ISSN 2472-1972

Pancreatic Neuroendocrine Tumor in a Young Child With Tuberous Sclerosis Complex 1

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Pancreatic neuroendocrine tumors (PNETs) occur in the context of tuberous sclerosis complex (TSC). To date, PNETs in association with TSC have been described almost exclusively in adults and in the context of TSC2. We present the evaluation of a PNET in a young child with TSC1. A 3-year, 6-month-old boy with TSC1 was found on surveillance to have a small pancreatic lesion measuring 0.4 cm on magnetic resonance imaging (MRI). The lesion showed interval enlargement to 1 cm on serial MRI studies during the ensuing 16 weeks. Endocrine laboratory tests did not reveal a functional tumor. The patient underwent enucleation of the pancreatic lesion. Microscopic examination defined a well-differentiated PNET, grade II/intermediate grade with a mitotic rate of two mitotic figures per 10 high-powered field and Ki-67 proliferation index of ~15%. The tumor was positive for the *TSC1* gene mutation. The patient was free of tumor recurrence at the 5-year follow-up examination, as determined by endocrine surveillance and annual MRI of the abdomen. In the reported data, PNET in patients with TSC has been primarily reported in association with TSC2. Our case demonstrates that patients with TSC1 can develop PNETs, even at an early age. The international TSC consensus group 2012 recommendation was to obtain MRI of the abdomen every 1 to 3 years for surveillance of renal angiomyolipomas and renal cystic disease. It might be beneficial to add a pancreatic protocol to the surveillance guidelines to evaluate for PNET.

Freeform/Key Words: tuberous sclerosis complex, pancreatic neuroendocrine tumor

Pancreatic neuroendocrine tumors (PNETs) are rare in children [1]. They are typically sporadic but are known to occur with genetic syndromes such as multiple endocrine neoplasia type 1, von Hippel-Lindau disease, neurofibromatosis 1, and tuberous sclerosis complex (TSC) [2]. They are infrequent in the context of TSC compared with other genetic syndromes and have been reported almost exclusively in patients with the TSC2 variant [3, 4].

TSC is inherited as an autosomal dominant multisystem disorder. However, only 20% to 30% of all reported cases have been familial, and the remaining 70% to 80% of cases have occurred in the context of a spontaneous mutation [5]. The prevalence of the disease has been 1:6000 to 1:10,000 [6]. TSC is caused by mutations within one of two tumor suppressor genes, *TSC1* on chromosome 9q34 or *TSC2* on chromosome 16p13.3, which encode for hamartin and tuberin, respectively [5]. Both genes are involved in regulating cell growth through inhibition of the mammalian target of

Abbreviations: mTOR, mechanistic target of rapamycin; PNET, pancreatic neuroendocrine tumor; SEGA, subependymal giant cell astrocytoma; TSC, tuberous sclerosis complex.

Received 4 February 2019
Accepted 23 April 2019
First Published Online 29 April 2019

Case Report
June 2019 | Vol. 3, Iss. 6
doi: 10.1210/js.2019-00051 | Journal of the Endocrine Society | 1201–1206

rapamycin (mTOR) pathway. TSC1 is twice as likely to be familial than spontaneous; however, TSC2 has more often been spontaneous than familial [7]. The existence of an important genotype–phenotype correlation in individuals bearing specific mutations in either TSC1 or TSC2 is controversial. Observations from both human and mouse models have suggested that a more severe phenotype will be associated with mutations in TSC2 rather than TSC1 [8].

The diagnosis of TSC is established by the identification of a heterozygous pathogenic variant in TSC1 or TSC2 on molecular genetic testing [6]. A clinical diagnosis of TSC is established in patients who meet the clinical diagnostic criteria for TSC, which include the presence of two major clinical features of TSC or one major and two minor clinical features of TSC. The major features include hypomelanotic macules, angiofibromas, ungual fibromas, shagreen patch, multiple retinal hamartomas, cortical dysplasia, subependymal nodules, subependymal giant cell astrocytoma (SEGA), cardiac rhabdomyoma, lymphangioleiomyomatosis, and angiomyolipomas. The minor features include "confetti" skin lesions, dental enamel pits, intraoral fibromas, retinal achromic patch, multiple renal cysts, and nonrenal hamartoma. However, PNETs have not been included in the diagnostic criteria of TSC, and, at present, screening has not been recommended.

To the best of our knowledge, only two cases of PNET in patients with the TSC1 variant have been reported, and both had developed in adults [3, 9]. We present the case of a 3-year-old child with TSC1 who had presented with a pancreatic lesion diagnosed as PNET.

