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Malignant pancreatic tumour within the spectrum of tuberous sclerosis complex in childhood

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Abstract A 12-year-old boy with tuberous sclerosis complex (TSC) presented with a large retroperitoneal tumour. Exploratory surgery revealed an infiltrative tumour originating from the pancreas, with local metastases to the lymph nodes. The histologal diagnosis was a malignant islet cell tumour. Retrospectively measured pancreatic hormone levels, however, were normal. A connection between the malignancy and TSC was demonstrated by loss of heterozygosity of the TSC2 gene in the tumour. The primary mutation Q478X in this patient was identified in exon 13 of the TSC2 gene on chromosome 16.

Conclusion Pancreatic islet cell tumours have been mainly associated with multiple endocrine neoplasia syndrome type 1. In our case we demonstrate a direct relationship of this tumour to tuberous sclerosis complex, in the absence of further signs of multiple endocrine neoplasia syndrome type 1.

Key words Tuberous sclerosis complex · TSC2 gene · Loss of heterozygosity · Pancreas · Islet cell tumour

Abbreviations *LOH* loss of heterozygosity · *MEN-1* multiple endocrine neoplasia syndrome type 1 · *TSC* tuberous sclerosis complex

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous disorder, with a birth prevalence of 1:6,000–10,000 [10], characterized by the growth of hamartomas that can appear in virtually any organ or tissue. Diagnostic criteria most widely used are those of Gomez [4]. The most frequently affected organs are skin, brain, kidneys, eyes, and heart. About 50% of patients with TSC present as new cases in their family (new mutations). Small children often present with epilepsy and mental retardation. Abdominal involvement in TSC usually involves the kidneys with bilateral multiple renal

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cysts, often in combination with angiomyolipomas. The frequency of renal involvement in TSC has been estimated to be between 40% and 80% [4]; in a small number of patients the liver and/or pancreas show changes like those in the kidneys. Malignant degeneration of these tumours and cysts is rare. Mutation in one of two separate genes can cause TSC. Both these genes have been isolated: TSC1 on chromosome 9q34.3 [12] and TSC2 on chromosome 16p13.3 [2]. The clinical picture of patients with TSC1 mutations or with TSC2 mutations is very similar, perhaps indistinguishable. In principle, each family has a separate mutation, but recurrence of a particular mutation in unrelated sibships has been reported [12,15].

In this report we demonstrate the case of a young boy with TSC, presenting with abdominal pain, caused by a malignant pancreatic islet cell tumour. Mutation analysis in blood cells of the patient resulted in the identification of the germ-line mutation in the TSC2 gene. The involvement of the TSC2 gene in the aetiology of the pancreatic tumour was shown by the demonstration of allelic loss of the non mutated allele of the TSC2 gene in tumour tissue.

Materials and methods

The surgical specimen was fixed for 24 h in 4% phosphate buffered formalin, 4 μm paraffin embedded sections were stained with haematoxylin and eosin. The used antibodies were directed against chromogranin-A (polyclonal, DAKO), insulin (polyclonal, DAKO), synaptophysin (monoclonal, Boehringer), gastrine (polyclonal, DAKO), islet amyloid polypeptide (gift from van Hulst [6]), somatostatin (polyclonal, DAKO), glucagon (polyclonal, DAKO) and MIB-1 (monoclonal, Immunotech). Subsequently streptavidin-biotin-peroxidase staining was done. Immunohistochemical procedures were performed as described previously [14].

Primer sequences for direct sequence analysis of the 41 exons of the TSC2 gene are available on request. PCR conditions for exon 13 for 100 μl volume were 10 mM Tris pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 100 μM mix of each deoxynucleotide and 2 U of Taq polymerase (Gibco BRL). For the amplification reaction of exon 13 thermal cycling conditions were 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, 90 s at 72°C with a final elongation of 5 min at 72°C. Cycle sequence reactions were performed using the ABI prism dye primer cycle sequence ready reaction kit (Perkin Elmer), gels were run on an ABI 377 automated DNA sequencer.

Loss of heterozygosity (LOH) studies were initiated by comparing DNA from peripheral leucocytes and DNA from tumour tissue, using markers D9S149 and D9S150 for the chromosome 9q34 TSC1 region [12], and KG8(intragenic in the PKD1 gene, adjacent to the TSC2 gene) and 16AC2.5(D16S291) for the chromosome 16p13 TSC2 region [2]. The LOH results were confirmed by allele specific oligonucleotide hybridization analysis (sequences available on request). Hybridization was performed at 37°C for 1 h, filters were washed to 0.3 × SSC for 10 min at 37°C.

