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CASE REPORT

Sharon L. Mount · Donald L. Weaver
Douglas J. Taatjes · Wendy C. McKinnon
James C. Hebert

Von Hippel-Lindau disease presenting as pancreatic neuroendocrine tumour

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Abstract A 21-year-old woman with a family history of von Hippel-Lindau disease presented with a mass in the head of the pancreas. Light microscopic features of the tumour suggested neuroendocrine differentiation and although it displayed positive immunostaining for the antigens expected in a neuroendocrine neoplasm, S-100 staining was also present. This unusual feature prompted further evaluation by routine and post-embedding protein-A gold immunoelectron microscopy, which demonstrated the presence of neuroendocrine granules. Tumour cell DNA content was normal by flow cytometry. Although this patient exhibited no other signs of von Hippel-Lindau disease, the presence of a pancreatic tumour with neuroendocrine differentiation demonstrated that she was affected. Future surveillance and genetic counselling will be influenced by this diagnosis.

Key words Von Hippel-Lindau disease · Neuroendocrine tumour · Electron microscopy · Flow cytometry · Immunohistochemistry

Introduction

Von Hippel-Lindau disease is an autosomal dominant disease which is characterized by a variety of benign and malignant neoplasms and visceral cysts, particularly of the pancreas, kidney and epididymis. The most characteristic tumours are retinal haemangioblastoma ("von Hippel's tumour") and haemangioblastoma of the cerebellum ("Lindau's tumour"). These are the most common presenting manifestations of the disease. Other commonly associated neoplasms include haemangioblastoma of the medulla oblongata or spinal cord, angiomas of the liver and kidney, adenomas of the kidney and epididymis, renal cell carcinomas and adrenal phaeochromocytomas.

Morphologically documented pancreatic manifestations of von Hippel-Lindau disease include simple cysts [19], multiple serous cystic lesions [21, 24] and islet cell tumours (neuroendocrine neoplasms) [4]. Although the association of neuroendocrine tumours of the pancreas and von Hippel-Lindau disease has been described [4, 7, 17, 23] this is the first case of a patient presenting with a pancreatic neuroendocrine tumour as the first and sole manifestation of von Hippel-Lindau disease.

S. Mount (✉) · D. Weaver · D. Taatjes
Department of Pathology,
University of Vermont College of Medicine, Burlington, Vermont, VT 05405-0068, USA

W. McKinnon
Department of Pediatrics,
University of Vermont College of Medicine, Burlington, Vermont, USA

J. Hebert
Department of Surgery,
University of Vermont College of Medicine, Burlington, Vermont, USA

Case report

A 21-year-old Caucasian woman presented with abdominal pain. The patient had a strong familial history of von Hippel-Lindau disease (Fig. 1). Although she herself had no previous manifestations of this autosomal dominant disorder, she had been followed by head CT scans. As follow up for kidney stones, which she had complicating a recent pregnancy, an abdominal CT scan was performed which revealed a mass in the head of the pancreas. Angiography confirmed the presence of a large mass exhibiting neovascularity and supplied by pancreaticoduodenal branches of the coeliac and mesenteric arteries. The patient was subsequently referred for surgical resection of the pancreatic mass.

Surgical exploration of the abdomen revealed a tumour in the head of the pancreas which was fixed to the duodenum and measured approximately 7 cm in greatest dimension. The tumour did not appear to involve adjacent anatomical structures and there was no evidence of metastatic disease in any intra-abdominal organs or lymph nodes. A pancreaticoduodenectomy (Whipple procedure) was performed. The patient's recovery was unremarkable with the exception of left lower lobe pneumonia which developed on post-operative day 5. This was treated with intravenous Cefazolin, and she was discharged from the hospital on post-operative day 14. A 6-month follow up CT scan of the abdomen was unremarkable and the patient is asymptomatic.