1. Case Report

A 3-year, 6-month-old male patient with TSC1 was identified by routine surveillance abdominal MRI to have a 0.4-cm hypointense lesion in the posterior body of the pancreas.

His medical history included epilepsy at 3 months of age, at which time several hypomelanotic lesions were noted on his trunk on physical examination. Using central nervous system MRI, multiple subependymal nodules were seen along the white matter and cortex, with the largest subependymal tuber (7 mm) seen along the posterolateral aspect of the right frontal horn, consistent with TSC. Molecular studies confirmed a mutation at exon 10 of the *TSC1* gene, with a heterozygous base-change mutation (c.989dupT, an abnormal TSC1 protein, hamartin, p.Ser331fs). Both parents tested negative for the mutation. Multiple small, nonobstructive, cardiac rhabdomyomas had been seen on echocardiography at 15 months. Renal ultrasonography at 3 years of age showed interval development of a small unilateral cortical cyst and bilateral nonshadowing punctate echogenic foci compatible with angiomyolipomas. MRI of the abdomen performed at 3 years, 3 months of age showed a 1.2-cm unilateral renal upper pole cyst with septation and innumerable tiny cysts and/or angiomyolipomas. Repeated brain MRI at 3 years, 6 months of age showed marked interval enlargement of a single subependymal nodule in the posterolateral aspect of the right frontal horn at the level of the foramen of Monro, with a mass effect and a localized right to left midline shift that was strongly suspicious for interval development of a SEGA. The patient underwent right frontal endoscopic resection of the SEGA. At that time, at 3 years and 6 months of age, abdominal MRI revealed a 0.4-cm hypointense lesion in the posterior body of the pancreas. Three months later, MRI showed a minimal increase of the pancreatic lesion to 0.45 cm. At 3 years, 10 months, MRI showed an interval increase of the pancreatic mass to 1.0 cm. The mass was hypointense on T1-weighted images and mildly hyperintense on T2-weighted images, with minimal central enhancement. The differential diagnosis included a neuroendocrine tumor or hamartoma. The endocrine laboratory test results were consistent with a nonfunctional tumor: glucose, 81 mg/dL; insulin, 4 μIU/mL (range 2.6 to 24.9 μIU/mL); gastrin, 83 pg/mL (fasting 3 to 4 hours; range, 2 to 168 pg/mL); serum neuron-specific enolase, 8.9 ng/mL (range, 0.0 to 12.5 ng/mL); chromogranin A, 2 nmol/L (range, 0 to 5 nmol/L); IGF-1, 59 ng/mL (range, 20 to 141 ng/mL); IGF binding protein-3, 1960 ng/mL (range, 972 to 4123 ng/mL). After careful review, the patient underwent open enucleation of the 1.0-cm pancreatic mass. Microscopic examination confirmed a well-differentiated PNET, intermediate grade (grade 2), with mitotic rate of 2 mitotic figures/10 high power field and Ki-67

proliferation index of ~15%. The tumor cells demonstrated diffuse positivity with chromogranin A, synaptophysin (cytoplasmic), and CD56 (membranous), consistent with neuroendocrine differentiation. The tumor cells were positive for CAM5.2 and CK19 (cytokeratin) and negative for CDX-2. Patchy PAX-8 positivity was present, with loss of progesterone receptor expression. The tumor cells were focally positive for glucagon but negative for insulin, somatostatin, gastrin, and pancreatic polypeptide. Molecular studies of the pancreatic tumor tissue revealed the identical mutation at exon 10 of the *TSC1* gene with a heterozygous base-change mutation (c.989dupT, an abnormal TSC1 protein, hamartin, p.Ser331fs). The pancreatic tumor was also positive for other variants of unknown significance (ASXL1:G1154R, IRF8:E225K, MLH1:E319K). Following five years of ongoing surveillance, the patient has not had recurrence of the PNET.

2. Discussion

PNETs are rare epithelial tumors that are often sporadic but are known to be strongly associated with endocrine neoplasia syndromes [3]. The relative frequency of PNETs associated with these diseases, in descending order, has been as follows: multiple endocrine neoplasia type 1 (80% to 100%), von Hippel-Lindau disease (range, 10% to 17%), neurofibromatosis type 1 (<10%), and TSC (range, 1.5% to 1.8%) [2].

