Are neuroendocrine tumours a feature of tuberous sclerosis? A systematic review

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Abstract

Tuberous sclerosis complex (TSC) is an autosomal dominant multisystem disorder characterised by the development of multiple hamartomas in numerous organs. It is caused by mutations of two tumour suppressor genes, *TSC1* on chromosome 9q34 and *TSC2* on chromosome 16p13.3, which encode for hamartin and tuberin respectively. The interaction between these two proteins, the tuberin–hamartin complex, has been shown to be critical to multiple intracellular signalling pathways, especially those controlling cell growth and proliferation. TSC may affect skin, central nervous system, kidneys, heart, eyes, blood vessels, lung, bone and gastrointestinal tract. Small series and case reports have documented that in tuberous sclerosis patients many endocrine system alterations might occur, affecting the function of the pituitary, parathyroid and other neuroendocrine tissue. There have been scattered reports of the involvement of such tissue in the pathological process of TSC, but no systematic review as to whether this is a true association. We have therefore systematically assessed all available published literature in this area. We conclude that there may be an association with pituitary and parathyroid tumours, and two recent descriptions of Cushing's disease are especially intriguing. However, the evidence seems more firm in the case of islet cell tumours, particularly insulinomas. As these latter may cause changes in mental state that may be confused with the cerebral manifestations of TSC *per se*, it is particularly important for physicians working with these patients to be aware of the putative and indeed likely association.

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Introduction

The term tuberous sclerosis complex (TSC) was first used by Bourneville in 1880, although an earlier case of a neonate with multiple cardiac rhabdomyomas and areas of cerebral sclerosis that might have represented TSC was originally reported by von Recklinghausen in 1865 ([chromosome 16 tuberous sclerosis consortium 1993](#), [van Slegtenhorst *et al.* 1997](#)). Tuberous sclerosis, occurring in ~ 1 in 6000–10 000 individuals ([Osborne *et al.* 1991](#)), is an autosomal dominant disorder with very high penetrance and manifest with variable phenotypes. However, only 20% of patients have a positive family history of TSC, and there is a very high spontaneous mutation rate such that ~ 70–80% of TSC patients appear as sporadic cases ([Osborne *et al.* 1991](#), [Astrinidis & Henske 2005](#), [Rosser *et al.* 2006](#)). Tuberous sclerosis is a systemic disease with protean

manifestations affecting multiple organ systems. From a physiopathological point of view, TSC is a disorder of cellular migration, proliferation and differentiation ([Kwiatkowski & Short 1994](#)).

TSC results in hamartomatous lesions primarily involving the skin, central nervous system, kidneys, eyes, heart and lungs, but the clinical findings and severity of TSC are highly variable ([Rosser *et al.* 2006](#)). In most patients with TSC, the initial management issue is related to making an appropriate diagnosis by identification of major and minor diagnostic features. The second important issue is management of TSC in long-term follow-up, in particular, the growth of angiomyolipomas or subependymal giant-cell tumours ([Weiner *et al.* 1998](#)). The current clinical diagnostic criteria for TSC were revised by a consortium in [Roach *et al.* \(1998\)](#), and

recommendations for the diagnostic evaluation of TSC were proposed in [Roach et al. \(1999\)](#)). The diagnosis of definite, probable or possible TSC is based on the presence of major and/or minor features of the disease ([Roach et al. 1998, 1999; Table 1](#)). It should be emphasised that no single feature of TSC is diagnostic ([Crino et al. 2006](#)). However, what has remained unclear as to whether neuroendocrine tumours (NETs) are also seen with increased frequency in TSC, although there are numerous case reports throughout the literature.

In order to review the literature for NETs in TSC patients, we searched the PubMed database (<http://www.ncbi.nlm.nih.gov/pubmed/>), dating articles back to 1950, including all languages of published papers. We used the key phrases in combinations including ‘tuberous sclerosis’ and ‘neuroendocrine tumours’, ‘endocrine neoplasia’, ‘pituitary adenoma’, ‘acromegaly’, ‘Cushing’s disease’, ‘hypercortisolemia’, ‘prolactinoma’, ‘hyperprolactinemia’, ‘non-functioning pituitary adenoma’, ‘parathyroid adenoma’, ‘hyperparathyroidism’, ‘pancreatic tumours’, ‘non-functioning islet cell tumour’, ‘gastrinoma’, ‘insulinoma’, ‘carcinoid’, ‘pheochromocytoma’, ‘adrenal adenoma’ and ‘medullary thyroid carcinoma’. We have included in this review all papers identified. The majority were published in English, but we have also reviewed those in German or French and one which was in Romanian.

Clinical manifestations of tuberous sclerosis

Cutaneous findings associated with TSC include hypomelanotic macules (‘ash-leaf’ spots), facial angiofibromata (adenoma sebaceum), ungula fibromas and the shagreen patch. Hypomelanotic macules are not specific

for tuberous sclerosis and, as such, these lesions are not considered as major features unless they are numerous ([Roach et al. 1998](#)). They may be detected in patients of all ages, including infants, and are identified more readily with the use of an u.v. (Wood’s) light ([Kwiatkowski & Short 1994](#)). Renal lesions occur in 55–75% of TSC patients, and are related to bilateral renal angiomyolipomas, which are usually multiple. These tumours have abnormal vasculature and are frequently complicated by spontaneous and life-threatening bleeding ([Ewalt et al. 1998](#)). The incidence of renal epithelial cysts in tuberous sclerosis is estimated to be 15–20%, most of which are first discovered in childhood. Renal cysts may be multiple in number and indistinguishable from polycystic kidney disease ([Stillwell et al. 1987](#)). Although the incidence of renal cell carcinoma in patients with TSC is far less than in von Hippel-Lindau disease (VHL) or acquired cystic kidney disease, its association does not appear to be merely coincidental, and has been reported in up to 2% of cases. The carcinomas typically are discovered at younger ages than expected for renal cell carcinoma in the general population ([Bjornsson et al. 1996](#)).

Pulmonary manifestation including lymphangiomyomatosis (LAM), which usually affects women between 20 and 40 years of age and is characterised by the widespread pulmonary proliferation of abnormal smooth-muscle cells and cystic changes within the lung parenchyma ([Ryu et al. 2006](#)). LAM usually presents with dyspnoea or pneumothorax; however, an asymptomatic course is also seen ([Johnson & Tattersfield 2002](#)).

TSC often causes disabling neurologic disorders including epilepsy and mental retardation as well as neurobehavioural abnormalities such as autism ([Crino et al. 2006](#)). Epilepsy remains the most prevalent and challenging clinical manifestation of TSC. Seizures have been reported in 78% of patients, frequently

Table 1 Diagnostic criteria for tuberous sclerosis complex Roach et al. (1998)

Major features	Minor features
Facial angiofibroma or forehead plaque	Multiple, randomly distributed pits in dental enamel
Non-traumatic ungula or periungual fibroma	Hamartomatous rectal polyps
Hypomelanotic macules (more than 3)	Bone cysts
Shagreen patch (connective tissue nervous)	Cerebral white-matter radial migration lines
Cortical tuber	Gingival fibromas
Subependymal nodule	Non-renal hamartoma
Sub endependymal giant cell astrocytoma	Retinal achromic patch
Multiple retinal nodular hamartomas	‘Confetti’ skin lesions
Cardiac rhabdomyoma, single or multiple lymphangiomyomatosis	Multiple renal cysts
Renal angiomyolipoma	
Definite TSC: either two major feature or one major feature plus two minor features	
Probable TSC: one major plus one minor feature	
Possible TSC: either one major or two or more minor features	

beginning before 1 year of age (69%) and occurring more commonly in males than females, regardless of age (Webb *et al.* 1996). Seizures are often refractory to treatment, even to polytherapy with antiepileptic drugs (Thiele 2004). The occurrence of subependymal brain nodules, brain cortical hamartomas (tubers) or cystic lesions, focal cortical dysplasia, calcification as well as subependymal giant-cell astrocytomas has also been reported (Kwiatkowski & Short 1994).