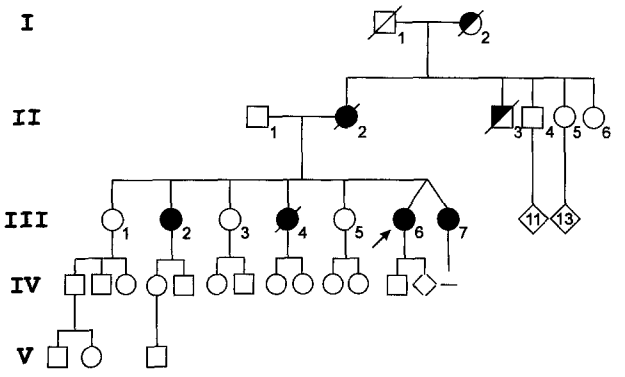

Fig. 1 Pedigree shows the positions of affected individuals *(filled symbols)* and of individuals with probable von Hippel-Lindau's disease *(half-filled symbols)*. Key is as follows: *circle* female, *square* male, *diamond* sex not specified, *number inside of diamond* number of children, sex not specified, I_2 brain tumour, death at approximately age 30, II_2 cerebellar astrocytoma, renal cell carcinoma, death at age 40, II_3 probable visceral tumour, refuses to be screened, III_2 cerebellar haemangioblastoma, III_4 retinal haemangioblastoma, renal cell carcinoma, death at age 31, III_6 pancreatic neuroendocrine tumour, III_7 renal tumour

Materials and methods

Tissue was obtained immediately after tumour resection for routine light microscopy, immunohistochemistry, electron microscopy, immunoelectron microscopy and flow cytometric DNA analysis. Tissue fixed in 10% neutral buffered formalin was first embedded in paraffin, then used for immunohistochemical studies and routine haematoxylin and eosin (H&E) stained light microscopic sections. The avidin-biotin immunoperoxidase technique was employed for immunohistochemical studies [30]. Because pancreatic polypeptide staining is not offered in our clinical histopathology laboratory, a paraffin block containing tumour was sent to IMPATH (New York, N.Y., USA), a reference laboratory, for immunostaining. See Table 1 for antibodies used.

For conventional electron microscopy, 1 mm^3 fresh tissue sections were fixed in half-strength Karnovsky's fixative (2.5% glutaraldehyde, 2.0% paraformaldehyde), postfixed in osmium tetroxide and embedded in Spurr's resin according to conventional protocols.

Immunoelectron microscopy was performed on 1 mm^3 tissue sections which were mildly fixed in 3% (para)formaldehyde, 0.1% glutaraldehyde in 0.01 M phosphate buffer, 0.15 M sodium chloride, pH 7.3 (PBS) for 6 h at 4° C. Following quenching of free aldehyde groups with 0.05 M ammonium chloride in PBS, the tissue pieces were dehydrated in a graded series of ethanols at progressively lower temperature, and finally infiltrated and embedded in the hydrophilic resin Lowicryl K4M (Polysciences, Eppleheim, Germany) at –35° C as previously described [22]. Antigenic sites were visualized on ultrathin sections mounted on parlodion/carbon coated grids using the protein A-gold technique [27, 29]. Colloidal gold particles with an average diameter of 14 nm were prepared according to the citrate reduction method of Frens [10] and complexed with protein A (Pharmacia LKB Biotechnology, Piscataway, N.J., USA) as described previously [27, 29]. Antibodies used for immunoelectron microscopic localization included anti-chromogranin A (1:2,000), anti-keratin (AE1/AE3; 1:2,000) and anti-cytokeratin cocktail (MAK-6; undiluted).

Fresh tissue and cell aspirates were procured for flow cytometric DNA content analysis. Cell suspensions were obtained by ex vivo needle aspiration of the fresh tumour. Cells and minced fresh tumour were then frozen in separate aliquots of 10% dimethyl sulphoxide in sucrose/citrate buffer. Cells were subsequently stained in 500 µl of a solution of propidium iodide (0.05 mg/dl, Sigma, St. Louis, Mo., USA), Triton X-100 (2%) and trisodium citrate (0.112%) for 15 min at 37° C. RNA was removed by the subsequent addition of 500 µl of RNAse (360 units/ml, Worthington Biochemical Corporation, Freehold, N.J., USA) in trisodium citrate for 15 min at 37° C. The sample was allowed to equilibrate for 1 h in the dark at 4° C prior to analysis on an EPICS Profile cytometer (Coulter Corporation, Hialeah, Fl., USA). The resulting DNA histograms were analysed using Verity:ModFit (Verity Software House, Topsham, Me., USA), a cell cycle analysis program.

Results

Gross Pathology

A 6.5×4.0×5.0 cm firm, somewhat lobulated mass was present within the head of the pancreas (Fig. 2). The tumour was directly adjacent to the duodenum and came within 3.5 cm of the surgical margin of the distal pancreas. The cut surface of the neoplasm revealed a variegated tan-yellow appearance punctuated by thick fibrous bands and foci of punctate haemorrhage. The bile duct was identified but was uninvolved by the tumour. Small cysts measuring up to 0.2 cm in diameter were also present within the mass.