Recently reported data have added to our understanding of the frequency of PNETs in patients with TSC. Larson *et al.* [10], in a retrospective study of 219 patients with TSC, reported the incidence of PNETs to be 1.5% to 1.8%. In their study, the PNETs in the context of TSC had developed at a younger mean age (range, 26 to 56 years) than has been reported in the general population. Also, all the studied patients with TSC were reported to have TSC2, rather than TSC1, variants [10]. Data from their cohort support the finding that PNETs represent the dominant pancreatic pathology in the setting of TSC [10]. In a retrospective study of 55 children with TSC by Koc *et al.* [11], 5 of the 55 children aged 5 to 14 years, had a diagnosis of PNET, a frequency of 9.9%. Of these five patients, only one had undergone genetic testing and was found to have a TSC2 variant [11]. On review of the reported data, we found only two case reports with PNET in TSC1, both in adults. Gustafson *et al.* [9], in 2008, reported the case of a 29-year-old woman with multifocal renal carcinoma and PNET. She was confirmed to have a pathogenic heterozygous TSC1 variant (Q343X TSC1 mutation and a heterozygous R1329H TSC2 polymorphism) [9]. Mortaji *et al.* [3], in 2018, reported the case of a 35-year-old woman with PNET in the TSC1 variant (c.1530_1531delCA; p.Asp510Glufs*24) that had been discovered by surveillance imaging studies after her daughter had presented with an incidental finding of subependymal nodules on central nervous system MRI [3].

PNETs can be functional or nonfunctional, depending on whether they cause hormonal hypersecretion syndromes. Functional PNETs can release insulin, glucagon, gastrin, vasoactive intestinal peptide, and somatostatin and present with symptoms caused by ectopic secretion of hormones. Less commonly, they can secrete adrenocorticotropic hormone, GHRH, PTH-related peptide, and/or calcitonin. Very rarely, PNETs will secrete LH, renin, IGF-2, or erythropoietin. Nonfunctioning PNETs also secrete a number of substances, such as chromogranin A, neuron-specific enolase, pancreatic polypeptide, and ghrelin. However, patients with nonfunctioning PNETs will not present clinically with a hormonal syndrome compared with those with functional PNETs [2]. Our patient was asymptomatic, in keeping with the presence of a nonfunctional PNET. The serum levels of glucose, insulin, gastrin, and pancreatic polypeptide were in the normal range in our patient.

The association between TSC and PNET can be explained by the common molecular pathway of upregulation of the phosphoinositide 3-kinase/AKT (protein kinase B) pathway, with cascade activation of MAPK and mTOR [12]. The TSC1 and TSC2 proteins form a complex and function as tumor suppressors by inhibiting mTOR, which regulates the major cellular processes required for normal cell growth and differentiation, metabolism, and survival [13]. Understanding of this pathway has led to the exploration of mTOR inhibitors such as everolimus (a sirolimus analog) as adjuvant therapy for PNETs.