Cardiac rhabdomyomas are very common and often multiple in neonates with tuberous sclerosis, and may be rarely associated with cardiac failure, dysrhythmias and thromboembolic complication (Smith *et al.* 1989, Smythe *et al.* 1990).

Ophthalmic lesions occur in up to 75% of all TSC patients, and include retinal involvement: these range from mulberry lesions near the margin of the optic disc to plaque-like hamartomas and depigmented lesions (achromic patches; Kiribuchi *et al.* 1986).

Molecular biology of TSC

The *TSC1* gene, which is located on chromosome 9q34, encodes a transcript of 8.6 kb, containing 21 exons and encompassing 55 kb genomic DNA (van Slegtenhorst *et al.* 1997). The *TSC2* gene, which is located on chromosome 16p13, encodes a transcript of 5.5 kb, containing 41 exons and encompassing 40 kb genomic DNA (Xu *et al.* 1995; Fig. 1). *TSC1* and *TSC2* genes encode hamartin (140 kDa protein) and tuberlin (200 kDa protein) respectively. Both hamartin and tuberlin interact physically with high affinity to form heterodimers, consistent with the known similarities in clinical presentation in patients with *TSC1* and *TSC2*

mutations (Plank *et al.* 1998). Despite many biochemical advances, exactly how mutations in *TSC1* or *TSC2* lead to the clinical manifestation of TSC remains unknown (Astrinidis & Henske 2005), and the existence of genotype–phenotype correlations are still debatable (van Slegtenhorst *et al.* 1999).

Extensive studies of the *TSC1* and *TSC2* genes in patients with TSC have revealed a wide spectrum of mutations, but there are no particular regions ('hot spots') within the *TSC1* or *TSC2* gene in which mutations occur at a high rate. More than 300 mutations have been reported for *TSC2*, including missense (less than 5% of known *TSC2* mutations) and nonsense (together with missense, about 20%), and frameshift deletions/insertions and splice junction mutation (Astrinidis & Henske 2005, Sancak *et al.* 2005). The most frequently missense mutations in TSC are at Arg611 (exon 16) and Pro1675Leu (exon 38), and an 18 bp in-frame deletion in exon 40 has been observed (Sancak *et al.* 2005). Regarding *TSC1* mutations, almost all reported cases present nonsense or frameshift mutations, causing premature protein truncation (Astrinidis & Henske 2005, Sancak *et al.* 2005). However, while there is no significant correlation between the TSC patient's genotype and phenotype, it seems that patients with *TSC1* mutations are less affected clinically than patients with *TSC2* mutations (Dabora *et al.* 2001).

In terms of Knudson's two-hit tumour suppressor gene model (Knudson 1971), the inactivation of two alleles of either *TSC1* or *TSC2* should be crucial in producing the clinical manifestation of TSC. Most 'second hits' are large deletions involving loss of surrounding loci and loss of heterozygosity (LOH) in *TSC1* or *TSC2*, which have

Figure 1 Structure of Hamartin (TSC1) and Tuberlin (TSC2). Hamartin (encoded by *TSC1*) is 1164 amino acid peptide with a molecular mass of 130 kDa. It interacts with tuberlin through amino acids 302–430. Tuberlin (encoded by *TSC2*) is 1807 amino acid peptide with a molecular mass 200 kDa. It interacts with hamartin through amino acids 1–418. The activity of *TSC1* and *TSC2* is regulated by both inhibitory and activating phosphorylation events at specific amino acid residues. CDK1, cyclin dependent kinase 1; GSK3B, glycogen synthase 3beta; ERK2, extracellular-related kinase 2; AKT/PKB, protein kinase B; RSK1, p90 ribosomal S6 kinase 1; AMPK, AMP kinase and GAP; GTPase-activating protein, reviewed by (Astrinidis & Henske 2005, Crino *et al.* 2006).

been reported in angiomyolipomas, rhabdomyomas and LAM cells (Astrinidis *et al.* 2000, Astrinidis & Henske 2005). On the other hand, in patients meeting the clinical criteria for a diagnosis of TSC, 15–20% have no identifiable mutation. These persons generally have milder clinical disease than patients with identified *TSC1* or *TSC2* mutations (Sancak *et al.* 2005). However, the most common TSC-associated lesions are limited to relatively few organs including the brain, heart, kidney, lungs and skin, while the *TSC* gene products (hamartin and tuberlin) are expressed in most tissues (Johnson *et al.* 2001). Tuberlin was present in abundance in control brains, but was lost from TSC tissues, such as cerebral cortices, cortical tubers and subependymal giant-cell astrocytomas (Mizuguchi *et al.* 1996). Additionally, its immunoreactivity was decreased in the TSC-associated hamartomas occurring in kidneys or heart in comparison to normal samples (Mizuguchi *et al.* 1997).

The TSC1–TSC2 complex interacts with several proteins, but in most cases the clinical relevance of these interactions remains unknown. The hamartin–tuberlin complex, through its GTPase-activating protein (GAP) activity towards the small G-protein Ras homologue enriched in brain, is a critical negative regulator of mammalian target of rapamycin (mTOR); Huang & Manning 2008). In normal cells, it has been shown that the TSC1–TSC2 complex inhibits the mTOR cascade (Fig. 2). Direct phosphorylation and inactivation of TSC2 by the serine–threonine kinase Akt (protein kinase B) induces mTOR activation (Manning *et al.* 2002, Tee *et al.* 2002); TSC2 is phosphorylated by Akt at multiple residues in response to mitogens, but the sites S939 and T1462 appear to represent the major loci of regulation as these residues are required

for maximal growth factor stimulation of S6K1 (Manning *et al.* 2002). The serine–threonine kinase mTOR is a crucial regulator involved in cell growth and proliferation due to phosphorylation of downstream regulators such as p70S6K kinase and eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1); in TSC-associated tumours, loss of TSC1 or TSC2 result in unregulated autonomous mTOR-dependent phosphorylation of p70S6K and EIF4EBP1 (El-Hashemite *et al.* 2003). Phosphorylation can also positively regulate tuberlin. AMPK (5'AMP-activated protein kinase), a sensor of cellular energy status, phosphorylates tuberlin at T1227 and S1345 and thereby activates tuberlin to downregulate S6K activity (Inoki *et al.* 2003). As a result of energy starvation, the tumour suppressor LKB1 phosphorylates and activates AMPK, which in turn phosphorylates and activates tuberlin, leading to inactivation of mTOR (Shaw *et al.* 2004). The mitogen-activated protein kinase (MAPK)/ extracellular signal-regulated kinase (ERK) pathway is situated upstream of the TSC complex. Activation of ERK1/2 occurs through phosphorylation by MAP kinase kinase (MEK1/2). Mitogenic stimuli or oncogenic Ras activates the Raf–MEK1/2–ERK1/2 signalling cascade leading to phosphorylation of tuberlin by ERK1/2, and the consequent functional inactivation of TSC1/TSC2 to regulate S6K. ERK1/2 was shown to interact with tuberlin and to phosphorylate it at S664 (Ma *et al.* 2005). In addition, the MAPK-activated kinase, p90 ribosomal S6 kinase (RSK) 1, was found to interact with and phosphorylate tuberlin at S1798. RSK1 phosphorylation leads to inactivation of tuberlin resulting in increased mTOR signalling to S6K (Roux *et al.* 2004, Astrinidis & Henske 2005, Crino *et al.* 2006; Figs 1 and 2).