Light Microscopy

H&E stained sections showed a well-encapsulated neoplasm displaying a mixture of acinar, nested, solid, and

Table 1 Antibodies used in this study

Antibody	Dilution	Source
Anti-chromogranin A (clone LK2H10)	1:400	Hybritech, San Diego, Calif., USA
Anti-neuron specific enolase	1:800	DAKO, Carpenteria, Calif., USA
Anti-keratin (clone AE1/AE3)	1:150	Boehringer-Mannheim, Indianapolis, Ind., USA
Anti-cytokeratin cocktail (MAK-6)	Undiluted	Triton Biosciences, Alameda, Calif., USA
Anti-S100 protein	1:2000	DAKO
Anti-insulin	Undiluted	DAKO
Anti-gastrin	1:1300	DAKO
Anti-serotonin (H209)	1:1600	DAKO
Anti-glucagon	Undiluted	DAKO
Anti-HMB-45	1:100	Enzo Diagnostics, New York, N.Y., USA
Anti-somatostatin	Undiluted	DAKO
Anti-neurofilament (2F11)	1:500	DAKO
Anti-pancreatic polypeptide	–	Performed by IMPATH

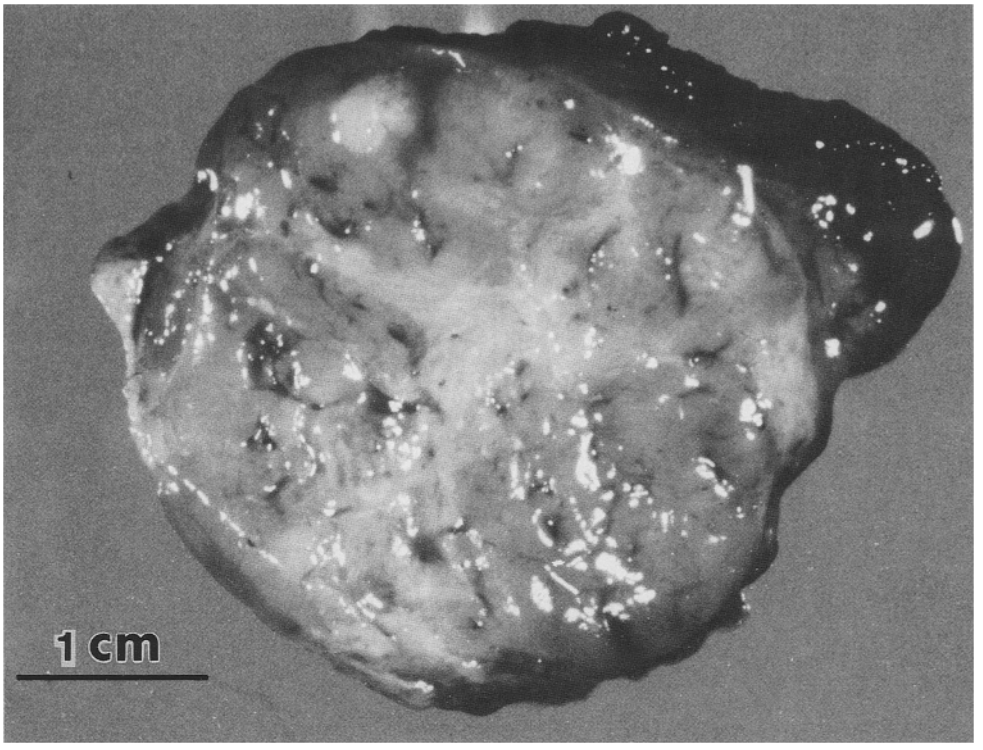

Fig. 2 Gross photo of cross section of pancreatic tumour demonstrating a variegated, somewhat lobulated appearance with fibrotic bands and small foci of haemorrhage

