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DISSERTAÇÃO COM VISTA À ATRIBUIÇÃO DO GRAU DE MESTRE NO ÂMBITO DO CICLO DE ESTUDOS DO MESTRADO INTEGRADO EM MEDICINA

MARIA CLARA COSTA BONIFÁCIO SELORES DOS SANTOS

ENVOLVIMENTO RENAL NA ESCLEROSE TUBEROSA

ARTIGO DE REVISÃO

ÁREA CIENTÍFICA DE NEFROLOGIA

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PROF. DOUTORA LUÍSA LOBATO

PROF. DOUTOR RUI ALVES

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ARTIGO DE REVISÃO

NEFROLOGIA

A ALUNA,

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(MARIA CLARA COSTA BONIFÁCIO SELORES DOS SANTOS)

ESCOLHA DO TEMA

A área nefrológica foi uma escolha natural pelo interesse na especialidade. A opção foi baseada na actualidade do tema, no sentido em que grandes avanços na abordagem e tratamento das manifestações renais da esclerose tuberosa estão a despertar crescentes interesse e curiosidade do mundo da investigação e motivam a sua discussão em encontros científicos. O idioma usado na elaboração deste artigo foi o inglês, com o intuito da posterior publicação em revista médica da especialidade.

INSTITUIÇÕES ENVOLVIDAS

O trabalho foi desenvolvido com a colaboração do Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, sob orientação da Prof. Doutora Luísa Lobato, ICBAS e Serviço de Nefrologia do Hospital de Santo António, Centro Hospitalar do Porto. O Prof. Doutor Rui Alves da Faculdade de Medicina, Universidade de Coimbra e Serviço de Nefrologia dos Hospitais da Universidade de Coimbra foi o co-orientador da dissertação. O Serviço de Nefrologia do Centro Hospitalar de Vila Nova de Gaia/Espinho estimulou a realização do artigo nesta área.

INDEX

RENAL MANIFESTATIONS OF TUBEROUS SCLEROSIS

Clara Santos

Faculdade de Medicina, Universidade de Coimbra, Portugal

Abstract

Tuberous sclerosis (TS), coined by Bourneville in 1880, is characterized by widespread hamartomas in several organs, including brain, heart, skin, eye, kidney and lung. This disease is transmitted in an autosomal dominant pattern although two-thirds of patients present sporadic mutations. Hamartin and tuberin, encoded by the two causative genes, TSC1 and TSC2 respectively, comprehend a complex, which inhibits the mammalian target of rapamycin (mTOR), a major regulator of cell growth and proliferation.

This review provides an up-date of TS, its incidence, epidemiology and phenotype. The primary objective is to illuminate the challenges encountered by clinicians who manage this disease, specially understanding the spectrum of renal manifestations. In addition, the current knowledge in the field of molecular biology and genetics is revised.

This work places special attention on current treatment options and highlights the most recent promising therapeutic trials with sirolimus. In conclusion, recent guidelines in screening, diagnosis and state-of-the art treatment are emphasized.

Keywords: tuberous sclerosis, kidney, angiomyolipoma, inheritance, diagnosis, management, treatment

Resumo

A esclerose tuberosa (ET), definida em 1880 por Bourneville, é caracterizada pela presença de hamartomas em diversos órgãos, incluindo cérebro, coração, pele, olho, rim e pulmão. Esta doença é transmitida segundo um padrão autossómico dominante, embora dois terços dos doentes apresentem mutações esporádicas. A hamartina e a tuberina, produtos proteicos dos dois genes responsáveis, TSC1 e TSC2 respectivamente, formam um complexo que inibe o *mammalian target of rapamycin* (mTOR), um dos principais reguladores do crescimento e proliferação celulares.

O presente artigo apresenta uma revisão da literatura relacionada com a ET, com referência à incidência, epidemiologia e fenótipo. O objectivo principal deste trabalho é elucidar sobre os desafios com que se deparam os clínicos que lidam com esta doença, nomeadamente compreendendo o espectro de manifestações renais. Além disso, é apresentada uma revisão do conhecimento mais recente no campo da biologia molecular e genética.

Este trabalho atenta de forma particular às principais opções terapêuticas disponibilizadas actualmente e realça os mais recentes e promissores estudos terapêuticos recorrendo ao uso do sirolimus. Como principais conclusões deste trabalho, são destacadas as orientações actualmente preconizadas no seguimento, diagnóstico e tratamento, com referência aos recentes avanços terapêuticos.

Palavras-chave: esclerose tuberosa, rim, angiomiolipoma, hereditariedade, diagnóstico, abordagem, tratamento

Conflits of interest: None declared. Adress correspondence to Clara Santos, Faculdade de Medicina, Universidade de Coimbra, Rua Larga 3004-504 Coimbra, Portugal and Nephrology Department, Centro Hospitalar de Vila Nova de Gaia/Espinho, Rua Conceição Fernandes, 4434-502 Vila Nova de Gaia, Portugal. E-mail: mclarinhasantos@hotmail.com.

1. INTRODUCTION

The Tuberous Sclerosis (TS [MIM 191100]) is a dominantly inherited disorder characterized by widespread hamartomas in several organs, including brain, heart, skin, eye, kidney and lung. This way, to emphasize the variability of the clinical features of the disease, some authors use the concept Tuberous Sclerosis Complex (TSC). However, both terms are contemporary and can be equally used. TS belongs to the neurocutaneous syndromes, or phacomatoses, as derivatives of neural crest cell constitute the chief elements involved in its lesions (Milliner and Torres 1998).