Figure 2 Interactions of the TSC1–TSC2 complex with multiple cellular pathways. Phosphorylation by Akt (Manning *et al.* 2002), ERK1/2 (Ma *et al.* 2005) and RSK1 (Roux *et al.* 2004) inhibits, whereas phosphorylation by AMPK (Inoki *et al.* 2003) activates, tuberlin's potential to negatively regulate S6K.

Neuroendocrine tumours

NETs in the broadest sense may be taken to include pituitary and parathyroid tumours, as well as gastro-entero-pancreatic and bronchial NETs and pheochromocytomas and paragangliomas. They may occur sporadically or in a familial context of autosomal dominant inherited syndromes such as multiple endocrine neoplasia (MEN1 and MEN2), VHL, Carney complex (principally somatotroph tumours) or familial isolated pituitary adenomas. Rarely, tumours of endocrine glands have been observed in other phacomatoses, such as Recklinghausen disease (NF1). However, it has been difficult to establish whether NETs are also characteristic of TSC (Calender *et al.* 2001).

Overexpression of the proto-oncogene Akt/PKB has been demonstrated in certain NETs, and Akt activates downstream proteins including mTOR and p70S6K, which play an important role in cell proliferation (Grozinsky-Glasberg *et al.* 2008). Upregulation of the pituitary tumour-transforming 1 gene (*Pttg1*; Zhang *et al.* 1999a,b) and the phosphatidylinositol kinase/protein kinase B (Akt) pathways, have been described in sporadic pituitary tumours (Musat *et al.* 2005). Overexpression of B-Raf mRNA and protein was found in non-functioning pituitary adenomas, highlighting overactivity of the Ras-B-Raf-MAP kinase pathway in these tumours (Ewing *et al.* 2007). Recently, in two NET cell lines (rat insulinoma cell line and human pancreatic BON cell line), it was shown that RAD001 – a new agent antagonising TSC2 and mTOR function – inhibited NET cell line proliferation (Zitzmann *et al.* 2007, Grozinsky-Glasberg *et al.* 2008) and promoted apoptosis (Zitzmann *et al.* 2007).

Animal model of TSC

The Eker rat is a useful model of TSC: it carries a spontaneous germ line mutation of the *TSC2* gene (*Tsc*^{EK/+} that functions as a null equivalent) and is predisposed to multiple neoplasias (Fukuda *et al.* 1999). It is the only animal model of TSC and the expression of *TSC1/TSC2* gene products remain quite similar to humans. In the *Tsc*^{EK/+} rats, pituitary adenomas are a common finding, occurring in 58% of adult cases. There is also evidence of TSC2 LOH in these tumours, suggesting a ‘two-hit’ pathogenetic mechanism (Yeung *et al.* 1997). It was found that rats with pituitary tumours had significantly shorter survival than those without pituitary pathology due to haemorrhage into the pituitary tumours, whereas treatment with rapamycin (a specific mTOR inhibitor) effectively improved their clinical state and prolonged their survival. Tumour response was accompanied by downregulation of ribosomal S6 kinase activity, reduction in cell size and induction of apoptosis (Kenerson *et al.* 2005).

Neuroendocrine tumours and TS – case reports

Patients with TSC have been reported to occasionally present with NETs, but these are mostly in the form of single case reports (summarised in Table 2). However, as both conditions are rare, and both may depend on aberrant TSC1/2–mTOR signalling, such correlations may be indicative of a true relationship. Early reports centred on endocrine adenomas, especially in the

pituitary, pancreas as well as parathyroid, but more recently single case reports have been included patients with gastrinoma, pheochromocytoma and carcinoids in this group of patients.

Pituitary

There are a few case reports addressing pituitary adenomas in TSC patients, producing GH-, prolactin- or ACTH-secreting tumours (Galaction-Nitelea *et al.* 1978, Hoffman *et al.* 1978). Somatotroph tumours were reported in two cases (Galaction-Nitelea *et al.* 1978, Hoffman *et al.* 1978). In one of them the clinical diagnosis of acromegalic gigantism was reported in a boy who, at the age of 6 years, was diagnosed with TSC. Clinically, at the age of 12 years he presented with headache, enlarging hands and a growth of 10 cm in the year prior to admission. His skull radiograph showed intracranial calcification and a markedly enlarged sella, and angiograms confirmed suprasellar extension of the pituitary tumour. This patient had elevated pre-treatment serum GH, prolactin and somatomedin-C insulin-like growth factor-1 (IGF-1) levels. Further endocrine studies showed that he was pre-pubertal. He underwent pituitary surgery and post-operative radiotherapy: light microscopy of the tumour revealed an eosinophilic adenoma. In his father’s family, TSC was present over four generations, but without a known history of any endocrinopathies. One of the patient’s siblings, a 10-year-old sister, was as mentally retarded with the typical skin findings of TSC; her height was appropriate to her age and her mean GH level on three separate fasting sera was ‘within the normal range’. Interestingly, her serum prolactin was elevated, but her plain skull radiology and tomograms of the sella were normal (Hoffman *et al.* 1978). In another case report, a female with TSC presenting with hyperprolactinaemia with amenorrhoea and galactorrhoea was reported; she was unresponsive to any treatment then available (including protirelin (TRH), chlorpromazine, levodopa, bromocriptine mesylate or oestrogens). There was no evidence of a pituitary or hypothalamic tumour, but imaging facilities were not sophisticated (Bloomgarden *et al.* 1981). Another report has been published in the Romanian literature (Galaction-Nitelea *et al.* 1978).