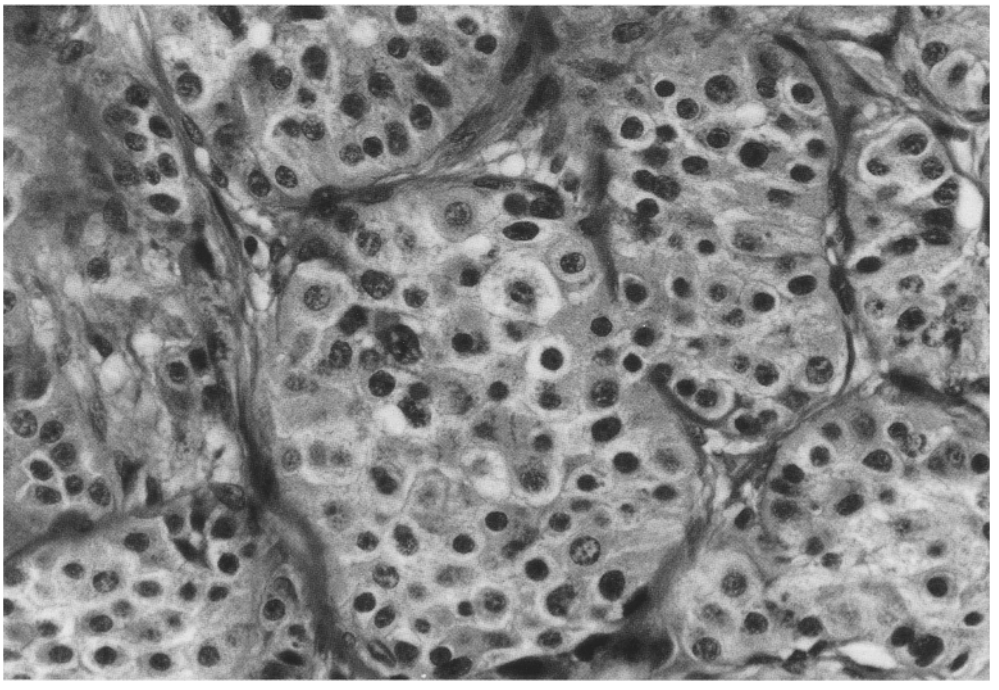

Fig. 3 Paraffin embedded section from pancreatic tumour showing nested arrangement of fairly uniform cells with round nuclei and abundant cytoplasm. Haematoxylin and eosin, ×600

trabecular patterns (Fig. 3). The tumour was separated by variably sized fibrous bands, and focal areas of necrosis and haemorrhage were identified. The majority of the tumour cells were uniform in size and shape with abundant eosinophilic cytoplasm and round nuclei with coarse chromatin. Mitoses were rare, averaging less than 2 per 50 high power fields. No atypical mitotic figures were identified. Rare pleomorphic cells with irregularly contoured, hyperchromatic nuclei were interspersed throughout the neoplasm. Scattered apoptotic cells were identified. There was no evidence of vascular or lymphatic invasion.

Immunohistochemistry

Strong positive cytoplasmic immunoreactivity for anti-chromogranin A, anti-keratin (AE1/AE3; MAK-6), anti-

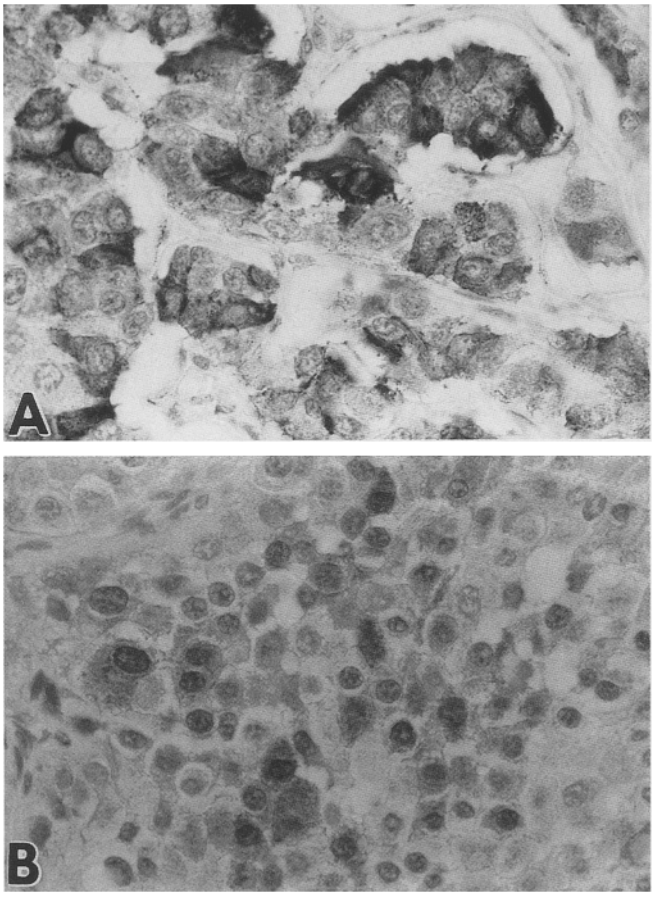

Fig. 4 Immunohistochemical demonstration of somatostatin (**A**) and S-100 protein (**B**) in tumour cells. ×400 (**A**), ×600 (**B**)