Case report

The patient was born in 1985 as the second child from healthy, non consanguineous, parents. Pregnancy and delivery had been normal. At 2 years a diagnosis of TSC was made on the basis of the presence of epilepsy, hypopigmented macules, mental retardation and CT scan abnormalities characteristic of TSC: subependymal calcified nodules, hypodense areas and left frontal atrophy. Ophthalmological examination and renal ultrasound were normal. By the age of 7 years, he had facial angiofibromas, a fibrous forehead plaque and ungual fibromas of the feet. At 9 years of age a large angiomyolipoma of the right kidney was embolized (both kidneys showed multiple smaller angiomyolipomas), and he underwent surgery for a subependymal giant cell astrocytoma, with placement of a ventriculoperitoneal shunt. Some months afterwards he had abdominal pains. Abdominal ultrasound showed no visible changes in his pre-existing renal TSC lesions. He had normal glucose levels, erythrocyte sedimentation rate, and liver and renal function parameters. The possibility of a psychosomatic component to his complaints was considered and a period of observation was allowed. Subsequently, an abdominal CT scan was made, showing a highly vascularized retroperitoneal tumour, growing from either the left kidney, stomach or pancreas. Needle biopsy was considered to be too risky. Explorative surgery proved the tumour to be of pancreatic origin, with infiltrative growth into the colon and stomach. Macroscopic subtotal resection was done, including the directly neighbouring lymph nodes. Post operative recovery was without complications.

Both his parents had been completely screened for TSC in the past and had shown no signs of the disease.

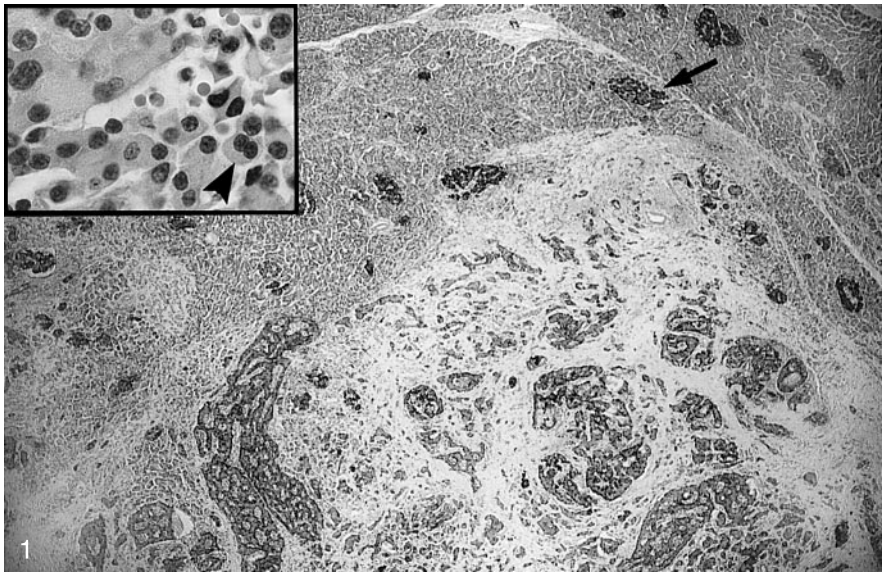
Results

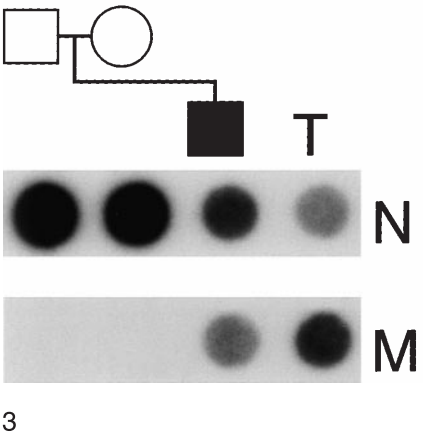
Pathology showed an islet cell tumour of the tail of the pancreas (maximum length 9.5 cm), with invasive growth into the posterior wall of the stomach and transverse colon (Fig. 1 A,B). Three of the resected lymph nodes showed metastases. Immunohistochemical staining was positive for the neuroendocrine markers synaptophysin and gastrine, and β-cell marker islet amyloid polypeptide, negative for insulin and somatostatin. Parts of the tumour were positive for chromogranin-A (a probable precursor for regulatory proteins), glucagon and monoclonal antibody MIB-1 (a marker for cell proliferation). Retrospectively, glucagon and glucose levels were determined in pre-operatively stored frozen plasma and were normal. The tumour thus appeared to have been non-hormone producing. Complete clinical screening of the patient for other endocrine neoplasias was negative, making a diagnosis of multiple endocrine neoplasia syndrome type 1 (MEN-1) improbable.

DNA analysis for LOH in the tumour of either TSC gene, using both chromosome 9 and 16 markers, showed LOH of chromosome 16 with both TSC2 markers, not with TSC1 markers (Fig. 2). The primary, germ-line mutation in the TSC2 gene appeared to be a nucleotide substitution C into T at position 1450 in exon 13 (Q478X). The LOH result was confirmed by allele specific oligonucleotide hybridization analysis for the mutation, showing that it was the normal, wild type allele that was lost in the pancreatic tumour (Fig. 3). The mutation was absent in DNA isolated from peripheral leucocytes of the parents and is therefore a de novo mutation.