There have been two cases of Cushing’s disease reported in TSC patients, one in an adult (Tigas *et al.* 2005) and another in a child (Nandagopal *et al.* 2007). The first related to 32 years old man with history of epilepsy from childhood and clinical features of TSC (periungual fibromas, adenoma sebaceum, shagreen patches, retinal astrocytoma and calcified tubers around the left-lateral ventricle). This patient was

Table 2 Neuroendocrine tumours in tuberous sclerosis complex patients – summary of case reports (PubMed 1950–2008)

Sex	TSC onset as	NET onset	Genetics	NET	Remarks	References
Pituitary						
m	32 years adult	33 years	–	ACTH-oma	Clinical examination consistent with Cushingoid features	Tigas <i>et al.</i> (2005)
m	Child	13.5 years	–	ACTH-oma	Short stature, a change in his appearance including weight gain, abnormal distribution of fat tissue and rounded face, plethora and acne	Nandagopal <i>et al.</i> (2007)
m	Child	12 years	–	GH-oma	Acromegalic gigantism	Hoffman <i>et al.</i> (1978)
f	16 years child	25 years	–	–	Hyperprolactinaemia with amenorrhoea and galactorrhoea after delivery of third child, no evidence of a pituitary or hypothalamic tumour	Bloomgarden <i>et al.</i> (1981)
Parathyroid						
m	Child	15 years	–	Parathyroid adenoma	Initial diagnosis – acute pancreatitis	Mortensen & Rungby (1991)
f	Child	20 years	–	Parathyroid hyperplasia	Additionally diagnosed with thyrotoxic. On autopsy multiple endocrine adenomatosis affecting, in addition to the parathyroid, the pituitary (a non-functioning pituitary adenoma), adrenals and pancreas (islet cell tumour)	Ilgren & Westmoreland (1984)
f	Child	14 years	–	Parathyroid adenoma	Anorexia, occasional nausea and vomiting, polydipsia, polyuria and constipation. Generalised osteoporosis	Yin <i>et al.</i> (1984)
Pancreas						
f	Child	24 years	–	Insulinoma	Symptomatic hypoglycaemia secondary to an insulinoma, a novel onset of seizures	Gutman & Leffkowitz (1959)
m	Child	23 years	–	Insulinoma	Recurrent seizures presented after 15 years of being seizure free	Davoren & Epstein (1992)
m	Child	28 years	–	Insulinoma	New symptoms included behavioural changes characterised by episodes of agitation and, at other times, lethargy	Kim <i>et al.</i> (1995)
f	Child	18 years	–	Insulinoma	Symptomatic hypoglycaemia secondary to an insulinoma	Boubaddi <i>et al.</i> (1997)
m	Child	43 years	–	Insulinoma	Episodes of sweating and dizziness, usually developed after periods of extended fasting and resolved by drinking fruit juices	Eledrisi <i>et al.</i> (2002)

Table 2 continued

Sex	TSC onset as	NET onset	Genetics	NET	Remarks	References
m	Child	34 years	–	Pancreatic gastrinoma	Reflux oesophagitis and massive weight lost. At diagnosis multiple metastases (liver, abdominal lymph node, lungs and thoracic/lumbar vertebral column)	Schwarzkopf & Pfisterer (1994)
m	Adult	39 years	Sequencing of the <i>TSC2</i> gene showed a novel 1bp insertion at position 45–46 leading to a frame shift	Pancreatic islet cell neoplasm	Lichenified hyperpigmented plaques suggesting a paraneoplastic process	Merritt <i>et al.</i> (2006)
m	Child	12 years	<i>TSC2</i> gene LOH in the tumour and the germ line mutation Q478X in exon 13 of the <i>TSC2</i> gene on chromosome 16. Screening for <i>MEN1</i> negative	Malignant islet cell tumour	A large infiltrative retroperitoneal tumour originating from the pancreas, with local metastases to the lymph nodes	Verhoef <i>et al.</i> (1999)
m	Child	6 years	Mutation analysis of DNA extracted from peripheral blood cells identified an R1459X <i>de novo</i> mutation in exon 33 of the <i>TSC2</i> gene. Tuberin staining in tumour cells was lost, with normal expression in residual normal pancreas. Chromosome 16p13 LOH in malignant pancreatic islet cell tumour, but not in normal pancreas	Malignant islet cell tumour of pancreas	In abdominal CT scan – a highly vascularised retroperitoneal tumour arising from pancreas	Francalanci <i>et al.</i> (2003)
Phaeochromocytoma f	Child	29 years	–	Pleo-morphic phaeo-chromo-cytoma	Recurrent fevers and pain located in upper right abdomen quadrant. Ultrasonography showed a lesion arising from the right adrenal. Some month later disease abdominal recurrence, involving the spinal cord	Stern <i>et al.</i> (1982)
Carcinoid f	Adult	34 years	A germ line nonsense mutation C165X in exon 6 of <i>TSC1</i> . <i>TSC1</i> LOH in LAM cells, lymph nodes, uterus and kidneys but not in carcinoid cells	Bronchial carcinoid	Two years after diagnosis of sporadic lymphangiomyomatosis (LAM) – haemoptysis related to a bronchial carcinoid	Sato <i>et al.</i> (2004)

referred at the age of 33 to an endocrine clinic as he had noted a change in his facial appearance, increasing central obesity, easy bruising and a fatty lump at the back of his neck; in addition, he was experiencing depression. Clinical examination was consistent with Cushingoid features including facial fullness with plethora, chemosis, proximal myopathy and hypertension. His laboratory results showed elevated 0900 h plasma ACTH and serum cortisol levels with raised urinary free cortisol. Further investigations confirmed ACTH-dependent Cushing disease. Computer tomography (CT) revealed bilateral adrenal enlargement with an unusual rounded area of low signal within the pituitary fossa on magnetic resonance imaging (MRI). As the patient's condition deteriorated rapidly he underwent bilateral adrenalectomy, followed by hydrocortisone and fludrocortisone replacement therapy. The subsequent histology confirmed adrenocortical hyperplasia. After a period of 10 years on appropriate replacement, he represented with headache, difficulty with walking and deterioration in his mental state. A giant-cell xanthoastrocytoma was diagnosed and removed transcranially. Six months after this surgery his ACTH was again noted to be elevated and an MRI brain showed a pituitary microadenoma. He underwent transsphenoidal hypophysectomy with histology consistent with ACTH-secreting adenoma (Tigas *et al.* 2005).

Recently, a second case of ACTH-secreting pituitary adenoma was reported in 13-year-old boy diagnosed with TSC from the age of 5 years (Nandagopal *et al.* 2007). His mother and maternal grandfather also had TSC. The diagnosis of TSC was made based on hypopigmented macules, a shagreen patch, adenoma sebaceum and intracranial lesions. This patient had been growing normally until the age of 7 years, when his high velocity started to decrease (with normal IGF1 and a normal GH response to the clonidine stimulation test). However, he was 13 years old, but his bone age was only 11 years. He was noted to show a change in his appearance including weight gain, the abnormal distribution of fat tissue and rounded face, plethora and acne. Laboratory investigation revealed an elevated urinary free cortisol as well as a high morning 0900 h serum cortisol. MRI of the pituitary was consistent with pituitary microadenoma, and petrosal sinus sampling confirmed a central source for his ACTH. The patient underwent transsphenoidal surgery with histology of the lesion showing adenoma tissue with regressive changes including cholesterol clefts, lymphocytic infiltration and siderophages (possible secondary to haemorrhage). Post-operatively, the patient remained

well with no recurrence of cluster of differentiation on hydrocortisone replacement (Nandagopal *et al.* 2007).

There is in addition in the literature a single report regarding a non-functioning pituitary adenoma, which was found in a TSC patient incidentally at autopsy (Ilgren & Westmoreland 1984).