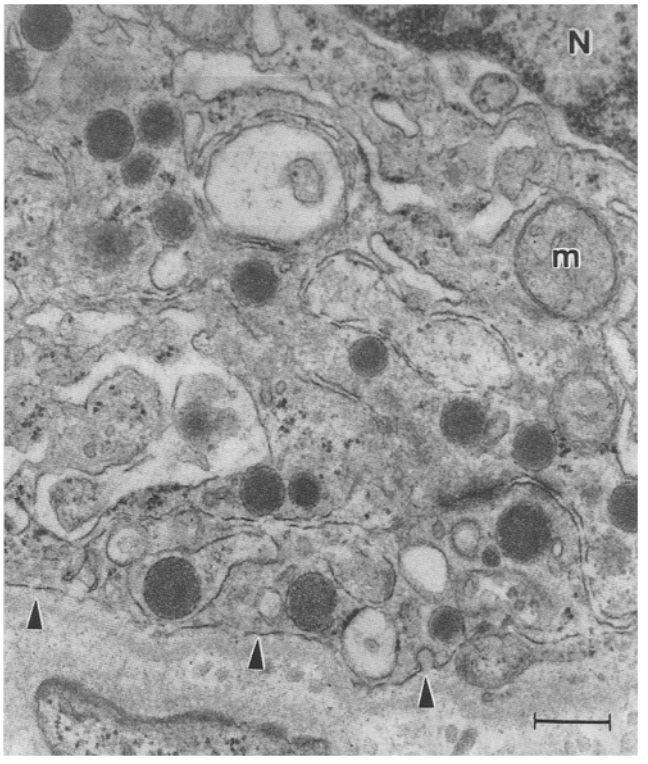

Fig. 5 Electron micrograph from Spurr's embedded tumour demonstrating numerous dense core granules, mitochondria and rough endoplasmic reticulum. Note the presence of a continuous basal lamina *(arrowheads)* that surrounds nests of cells; *m* mitochondria, *N* nucleus. ×36,000. *Bar* = 0.26 μm

somatostatin (Fig. 4A) and anti-neuron specific enolase (not shown) was demonstrated in virtually every tumour cell. Positive immunoreactivity for S-100 protein (Fig. 4B) was demonstrated in the majority of neoplastic cells ranging from strong (in approximately 50% of the cells) to extremely weak immunoreactivity. No immunoreactivity was demonstrated for anti-pancreatic polypeptide, anti-insulin, anti-gastrin, anti-serotonin, anti-glucagon, anti-neurofilament or HMB-45.

Conventional Electron Microscopy

The tumour was composed of cells with oval nuclei, some with prominent nucleoli and others with a diffuse chromatin pattern. The cytoplasm of the tumour cells was filled with many mitochondria, rough endoplasmic reticulum, rare microfilaments and numerous diagnostic dense core secretory granules (Fig. 5). The dense core granules had a medium dense centre surrounded by a thin clearing and dense peripheral rim. The granules were round to oval and varied slightly in size. No polygonal crystals characteristic of a beta cell tumour were present. A continuous basal lamina surrounded nests of cells (Fig. 5) and desmosomes were present between individual tumour cells.

Immunoelectron microscopy

Numerous dense core granules within tumour cells labelled with colloidal gold particles indicative of chromogranin A immunoreactivity (not shown). Scattered intermediate filaments and tonofilaments were decorated by colloidal gold particles indicative of cytokeratin AE1/AE3 immunoreactivity (not shown) and MAK-6 immunoreactivity (not shown).

Flow Cytometry

The DNA content of the fresh/frozen tumour and tumour cell aspirates was almost entirely diploid (92% and 93%, respectively; not shown). Cell cycle activity (S-phase) was 12% in the fresh frozen tissue and 13% in the cell aspirate. Both preparations exhibited a small population (approximately 7%) of hyperdiploid cells (DNA index: 1.15) which may be explained by the component of slightly larger cells observed within the neoplasm on light microscopic sections. An alternative explanation is differential propidium iodide uptake by a small proportion of the tumour cells.