Discussion

We present a mentally retarded boy with tuberous sclerosis who developed an unexpected malignant pancreatic





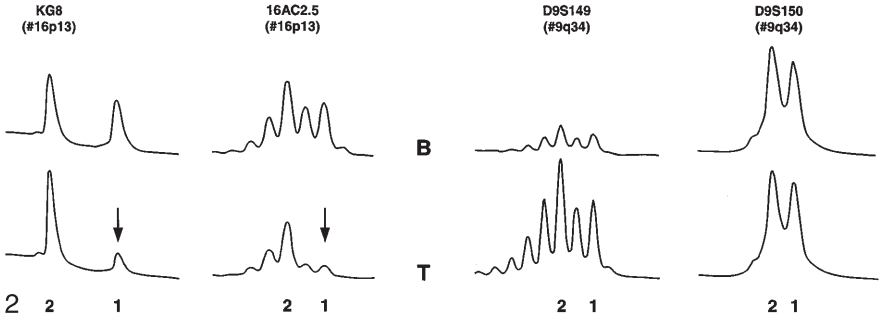


Fig. 1 A Low magnification (42×, chromogranin-A) to demonstrate the infiltrative growth of the islet cell tumour of the pancreas in a 12-year-old tuberous sclerosis patient. Note a similar staining with chromogranin-A of normal pancreatic islet cells (*arrow*) and tumour cells. **B** (*inset*) High magnification (500×, H & E) of the tumour cells. Note the mild degree of nuclear pleomorphism and the occurrence of binucleate tumour cells (*arrowhead*)

Fig. 2 Loss of heterozygosity (LOH) studies of blood (*B*) versus pancreas tumour (*T*) DNA of the index patient with tuberous sclerosis. For chromosome 16 (*left*) markers KG8 and 16AC2.5 clearly show partial loss of one allele (*arrows*) in DNA isolated from tumour (T) versus blood cells (B). For chromosome 9, markers D9S149 and D9S150 show no loss. (Although the overall signal in blood for D9S149 is reduced, the relative ratio of the alleles 1 and 2 remains unchanged)

Fig. 3 Demonstration of the Q478X mutation in exon 13 of the TSC2 gene in the patients blood (*black square*) and tumour tissue (*T*) by allele specific oligonucleotide hybridization. [*N* the normal allele, which is also present in the parents (*left two dots*), *M* mutated allele.] The relative intensities of the normal versus the mutated signal are clearly reversed in the tumour tissue, indicating LOH of the wild type TSC2 allele

tumour, with infiltrative growth and local metastases to the lymph nodes. Since radical excision was not possible, follow up by regular CT scan will be done, as recurrence is possible. The tumour was classified as a non-hormone producing islet cell tumour, the boy did not show clinical signs of a hormonal imbalance. Neuroendocrine tumours in children are found in MEN-1 syndrome, which has been mapped to chromosome 11. Screening for other neuroendocrine tumours showed no other manifestations of a possible co-existing MEN-1 syndrome. In MEN tumours loss of chromosome 16p13 has not been described. To our knowledge, no other reports on malignant pancreatic tumours in children with TSC have been published. If affected, the pancreas usually shows cysts and angiomyolipomas. Islet cell tumours and gastrinoma have been incidentally reported in adult TSC patients [1, 7, 11].

Malignancies do sometimes arise in association with tuberous sclerosis [3]. LOH has been reported in non metastasizing hamartomas of TSC patients [5]. At present it is unknown how the relatively rare transition from benign hamartoma to metastasizing malignant tumour should be viewed. Little is known about the interactions between the TSC1 and TSC2 gene products, called hamartin and tuberin respectively [13]. The demonstration of loss of the wild type TSC-2 allele in a neuroendocrine pancreatic tumour of this TSC patient strongly suggests a role for TSC2 as a tumour suppressor gene in its aetiology, in line with the proposed tumour suppression function of tuberin [5]. This loss of the non mutated allele, or 'second hit' is considered to be a decisive step in tumourigenesis [8]. Mutation analysis resulted in the identification of the Q478X germ-line

mutation or 'first hit' in the patient, likely to produce a severely truncated tuberin protein, instable and/or inactive, from that allele. A naturally occurring rat strain with a germ-line insertion in the TSC2 gene is the 'Eker rat', which is prone to develop renal carcinoma. In vitro, tumour growth can be inhibited by introducing an active TSC2 gene into cultured tumour cells of this rat [9], supporting the hypothesis that the TSC2 gene is a tumour suppressor gene.

Our findings show that the TSC2 gene can be involved in tumours that are not specifically associated with TSC, and might play a more general role in the suppression of tumour growth in different kinds of tissues.

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