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 protein 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 angiomylipomas 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 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 lymphangiomatosis (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>
<thead>
<tr>
<th>Table 1 Diagnostic criteria for tuberous sclerosis complex Roach et al. (1998)</th>
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<tr>
<td><strong>Major features</strong></td>
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<td>Facial angiofibroma or forehead plaque</td>
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<td>Non-traumatic ungula or periungual fibroma</td>
</tr>
<tr>
<td>Hypomelanotic macules (more than 3)</td>
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<td>Shagreen patch (connective tissue nervous)</td>
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<tr>
<td>Cortical tuber</td>
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<td>Subependymal nodule</td>
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<td>Subependymal giant cell astrocytoma</td>
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<tr>
<td>Multiple retinal nodular hamartomas</td>
</tr>
<tr>
<td>Cardiac rhabdomyoma, single or multiple lymphangiomyomatosis</td>
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<td>Renal angiomyolipoma</td>
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</tbody>
</table>

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 tuberin (200 kDa protein) respectively. Both hamartin and tuberin 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 Tuberin (TSC2). Hamartin (encoded by TSC1) is 1164 amino acid peptide with a molecular mass of 130 kDa. It interacts with tuberin through amino acids 302–430. Tuberin (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, kidneys and skin, while the TSC gene products (hamartin and tuberin) are expressed in most tissues (Johnson et al. 2001). Tuberin 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–tuberin 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 down-stream 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 tuberin. AMPK (5′-AMP-activated protein kinase), a sensor of cellular energy status, phosphorylates tuberin at T1227 and S1345 and thereby activates tuberin 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 tuberin, 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 tuberin by ERK1/2, and the consequent functional inactivation of TSC1/TSC2 to regulate S6K. ERK1/2 was shown to interact with tuberin 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 tuberin at S1798. RSK1 phosphorylation leads to inactivation of tuberin resulting in increased mTOR signalling to S6K (Roux et al. 2004, Astrinidis & Henske 2005, Crino et al. 2006; Figs 1 and 2).