Discussion

The prevalence of pancreatic islet cell tumours in von Hippel-Lindau disease has been difficult to assess from the literature because most of the published data are in the form of case reports or, by necessity, limited to kindred studies [4, 5, 7, 14, 15, 16, 17, 18, 19, 21, 23, 24, 26]. Moreover, there is marked variability of pancreatic involvement in the disease within families [19]. Binkovitz and colleagues [4] recently reported an incidence of 17% in their study of 43 patients with von Hippel-Lindau disease from over 25 kindreds. In our particular case, the confirmation that this tumour was indeed a neuroendocrine tumour was essential, in that it was the first manifestation of disease in this patient and thus the first indication that she was affected by von Hippel-Lindau disease. In addition, the association in von Hippel-Lindau disease of pancreatic islet cell tumours with higher prevalence rates of renal cell carcinoma (67% versus 31%) and phaeochromocytomas (33% versus 7%), but a lower prevalence of central nervous system haemangioblastomas (33% versus 72%) [4] has significant impact on the future clinical surveillance of this patient.

The diagnosis of neuroendocrine tumour was suggested by the H&E light microscopic appearance. Much of the tumour was composed of uniform cells with abundant cytoplasm and low grade nuclei arranged in trabecular, acinar and nested growth patterns characteristic of a neuroendocrine tumour. However, focal areas exhibited greater degrees of nuclear enlargement and pleomorphism suggesting that adenocarcinoma and acinar cell carcinoma be included in the differential diagnosis. Although gangliocytic paraganglioma could enter into the differential diagnosis, this was ruled out due to the occurrence of the tumour in the pancreas proper, as opposed to the small bowel, the most common site of gangliocytic paraganglioma [6, 14, 25, 28]. Moreover, gangliocytic and spindle cell components were not identified.

Although immunohistochemistry supported the neuroendocrine origin of the neoplasm with strong chromogranin A, somatostatin and neuron specific enolase immunoreactivity, no staining was obtained with anti-pancreatic polypeptide, anti-insulin and anti-glucagon antibodies. Interestingly, the tumour also demonstrated positive S-100 staining, a result not commonly associated with tumours of neuroendocrine differentation. Conventional electron microscopy confirmed the diagnosis of a neuroendocrine tumour by the identification of dense core granules which were subsequently demonstrated to be strongly labelled with gold particles indicative of chromogranin A immunoreactivity by immunoelectron microscopy. In addition, intermediate filaments and tonofilaments were labelled with anti-keratin antibodies.

Although a high proportion of pancreatic neuroendocrine tumours exhibit an aneuploid DNA content [1, 2], this case exhibited a primarily diploid cell population with a small population of hyperdiploid cells (DNA index: 1.15). Some evidence suggests that metastatic pancreatic neuroendocrine tumours may have a higher incidence of DNA aneuploidy [9]. The presence of aneuploidy in pancreatic neuroendocrine tumours does not appear to affect clinical outcome unless there is substantial excess DNA (DNA index >1.8) [2]. DNA aneuploidy, as measured by image cytometry, may also have prognostic significance in pancreatic endocrine tumours [8].

Recently, the von Hippel-Lindau gene has been identified using positional cloning strategies and has been mapped to the short arm of chromosome 3 at p25–26. The von Hippel-Lindau disease gene appears to function as a tumour suppressor gene. Variable expressivity is a hallmark of von Hippel-Lindau disease and penetrance appears to be complete by age 65 [20]. Prior to the cloning of the von Hippel-Lindau disease gene, it was suggested that the disease's phenotypic expression might result from allelic heterogeneity. The cloning efforts have identified a number of different types of mutations including deletions, insertions and point mutations [11]. Whether the variety of mutations explains the variable expression of von Hippel-Lindau disease remains to be seen. Identification of this gene has enabled the development of a clinically useful test to identify von Hippel-Lindau disease gene carriers in families who have an identifiable rearrangement by Southern blot analysis. For those families who do not have an obvious rearrangement by Southern blotting, linkage analysis using polymorphic markers may be useful in identifying gene carriers [12].

In summary, we report a case of a pancreatic neuroendocrine tumour investigated by immunohistochemical, electron microscopic, immunoelectron microscopic and flow cytometric techniques, arising in a 21-year-old patient with a family history of von Hippel-Lindau disease. The incidence of pancreatic neuroendocrine tumours is 17% in patients with known von Hippel-Lindau disease but only 0.5%–1.5% in unselected autopsies [13]. Unlike many cases of von Hippel-Lindau disease in which the patient commonly presents with retinal angiomas and cerebellar haemangioblastomas, in the case presented here the pancreatic neuroendocrine tumour was the first and only manifestation.

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