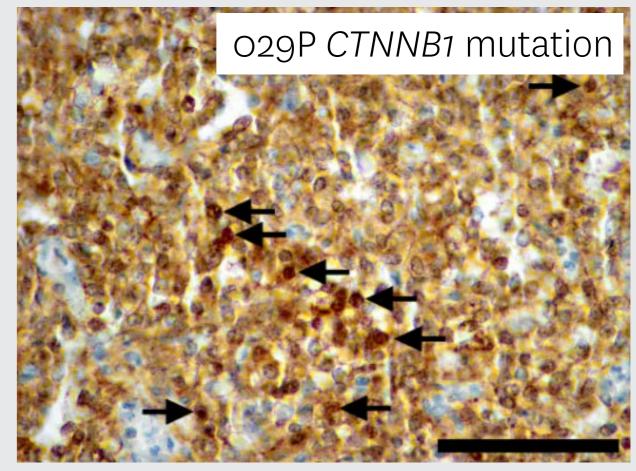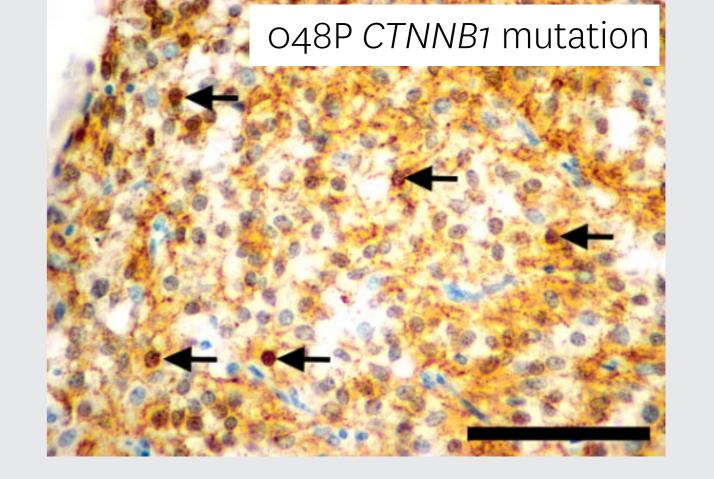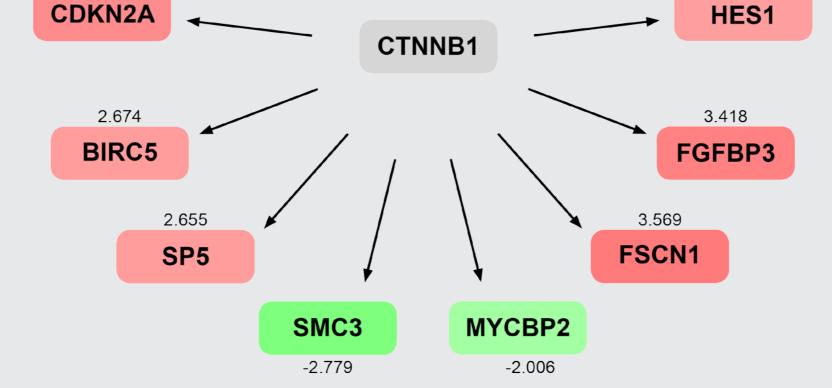
Revealed cellular relocalisation of β-catenin (brown) to the nucleus, concordant with SPN status and transcription factor function.

030P *CTNNB1* wild type

029P *CTNNB1* mutation

048P *CTNNB1* mutation

Homing in on precise diagnoses

- While differentiation of SPN from pNET can be challenging, it is important as the diagnosis has different prognostic implications. Genomic analysis provides a further tool for making this critical distinction. We believe that combining genomic information with traditional pathological information is likely to generate more precise diagnoses for many tumour types[6]
- Tighter diagnoses will enable not only more accurate clinical practice but also better research — tighter tumour cohorts may lead to more real findings

1. Cherenfant et al. (2013(PMID 24074416   2. Ohara et al. (2016(PMID 27784972   3. Moreno-Bueno et al. (2001(PMID 11703283
4. Abraham et al. (2002(PMID 11943721   5. Herbst et al. (2014(PMID 24467841   6. Harris et al. 2017 PMID 27556576
7. Wikipedia.com - Michael Bonert

Tamsin Robb
t.robb@auckland.ac.nz