Parathyroid

There are several reports related to hypercalcaemia and parathyroid adenomas in TSC patients (Ilgren & Westmoreland 1984, Yin *et al.* 1984, Mortensen & Rungby 1991). One patient with TSC was admitted to hospital with abdominal pain in the age of 15 years; his sister and mother also had confirmed TSC. He was diagnosed with acute pancreatitis and further examination showed hypercalcaemia due to hyperparathyroidism. Combined thallium–technetium scintigrams and ultrasonography indicated a parathyroid adenoma located on the right side of the neck; this lesion was removed and the diagnosis was verified pathologically, and post-operative his serum calcium was normal (Mortensen & Rungby 1991). A second patient was found to have hypercalcaemia at the age of 20 years. She was diagnosed with TSC in childhood with adenoma sebaceum, epilepsy, pneumothorax and bilateral ‘polycystic’ kidneys. The elevated calcium level was not decreased during a standard hydrocortisone suppression test. This patient also had a reduced creatinine clearance. The patient underwent parathyroidectomy and four hyperplastic parathyroid glands were found (histology showed chief cell hyperplasia). Six days after surgery she was normocalcaemic. This patient later developed haemoptyses and congestive heart failure and was noted to be thyrotoxic: she died of sepsis. Autopsy examination revealed showed multiple endocrine adenomatosis affecting, in addition to the parathyroid, the pituitary (the patient noted above with a non-functioning pituitary adenoma), adrenals and pancreas (islet cell tumour). This patient's mother (who had a mild degree of adenoma sebaceum) was found to be hypercalcaemic at the age of 51 years, and as with her daughter, developed hyperparathyroidism with four hyperplastic parathyroid glands (Ilgren & Westmoreland 1984). A third patient with TSC and with primary hyperparathyroidism was reported in China (Yin *et al.* 1984). A 14-year-old girl (born of a healthy family, diagnosed with TSC at the age of 2 years) was admitted to hospital because of increasing pain in the soles of her feet, aching pain over hips, legs and lumbar region, and gradually progressive difficulty in walking. This was associated with anorexia, occasional nausea and vomiting, polydipsia, polyuria and constipation. However, she

was normocalcaemic. Her radiological bone examination revealed generalised osteoporosis, marked thinning cortices of long bone, lumbar vertebrae biconcave deformity and phalangeal subperiosteal resorption. She underwent neck exploration, which showed a parathyroid adenoma of the left lower parathyroid gland, which was excised. Another three parathyroid glands were found to be normal and were not resected. Histology of the adenoma showed a typical encapsulated clear cell adenoma. After surgery she developed tetany, but on calcium and vitamin D therapy she was discharged home without complications.

Insulinoma

In the published literature there are at least five independent case reports demonstrating an insulinoma in TSC patients (Gutman & Leffkowitz 1959, Davoren & Epstein 1992, Kim *et al.* 1995, Boubaddi *et al.* 1997, Eledrisi *et al.* 2002). In all cases, TSC was found in childhood and neurological abnormalities were one of the main disease features. These patients developed symptomatic hypoglycaemia secondary to an insulinoma. The first case was reported in 1959 by Gutman & Leffkowitz (1959); a 24-year-old woman with mental retardation, diagnosed with TSC in childhood, at the age of 18 years developed seizures. These seizures occurred early in the morning and began with agitation and groaning; it was noted that she sweetened her tea excessively (Gutman & Leffkowitz 1959). In a 28-year-old mentally retarded man, new symptoms included behavioural changes characterised by episodes of agitation and, at other times, lethargy (Kim *et al.* 1995). In another case, recurrent seizures presented after 15 years of being seizure free: these continued despite therapeutic levels of carbamazepine and primidone. Additionally, this patient was suffering from tiredness, sleepiness after exertion and had an increased appetite for sweet food (Davoren & Epstein 1992). Finally, a 43-year-old man with TSC presented with mental confusion, slurred speech and abnormal behaviour. Over several months he had experienced episodes of sweating and dizziness, which usually developed after periods of extended fasting and were resolved by drinking fruit juices (Eledrisi *et al.* 2002). In all above cases hypoglycaemia with inappropriate hyperinsulinaemia was found. In two of the cases computed tomography did not demonstrate any pancreatic lesion, but coeliac axis angiography revealed hypervascular lesions located in the pancreas (Davoren & Epstein 1992, Kim *et al.* 1995). In one case, a large abdominal mass originated from pancreas presented on CT, and also showed increase uptake on octreotide

scanning (Eledrisi *et al.* 2002). All these five patients were treated with partial or total pancreatectomy, the histology being consistent with an insulinoma (Gutman & Leffkowitz 1959, Davoren & Epstein 1992, Kim *et al.* 1995, Boubaddi *et al.* 1997, Eledrisi *et al.* 2002). After successful surgery, glucose normalised and all new symptoms resolved in each patient.

Gastrinoma

There is one case report related to the presence of a gastrinoma in a TSC patient (Schwarzkopf & Pfisterer 1994). A 34-year-old man (with a diagnosis of TSC in childhood and a negative family history of TSC) was admitted to hospital due to reflux oesophagitis and massive weight lost. Based on biopsy of a pancreatic tumour that showed a gastrinoma, he was diagnosed with the Zollinger–Ellison syndrome. At the time of diagnosis he already had liver metastases. He was started on omeprazole, but his reflux oesophagitis and multiple stomach ulceration continued. This patient died at the age of 34 years having multiple metastases (liver, abdominal lymph node, lungs and thoracic/lumbar vertebral column), ascites and neoplastic cachexia. His autopsy examination confirmed a well-differentiated pancreatic gastrinoma, with positive immunostaining for gastrin and chromogranin-A, but negative for insulin.

Non-functioning islet cell tumour

In one case of TSC, a non-functioning islet cell tumour was discovered incidentally at autopsy (Ilgen & Westmoreland 1984). In another TSC patient, extensive lichenified hyperpigmented plaques suggesting a paraneoplastic process were thought to be related to a pancreatic islet cell neoplasm (weakly staining for glucagon on immunohistochemistry); in this case analysis sequencing of the *TSC2* gene showed a novel 1 bp insertion at position 45–46, leading to a frame shift (Merritt *et al.* 2006). There are two further case reports demonstrating malignant pancreatic tumours in TSC patients (Verhoef *et al.* 1999, Francalanci *et al.* 2003). One of them was related to child born in a healthy family, who at the age of 2 years was diagnosed for TSC based on epilepsy, hypopigmented macules and mental retardation. At the age of 7 years he developed facial angiofibromas, a fibrous forehead plaque and ungual fibromas of the feet. At the age of 9 years he underwent embolisation of a large angiomyolipoma of the right kidney (however, both kidneys showed multiple smaller angiomyolipomas) and surgery for a subependymal giant-cell astrocytoma. Some months after neurosurgery