**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 phaeochromocytomas 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). Over-expression of B-Raf mRNA and protein was found in non-functioning pituitary adenomas, highlighting over-activity 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 (TscEK/− 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 TscEK/− 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, phaeochromocytoma 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>
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<tr>
<th>Sex</th>
<th>TSC onset as</th>
<th>NET onset</th>
<th>Genetics</th>
<th>NET</th>
<th>Remarks</th>
<th>References</th>
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<td>Pituitary</td>
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<tr>
<td>m</td>
<td>32 years adult</td>
<td>33 years</td>
<td>–</td>
<td>ACTH-oma</td>
<td>Clinical examination consistent with Cushingoid features</td>
<td>Tigas <em>et al.</em> (2005)</td>
</tr>
<tr>
<td>m</td>
<td>Child</td>
<td>13.5 years</td>
<td>–</td>
<td>ACTH-oma</td>
<td>Short structure, a change in his appearance including weight gain, abnormal distribution of fat tissue and rounded face, plethora and acne</td>
<td>Nandagopal <em>et al.</em> (2007)</td>
</tr>
<tr>
<td>m</td>
<td>Child</td>
<td>12 years</td>
<td>–</td>
<td>GH-oma</td>
<td>Acromegalic gigantism</td>
<td>Hoffman <em>et al.</em> (1978)</td>
</tr>
<tr>
<td>f</td>
<td>16 years child</td>
<td>25 years</td>
<td>–</td>
<td>–</td>
<td>Hyperprolactinaemia with amenorrhoea and galactorrhoea after delivery of third child, no evidence of a pituitary or hypothalamic tumour</td>
<td>Bloomgarden <em>et al.</em> (1981)</td>
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<tr>
<td>Parathyroid</td>
<td></td>
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<tr>
<td>m</td>
<td>Child</td>
<td>15 years</td>
<td>–</td>
<td>Parathyroid adenoma</td>
<td>Initial diagnosis – acute pancreatitis</td>
<td>Mortensen &amp; Rungby (1991)</td>
</tr>
<tr>
<td>f</td>
<td>Child</td>
<td>20 years</td>
<td>–</td>
<td>Parathyroid hyperplasia</td>
<td>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)</td>
<td>Ilgren &amp; Westmoreland (1984)</td>
</tr>
<tr>
<td>f</td>
<td>Child</td>
<td>14 years</td>
<td>–</td>
<td>Parathyroid adenoma</td>
<td>Anorexia, occasional nausea and vomiting, polydipsia, polyuria and constipation. Generalised osteoporosis</td>
<td>Yin <em>et al.</em> (1984)</td>
</tr>
<tr>
<td>Pancreas</td>
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<tr>
<td>f</td>
<td>Child</td>
<td>24 years</td>
<td>–</td>
<td>Insulinoma</td>
<td>Symptomatic hypoglycaemia secondary to an insulinoma, a novel onset of seizures</td>
<td>Gutman &amp; Leffkowitz (1959)</td>
</tr>
<tr>
<td>m</td>
<td>Child</td>
<td>23 years</td>
<td>–</td>
<td>Insulinoma</td>
<td>Recurrent seizures presented after 15 years of being seizure free</td>
<td>Davoren &amp; Epstein (1992)</td>
</tr>
<tr>
<td>m</td>
<td>Child</td>
<td>28 years</td>
<td>–</td>
<td>Insulinoma</td>
<td>New symptoms included behavioural changes characterised by episodes of agitation and, at other times, lethargy</td>
<td>Kim <em>et al.</em> (1995)</td>
</tr>
<tr>
<td>f</td>
<td>Child</td>
<td>18 years</td>
<td>–</td>
<td>Insulinoma</td>
<td>Symptomatic hypoglycaemia secondary to an insulinoma</td>
<td>Boubaddi <em>et al.</em> (1997)</td>
</tr>
<tr>
<td>m</td>
<td>Child</td>
<td>43 years</td>
<td>–</td>
<td>Insulinoma</td>
<td>Episodes of sweating and dizziness, usually developed after periods of extended fasting and resolved by drinking fruit juices</td>
<td>Eledrisi <em>et al.</em> (2002)</td>
</tr>
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<td>Sex</td>
<td>TSC onset as</td>
<td>NET onset</td>
<td>Genetics</td>
<td>NET</td>
<td>Remarks</td>
<td>References</td>
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<tr>
<td>m</td>
<td>Child</td>
<td>34 years</td>
<td>–</td>
<td>Pancreatic gastrinoma</td>
<td>Reflux oesophagitis and massive weight loss. At diagnosis multiple metastases (liver, abdominal lymph node, lungs and thoracic/lumbar vertebral column)</td>
<td>Schwarzkopf &amp; Pfisterer (1994)</td>
</tr>
<tr>
<td>m</td>
<td>Adult</td>
<td>39 years</td>
<td>Sequencing of the TSC2 gene showed a novel 1bp insertion at position 45–46 leading to a frame shift</td>
<td>Pancreatic islet cell neoplasm</td>
<td>Lichenified hyperpigmented plaques suggesting a paraneoplastic process</td>
<td>Merritt et al. (2006)</td>
</tr>
<tr>
<td>m</td>
<td>Child</td>
<td>12 years</td>
<td>TSC2 gene LOH in the tumour and the germ line mutation Q478X in exon 13 of the TSC2 gene on chromosome 16. Screening for MEN1 negative</td>
<td>Malignant islet cell tumour</td>
<td>A large infiltrative retroperitoneal tumour originating from the pancreas, with local metastases to the lymph nodes</td>
<td>Verhoef et al. (1999)</td>
</tr>
<tr>
<td>m</td>
<td>Child</td>
<td>6 years</td>
<td>Mutation analysis of DNA extracted from peripheral blood cells identified an R1459X de novo mutation in exon 33 of the TSC2 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</td>
<td>Malignant islet cell tumour of pancreas</td>
<td>In abdominal CT scan – a highly vascularised retroperitoneal tumour arising from pancreas</td>
<td>Francalanci et al. (2003)</td>
</tr>
<tr>
<td>f</td>
<td>Child</td>
<td>29 years</td>
<td>–</td>
<td>Pleo-morphic phaeo-chromo-cytoma</td>
<td>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</td>
<td>Stern et al. (1982)</td>
</tr>
<tr>
<td>f</td>
<td>Adult</td>
<td>34 years</td>
<td>A germ line nonsense mutation C165X in exon 6 of TSC1. TSC1 LOH in LAM cells, lymph nodes, uterus and kidneys but not in carcinoid cells</td>
<td>Bronchial carcinoid</td>
<td>Two years after diagnosis of sporadic lymphangiomatosis (LAM) – haemoptysis related to a bronchial carcinoid</td>
<td>Sato et al. (2004)</td>
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</table>
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 a 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 had also confirmed TSCI. 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 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 (Ilgren & Westmoreland 1984). In another TSC patient, extensive lichenified hyperpigmented plaques suggesting a para-neoplastic 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 angiomylipoma of the right kidney (however, both kidneys showed multiple smaller angiomylipomas) 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 consisted 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, 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 women 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 TSCI gene. Furthermore, her LAM cells, lymph nodes, uterus and kidneys showed TSCI LOH, although surprisingly this was not found in microdissected tumour cells from the bronchial carcinoid tissue (Sato et al. 2004).

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 anemic, 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 angiomylipomas located bilaterally, with one dominant lesion in the right adrenal, which was partially necrotic in the central area. This patient underwent radiological surgery and histology was consistent with a pleomorphic pheochromocytoma. A renal angiomylipoma 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.

D Dworakowska and A B Grossman: Tuberous sclerosis and NETs
**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-entero-pancreatic 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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