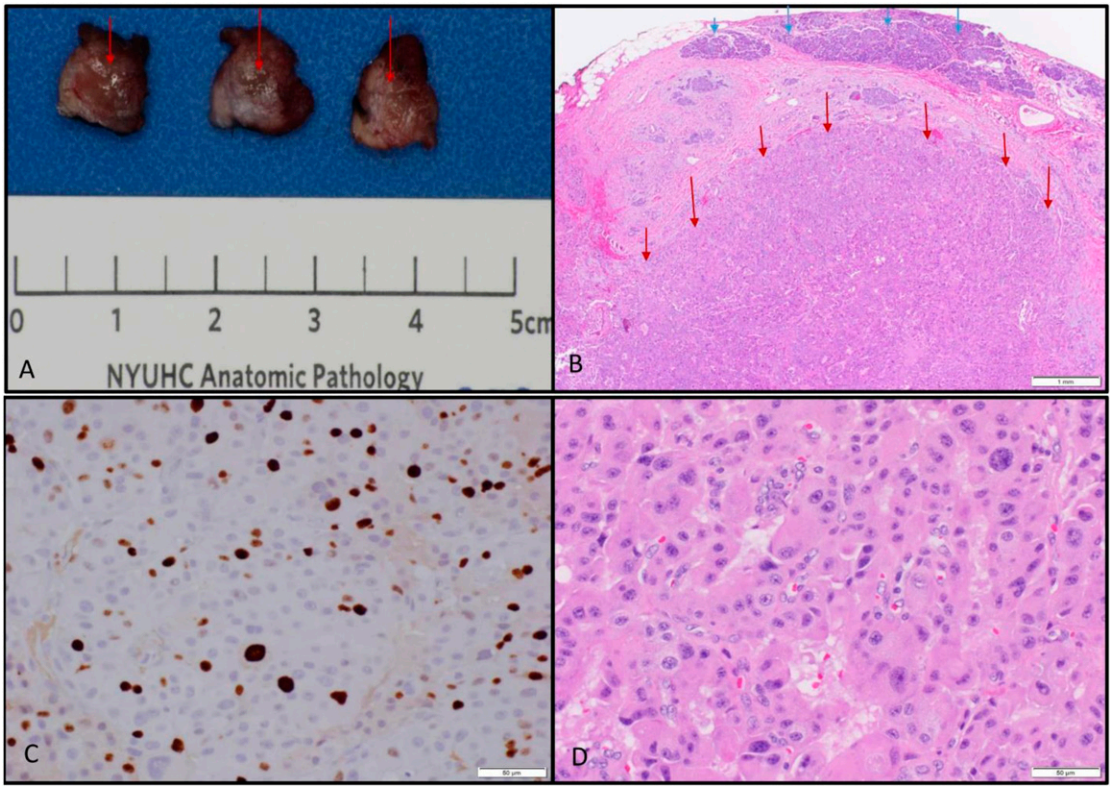

Figure 1. (A) Gross examination of the enucleated tumor showing in cross section, a 1.0 × 0.8 × 0.6-cm, fairly well-circumscribed, tan-brown, glistening nodule (*red arrows*). (B) Low power showing the tumor (*red arrows*) and a thin rim of normal pancreas parenchyma (*blue arrows*) (hematoxylin and eosin stain, original magnification ×40). (C) Proliferation index by Ki-67 was ~15% (original magnification ×400). (D) The tumor had a trabecular and nesting growth pattern, and the tumor cells showed granular eosinophilic cytoplasm and moderate nuclear pleomorphism (hematoxylin and eosin stain, original magnification ×400).

Just as in our patient, on gross examination (Fig. 1), PNETs will be red to tan, reflecting the abundant vasculature. Morphologically, well-differentiated PNETs will have the characteristic organoid arrangements of tumor cells in nests and trabeculae that are relatively uniform, with round to oval nuclei, coarsely granular with stippled chromatin, and variable, from pale to moderately eosinophilic, cytoplasm [14]. These cells produce abundant neurosecretory granules as reflected in the immunohistochemical expression of neuroendocrine markers, such as synaptophysin and chromogranin A. The mitotic rate is an important measure of the aggressiveness of PNETs. Mitotic rates <20% are consistent with low-grade well-differentiated PNETs [15]. Confirmation of neuroendocrine markers (synaptophysin and chromogranin), followed by epithelial markers (*e.g.,* CK19, CAM5.2), are important for the diagnosis of PNET [16]. Normal islet cells express progesterone receptors and will be positive for PAX8. Patchy PAX-8 positivity supports a pancreatic origin, and the loss of progesterone receptors might be associated with a worse prognosis [17]. Most tumor cells will be positive by immunohistochemistry for glucagon but negative for insulin, somatostatin, gastrin, and pancreatic polypeptide, with a predominance of α cells.

Our patient had a heterozygous germline mutation in the TSC1 gene, which was likely a *de novo* pathogenic variant, because this mutation was not found in his parents. Loss of heterozygosity for the TSC1 was not found in the PNET, and the mutation was heterozygous in both the germline and the tumor. It has been known that TSC1 mutations in the absence of loss of heterozygosity can cause TSC1 lesions by (i) subtle, second-hit mutations, (ii) haploinsufficiency alone is sufficient, or (iii) other genetic/epigenetic events involved in TSC lesion development [18, 19]. These mechanisms have been reported in other TSC lesions, such

as tubers, hamartomas, and SEGA, but, to the best of our knowledge, has not yet been described for PNET, possibly owing to the paucity of cases of PNET associated with TSC1 [19–21].

Treatment decisions for PNETs must be individualized, and it is important to distinguish functional and nonfunctional tumors. Functional tumors should be medically controlled to decrease the symptoms before surgical resection. The wait and watch approach is appropriate for nonfunctional, small (<2 cm), low-grade tumors, given their indolent nature. For patients with metastasis, other treatment modalities have included surgical debulking, systemic therapy, including chemotherapy or molecular targeted therapy (tyrosine kinase inhibitors, mTOR inhibitors), liver-directed therapy, and peptide radionuclide therapy [19].

In conclusion, PNET is considered rare in patients with TSC and has been mostly known to occur in association with the TSC2 variant. We have presented the case of a young child with TSC1 and PNET. It is important to be aware that PNETs can also occur in those with TSC1. It has been suggested that patients with TSC undergo surveillance studies as outlined by the 2012 international TSC consensus, which includes cross-sectional abdominal imaging to assess for the presence of renal angiomyolipomas and cysts [6]. It might be beneficial to add a pancreatic protocol to the cross-sectional abdominal imaging for the early diagnosis of PNET. The findings from the present case report have further strengthened the possibility of an association between TSC1 and PNET. It is an important association; however, a causative relationship remains to be proved.

Acknowledgments

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Disclosure Summary: The authors have nothing to disclose.

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