he presented with abdominal pain: CT scanning revealed a large, highly vascularised retroperitoneal tumour. Explorative surgery proved the tumour to be of pancreatic origin, with colonic and stomach infiltration and lymph node metastases. Histology was consistent with a malignant islet cell tumour. Immunohistochemical staining was positive for the neuroendocrine markers – synaptophysin and gastrin, and β -marker islet amyloid polypeptide, but negative for insulin and somatostatin; parts of the tumour were positive for chromogranin A, glucagon and MIB-1 (mindbomb homolog 1, a monoclonal antibody that is immunoreactive with Ki-67). His serum pancreatic hormone levels were normal. Screening for MEN1 was negative. Mutation analysis in blood cells resulted in the identification of the germ line mutation in the *TSC2* gene (Q478X in exon 13). The probable involvement of the *TSC2* gene in the aetiology of the pancreatic tumour was confirmed by the demonstration of LOH of the non-mutated allele of the *TSC2* gene in tumour tissue (Verhoef *et al.* 1999). Another case report related to a malignant islet cell tumour of pancreas was reported in a 6-year-old boy with TSC (Francalanci *et al.* 2003). He was born to a healthy family, and was diagnosed with TSC based on presence of hypopigmented cutaneous macules, epilepsy, mental retardation, intramyocardial masse, subependymal nodules within lateral ventricle and facial angiomyolipomas. At the age of 6 years his abdominal CT scan showed a highly vascularised retroperitoneal tumour arising from pancreas. This patient underwent radical surgery. Pathology showed a malignant islet cell tumour of pancreas with neoplastic thrombosis. Immunostaining was positive for the neuroendocrine markers including chromogranin, synaptophysin, neuron specific enolase and cytokeratin 8, but not insulin. Tuberin staining in tumour cells was lost, with normal expression in residual normal pancreas. Mutation analysis of his DNA extracted from peripheral blood cells showed a heterozygous C-to-T substitution at position 4375 in exon 33 of *TSC2*. The mutation replaced arginine 1459 for a stop codon, leading to a prematurely truncated tuberin. DNA analysis of tumour and normal cells was performed. Normal pancreatic tissue showed the heterozygous R1459X mutation. 16p13 LOH was found in malignant pancreatic islet cell tumour, but not in normal pancreas (Francalanci *et al.* 2003).

Phaeochromocytoma

There is currently a single case report of the occurrence of a phaeochromocytoma in a TSC patient (Stern *et al.* 1982). It was reported in 29 years old woman with TSC diagnosed at the age of 3 years, who was admitted to a

urology clinic due to recurrent fevers and pain located in upper right abdomen quadrant. She was anaemic, with an elevated white count and a raised erythrocyte sedimentation rate (ESR), but normal urinalysis. Ultrasonography showed a lesion, located on the right side, apparently arising from the right adrenal. Further angiography and CT scanning showed multiple angiomyolipomas located bilaterally, with one dominant lesion in the right adrenal, which was partially necrotic in the central area. This patient underwent radical surgery and histology was consistent with a pleomorphic pheochromocytoma. A renal angiomyolipoma was also confirmed. After surgery patient was well without fever and was discharged home. However, she was readmitted to hospital 1 year later because of back pain, located in right side and recurrence of fever. On examination a recurrent abdominal mass was found, which was confirmed by CT scanning. Because the tumour involved the spinal cord, a second operation was not possible and she was treated conservatively and symptomatically: she died at the age of 29 years, 9 months after her first surgery for a phaeochromocytoma.

‘Carcinoids’

In one case of TSC limited only to lymphangioleiomyomatosis (LAM), a bronchial carcinoid was reported (Sato *et al.* 2004). It was a case of 34-year-old Japanese woman with LAM who underwent surgical treatment for bilateral pneumothoraces. She suffered from chronic renal dysfunction and her ultrasonography and CT scans showed both kidneys normal in sizes but with multiple bilateral renal cysts (renal biopsy was not performed). Two years after diagnosis of sporadic LAM, she was admitted to hospital because of haemoptysis related to a bronchial carcinoid. Because of severely impaired renal function, surgery was not possible and she died due to massive haemoptysis. Her autopsy examination revealed the presence of LAM lesions in the lungs, mediastinal lymph nodes, kidneys and uterus (Sato *et al.* 2004). Mutation analysis of the *TSC* gene revealed that she had a germ line nonsense mutation C165X resulting from a base substitution (TGC → TGA) in exon 6 of *TSC1*. Sequencing of the same exon in her family revealed the wild-type unmutated sequence only, suggesting that she carried a *de novo* mutation in the *TSC1* gene. Furthermore, her LAM cells, lymph nodes, uterus and kidneys showed *TSC1* LOH, although surprisingly this was not found in microdissected tumour cells from the bronchial carcinoid tissue (Sato *et al.* 2004).

Medullary thyroid carcinoma

In current literature there is no case report addressing the occurrence of medullary thyroid carcinoma in a TSC patient.

Conclusion

The current clinical diagnostic criteria for TSC were revised by a consortium in 1998, and recommendations for the diagnostic evaluation of TSC were proposed in 1999. In addition, gene mutation analysis is now commercially available and may be used for genetic counselling. Couples with more than one child with TSC, no extended family history, and no clinical features of TSC are more likely to have germ line mosaicism for tuberous sclerosis than non-expression of the mutation. Germ line mosaicism, while fortunately rare, will not be suspected from either diagnostic criteria or molecular testing until a couple has multiple affected children. Genetic counselling for families with one affected child should include a small (1–2%) possibility of recurrence in subsequent children, even for parents who have no evidence of TSC after a thorough diagnostic evaluation (Roach *et al.* 1998).

At presentation, all children with suspected TSC should be thoroughly screened for seizures, developmental delay, and autism spectrum disorders. An ophthalmologic examination, brain imaging (CT or MRI), electrocardiogram (ECG) and renal ultrasound should also be performed. Subsequent evaluations depend on the patient's age and extent of organ involvement (Roach *et al.* 1999).

Because of the clinical complexity of the tuberous complex, the diagnosis and care of afflicted individuals should ideally be carried out by experienced physicians at specialised centres. Causes of death include cerebral complications (status epilepticus, obstructive hydrocephalus), renal involvement (renal haemorrhage, kidney failure, carcinoma), early cardiac failure and pulmonary involvement (recurrent spontaneous pneumothoraces and progressive respiratory failure; Kwiatkowski & Short 1994). However, it has been unclear as to whether endocrine tumours, more specifically NETs including pituitary adenomas, parathyroid adenomas and gastro-enteropancreatic and adrenomedullary NETs should also be considered a feature of TSC. Current recommendations for TSC do not include standard investigation for NETs. As noted above recent molecular studies suggest that the TSC1/2/mTOR pathway is aberrant in both TSC and sporadic NETs, such that there is a theoretical rationale as to why such tumours should

be more common in TSC. Nevertheless, the literature is redundant in scattered case reports which may represent coincidental and unassociated tumours, and which may account for some of the reports of the relatively common co-existing pituitary and parathyroid tumours, although the two recent case reports of Cushing's disease are intriguing. Even so, the multiple reports of co-existing NETs such as insulinomas, supported in some cases by LOH studies in the tumours themselves, do suggest that an increase in at least certain types of endocrine tumour is probable. This series of case reports suggest that at least in some cases of TSC it might be of value to extend the diagnostic approach and to add to the algorithm investigation for NETs. One group of TSC patient which might profit from broader investigations are patients with worsening of neurological symptoms, which might suggest hypoglycaemia. Additionally, a single plasma calcium assessment might also be useful. In centres specialising in TSC, the prospective ascertainment for endocrine tumours might be particularly useful. The new therapeutic approach related to mTOR inhibitors may also put the management of tuberous sclerosis patients as well as patients with NETs into a new perspective.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- Astrinidis A & Henske EP 2005 Tuberous sclerosis complex: linking growth and energy signaling pathways with human disease. *Oncogene* **24** 7475–7481.
- Astrinidis A, Khare L, Carsillo T, Smolarek T, Au KS, Northrup H & Henske EP 2000 Mutational analysis of the tuberous sclerosis gene TSC2 in patients with pulmonary lymphangioleiomyomatosis. *Journal of Medical Genetics* **37** 55–57.

- Bjornsson J, Short MP, Kwiatkowski DJ & Henske EP 1996 Tuberous sclerosis-associated renal cell carcinoma. Clinical, pathological, and genetic features. *American Journal of Pathology* **149** 1201–1208.
- Bloomgarden ZT, McLean GW & Rabin D 1981 Autonomous hyperprolactinemia in tuberous sclerosis. *Archives of Internal Medicine* **141** 1513–1515.
- Boubaddi NE, Imbert Y, Tissot B, Chapus JJ, Dupont E, Gallouin D, Masson B & De Mascarel A 1997 Secreting insulinoma and Bourneville's tuberous sclerosis. *Gastroenterologie Clinique et Biologique* **21** 343.
- Calender A, Vercherat C, Gaudray P & Chayvialle JA 2001 Deregulation of genetic pathways in neuroendocrine tumors. *Annals of Oncology* **12** (Supplement 1) S3–S11.
- Chromosome 16 Tuberous Sclerosis Consortium 1993 Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell* **75** 1305–1315.
- Crino PB, Nathanson KL & Henske EP 2006 The tuberous sclerosis complex. *New England Journal of Medicine* **355** 1345–1356.
- Dabora SL, Jozwiak S, Franz DN, Roberts PS, Nieto A, Chung J, Choy YS, Reeve MP, Thiele E, Egelhoff JC *et al.* 2001 Mutational analysis in a cohort of 224 tuberous sclerosis patients indicates increased severity of TSC2, compared with TSC1, disease in multiple organs. *American Journal of Human Genetics* **68** 64–80.
- Davoren PM & Epstein MT 1992 Insulinoma complicating tuberous sclerosis. *Journal of Neurology, Neurosurgery, and Psychiatry* **55** 1209.
- Eledrisi MS, Stuart CA & Alshanti M 2002 Insulinoma in a patient with tuberous sclerosis: is there an association? *Endocrine Practice* **8** 109–112.
- El-Hashemite N, Zhang H, Henske EP & Kwiatkowski DJ 2003 Mutation in TSC2 and activation of mammalian target of rapamycin signalling pathway in renal angiomyolipoma. *Lancet* **361** 1348–1349.
- Ewalt DH, Sheffield E, Sparagana SP, Delgado MR & Roach ES 1998 Renal lesion growth in children with tuberous sclerosis complex. *Journal of Urology* **160** 141–145.
- Ewing I, Pedder-Smith S, Franchi G, Ruscica M, Emery M, Vax V, Garcia E, Czirjak S, Hanzely Z, Kola B *et al.* 2007 A mutation and expression analysis of the oncogene BRAF in pituitary adenomas. *Clinical Endocrinology* **66** 348–352.
- Francalanci P, Diomedi-Camassei F, Purificato C, Santorelli FM, Giannotti A, Dominici C, Inserra A & Boldrini R 2003 Malignant pancreatic endocrine tumor in a child with tuberous sclerosis. *American Journal of Surgical Pathology* **27** 1386–1389.
- Fukuda T, Kobayashi T, Yasui H, Tsutsumi M, Konishi Y & Hino O 1999 Distribution of Tsc2 protein in various normal rat tissues and renal tumours of Tsc2 mutant (Eker) rat detected by immunohistochemistry. *Virchows Archiv* **434** 341–350.
- Galaction-Nitelea O, Docui I & Murgu V 1978 A case of tuberous sclerosis with acromegaly. *Revista de Medicină Internă, Neurologie, Psihiatrie, Neurochirurgie, Dermato-Venerologie. Neurologie, Psihiatrie, Neurochirurgie* **23** 253–262.
- Grozinsky-Glasberg S, Franchi G, Teng M, Leontiou CA, Ribeiro de Oliveira A Jr, Dalino P, Salahuddin N, Korbonits M & Grossman AB 2008 Octreotide and the mTOR inhibitor RAD001 (everolimus) block proliferation and interact with the Akt-mTOR-p70S6K pathway in a neuro-endocrine tumour cell Line. *Neuroendocrinology* **87** 168–181.
- Gutman A & Leffkowitz M 1959 Tuberous sclerosis associated with spontaneous hypoglycaemia. *BMJ* **2** 1065–1068.
- Hoffman WH, Perrin JC, Halac E, Gala RR & England BG 1978 Acromegalic gigantism and tuberous sclerosis. *Journal of Pediatrics* **93** 478–480.
- Huang J & Manning BD 2008 The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochemical Journal* **412** 179–190.
- Ilgren EB & Westmoreland D 1984 Tuberous sclerosis: unusual associations in four cases. *Journal of Clinical Pathology* **37** 272–278.
- Inoki K, Zhu T & Guan KL 2003 TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **115** 577–590.
- Johnson SR & Tattersfield AE 2002 Lymphangioleiomyomatosis. *Seminars in Respiratory and Critical Care Medicine* **23** 85–92.
- Johnson MW, Kerfoot C, Bushnell T, Li M & Vinters HV 2001 Hamartin and tuberin expression in human tissues. *Modern Pathology* **14** 202–210.
- Kenerson H, Dundon TA & Yeung RS 2005 Effects of rapamycin in the Eker rat model of tuberous sclerosis complex. *Pediatric Research* **57** 67–75.
- Kim H, Kerr A & Morehouse H 1995 The association between tuberous sclerosis and insulinoma. *American Journal of Neuroradiology* **16** 1543–1544.
- Kiribuchi K, Uchida Y, Fukuyama Y & Maruyama H 1986 High incidence of fundus hamartomas and clinical significance of a fundus score in tuberous sclerosis. *Brain & Development* **8** 509–517.
- Knudson AG Jr 1971 Mutation and cancer: statistical study of retinoblastoma. *PNAS* **68** 820–823.
- Kwiatkowski DJ & Short MP 1994 Tuberous sclerosis. *Archives of Dermatology* **130** 348–354.
- Ma L, Chen Z, Erdjument-Bromage H, Tempst P & Pandolfi PP 2005 Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. *Cell* **121** 179–193.
- Manning BD, Tee AR, Logsdon MN, Blenis J & Cantley LC 2002 Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Molecular Cell* **10** 151–162.
- Merritt JL II, Davis DM, Pittelkow MR & Babovic-Vuksanovic D 2006 Extensive acrochordons and

- pancreatic islet-cell tumors in tuberous sclerosis associated with TSC2 mutations. *American Journal of Medical Genetics. Part A* **140** 1669–1672.
- Mizuguchi M, Kato M, Yamanouchi H, Ikeda K & Takashima S 1996 Loss of tuberin from cerebral tissues with tuberous sclerosis and astrocytoma. *Annals of Neurology* **40** 941–944.
- Mizuguchi M, Kato M, Yamanouchi H, Ikeda K & Takashima S 1997 Tuberin immunohistochemistry in brain, kidneys and heart with or without tuberous sclerosis. *Acta Neuropathologica* **94** 525–531.
- Mortensen LS & Rungby J 1991 Tuberous sclerosis and parathyroid adenoma. *Journal of Clinical Pathology* **44** 961–962.
- Musat M, Korbonits M, Kola B, Borboli N, Hanson MR, Nanzer AM, Grigson J, Jordan S, Morris DG, Gueorguiev M *et al.* 2005 Enhanced protein kinase B/Akt signalling in pituitary tumours. *Endocrine-Related Cancer* **12** 423–433.
- Nandagopal R, Vortmeyer A, Oldfield EH, Keil MF & Stratakis CA 2007 Cushing's syndrome due to a pituitary corticotropinoma in a child with tuberous sclerosis: an association or a coincidence? *Clinical Endocrinology* **67** 639–641.
- Osborne JP, Fryer A & Webb D 1991 Epidemiology of tuberous sclerosis. *Annals of the New York Academy of Sciences* **615** 125–127.
- Plank TL, Yeung RS & Henske EP 1998 Hamartin, the product of the tuberous sclerosis 1 (TSC1) gene, interacts with tuberin and appears to be localized to cytoplasmic vesicles. *Cancer Research* **58** 4766–4770.
- Roach ES, Gomez MR & Northrup H 1998 Tuberous sclerosis complex consensus conference: revised clinical diagnostic criteria. *Journal of Child Neurology* **13** 624–628.
- Roach ES, DiMario FJ, Kandt RS & Northrup H 1999 Tuberous Sclerosis Consensus Conference: recommendations for diagnostic evaluation. National Tuberous Sclerosis Association. *Journal of Child Neurology* **14** 401–407.
- Rosser T, Panigrahy A & McClintock W 2006 The diverse clinical manifestations of tuberous sclerosis complex: a review. *Seminars in Pediatric Neurology* **13** 27–36.
- Roux PP, Ballif BA, Anjum R, Gygi SP & Blenis J 2004 Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase. *PNAS* **101** 13489–13494.
- Ryu JH, Moss J, Beck GJ, Lee JC, Brown KK, Chapman JT, Finlay GA, Olson EJ, Ruoss SJ, Maurer JR *et al.* 2006 The NHLBI lymphangioleiomyomatosis registry: characteristics of 230 patients at enrollment. *American Journal of Respiratory and Critical Care Medicine* **173** 105–111.
- Sancak O, Nellist M, Goedbloed M, Elfferich P, Wouters C, Maat-Kievit A, Zonnenberg B, Verhoef S, Halley D & van den Ouweland A 2005 Mutational analysis of the TSC1 and TSC2 genes in a diagnostic setting: genotype–phenotype correlations and comparison of diagnostic DNA techniques in tuberous sclerosis complex. *European Journal of Human Genetics* **13** 731–741.
- Sato T, Seyama K, Kumasaka T, Fujii H, Setoguchi Y, Shirai T, Tomino Y, Hino O & Fukuchi Y 2004 A patient with TSC1 germline mutation whose clinical phenotype was limited to lymphangioleiomyomatosis. *Journal of Internal Medicine* **256** 166–173.
- Schwarzkopf G & Pfisterer J 1994 Metastasizing gastrinoma and tuberous sclerosis complex. Association or coincidence? *Zentralblatt für Pathologie* **139** 477–481.
- Shaw RJ, Bardeesy N, Manning BD, Lopez L, Kosmatka M, DePinho RA & Cantley LC 2004 The LKB1 tumor suppressor negatively regulates mTOR signaling. *Cancer Cell* **6** 91–99.
- van Slegtenhorst M, de Hoogt R, Hermans C, Nellist M, Janssen B, Verhoef S, Lindhout D, van den Ouweland A, Halley D, Young J *et al.* 1997 Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. *Science* **277** 805–808.
- van Slegtenhorst M, Verhoef S, Tempelaars A, Bakker L, Wang Q, Wessels M, Bakker R, Nellist M, Lindhout D, Halley D *et al.* 1999 Mutational spectrum of the TSC1 gene in a cohort of 225 tuberous sclerosis complex patients: no evidence for genotype–phenotype correlation. *Journal of Medical Genetics* **36** 285–289.
- Smith HC, Watson GH, Patel RG & Super M 1989 Cardiac rhabdomyomata in tuberous sclerosis: their course and diagnostic value. *Archives of Disease in Childhood* **64** 196–200.
- Smythe JF, Dyck JD, Smallhorn JF & Freedom RM 1990 Natural history of cardiac rhabdomyoma in infancy and childhood. *American Journal of Cardiology* **66** 1247–1249.
- Stern J, Friesen A, Bowering R & Babaryka I 1982 Multiple bilateral angiomyolipomas of the kidneys in tuberous brain sclerosis in association with pleomorphic pheochromocytoma. *Fortschritte der Medizin* **100** 1809–1812.
- Stillwell TJ, Gomez MR & Kelalis PP 1987 Renal lesions in tuberous sclerosis. *Journal of Urology* **138** 477–481.
- Tee AR, Fingar DC, Manning BD, Kwiatkowski DJ, Cantley LC & Blenis J 2002 Tuberous sclerosis complex-1 and -2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling. *PNAS* **99** 13571–13576.
- Thiele EA 2004 Managing epilepsy in tuberous sclerosis complex. *Journal of Child Neurology* **19** 680–686.
- Tigas S, Carroll PV, Jones R, Bingham E, Russell-Jones D, Powell M & Scobie IN 2005 Simultaneous Cushing's disease and tuberous sclerosis; a potential role for TSC in pituitary ontogeny. *Clinical Endocrinology* **63** 694–695.
- Verhoef S, van Diemen-Steenvoorde R, Akkersdijk WL, Bax NM, Ariyurek Y, Hermans CJ, van Nieuwenhuizen O, Nikkels PG, Lindhout D, Halley DJ *et al.* 1999 Malignant

- pancreatic tumour within the spectrum of tuberous sclerosis complex in childhood. *European Journal of Pediatrics* **158** 284–287.
- Webb DW, Fryer AE & Osborne JP 1996 Morbidity associated with tuberous sclerosis: a population study. *Developmental Medicine and Child Neurology* **38** 146–155.
- Weiner DM, Ewalt DH, Roach ES & Hensle TW 1998 The tuberous sclerosis complex: a comprehensive review. *Journal of the American College of Surgeons* **187** 548–561.
- Xu L, Sterner C, Maheshwar MM, Wilson PJ, Nellist M, Short PM, Haines JL, Sampson JR & Ramesh V 1995 Alternative splicing of the tuberous sclerosis 2 (TSC2) gene in human and mouse tissues. *Genomics* **27** 475–480.
- Yeung RS, Katsetos CD & Klein-Szanto A 1997 Subependymal astrocytic hamartomas in the Eker rat model of tuberous sclerosis. *American Journal of Pathology* **151** 1477–1486.
- Yin W, Zhu DM, Wang DS, Wang DX, Wu EH & Zhu XY 1984 Parathyroid adenoma with primary hyperparathyroidism and tuberous sclerosis. A case report. *Chinese Medical Journal* **97** 599–602.
- Zhang X, Horwitz GA, Heaney AP, Nakashima M, Prezant TR, Bronstein MD & Melmed S 1999a Pituitary tumor transforming gene (PTTG) expression in pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* **84** 761–767.
- Zhang X, Horwitz GA, Prezant TR, Valentini A, Nakashima M, Bronstein MD & Melmed S 1999b Structure, expression, and function of human pituitary tumor-transforming gene (PTTG). *Molecular Endocrinology* **13** 156–166.
- Zitzmann K, De Toni EN, Brand S, Goke B, Meinecke J, Spottl G, Meyer HH & Auernhammer CJ 2007 The novel mTOR inhibitor RAD001 (everolimus) induces antiproliferative effects in human pancreatic neuroendocrine tumor cells. *Neuroendocrinology* **85** 54–60.