2. NOMENCLATURE

The disease was coined as “tuberous sclerosis” regarding the presence of multiple sclerotic masses scattered throughout the cerebrum and the potato-like consistency of the hypertrophic cerebral gyri (Milliner and Torres 1998; Leung and Robson 2007).

3. HISTORICAL REMARKS

The TS condition was first recognized by von Recklinghausen in 1862 when he documented the autopsy examination of a stillborn with multiple cardiac and brain tumors (Leung and Robson 2007). However, the term TS was only coined 18 years later, in 1880, when Désire-Magloire Bourneville, an expert in childhood retardation, described the pathologic features of the sclerotic cortical tubers and the multiple rounded lesions on the kidney surface found *at post mortem* in patients with epilepsy and mental retardation (Milliner and Torres 1998; Online Mendelian Inheritance in Man 2003).

In 1908, Vogt published his understanding of the link among seizures, mental retardation and adenoma sebaceum, later known as the Vogt triad, observed in only a third of cases (Józwiak et al. 2000).

In 1993, the European Chromosome 16 Tuberous Sclerosis Consortium (The European Chromosome 16 Tuberous Sclerosis Consortium 1993) identified TSC2 gene and designated its protein product as tuberin. TSC1 was identified and the predicted TSC1 protein was called hamartin by van Slegtenhorst et al. (1997).

In 1998, a panel of clinicians and geneticists revised the diagnostic criteria for TS at the Tuberous Sclerosis Complex Consensus Conference, based solely on the clinical features and designed to establish a consistent and reliable standard for the diagnosis (Roach et al. 1998).

4. EPIDEMIOLOGY

TS affects about 1 million individuals worldwide, with an estimated incidence of up to 1 in 6000 newborns (Leung and Robson 2007; Krueger and Franz 2008). However its real prevalence is not known because of undiagnosed cases consisting mostly of mildly affected or asymptomatic individuals (Józwiak et al. 2000). TS affects both genders and all ethnic groups (Krueger and Franz 2008). Nevertheless, there are no large-cohort studies in the literature that report if the incidence of TS is correlated with gender, ethnia or geographic area.

5. GENETIC TRANSMISSION

TS is transmitted in an autosomal dominant pattern although in approximately two-thirds of all cases neither parent has signs of the disease and it is caused by a sporadic mutation (Sancak et al. 2005), indicating a high rate of spontaneous mutation in the TSC genes, TSC1 and TSC2. TSC1 gene is located on chromosome 9q34, encoding hamartin (van Slegtenhorst

M et al. 1997) and TSC2 gene is located on chromosome 16p13.3, encoding tuberin (The European Chromosome 16 Tuberous Sclerosis Consortium 1993).

Initially, linkage trials have suggested that the number of families with mutations in each gene would be equivalent (Crino et al. 2006). However, several additional studies have been shown that the majority of TS patients have a mutation in the TSC2 gene (Dabora et al. 2001; Sancak et al. 2005). Approximately 70% of the sporadic cases are found in TSC2 gene. In contrast, in familial cases, approximately half of them show linkage to TSC1 and half to TSC2 (Jansen et al. 2008).

Extensive studies of the TSC1 and TSC2 genes in patients with TS have revealed a wide spectrum of mutations, but there are no particular regions (hot spots) within the TSC1 or TSC2 genes in which mutations occur at a high rate (Rosner et al. 2008). The overall mutation detection rate in patients with TS is around 85-90%. Therefore, mutations are not identified in 10-15% of patients (Kwiatkowski 2005; Curatolo et al. 2008). This fact could be due to (Curatolo et al. 2008): 1) methods that are not sensitive enough; 2) mutations in intronic and promoter regions, which might disrupt gene expression and be missed by most mutation screening methods; 3) mosaicism and 4) the possibility of mutations in an unknown third gene.

Mosaicism is the phenomenon in which a fraction of, rather than all, germ-line and somatic cells contain a mutation or chromosomal abnormality (Kwiatkowska et al. 1999). Somatic mosaicism occurs when an individual has a mutation in some, but not all, cells and tissues. Such patient has often milder manifestations of TS or manifestations limited to a single organ system but can have more severely affected progeny (Kwiatkowska et al. 1999; Kwiatkowski 2005). Somatic mosaicism undoubtedly explains some, if not most, of the cases where mutations cannot be identified (Kwiatkowski 2005). Germline mosaicism occurs when an individual carries a mutation only in the germ cells and has no other signs or symptoms of

the TS disease. It probably accounts for about 2% of the patients, making genetic counseling more difficult even when a specific mutation has been identified (Roach et al. 1998). It may be suspected when apparently healthy parents have two or more children with TS.

TSC2 lies immediately adjacent to PKD1, the major gene for Autosomal Dominant Polycystic Kidney Disease (ADPKD) and deletions involving both genes are identified in 2-3% of patients with TS, resulting in a polycystic kidney phenotype (Sampson et al. 1997).

6. GENOTYPE-PHENOTYPE CORRELATIONS

A major goal in TS research is to define genotype-phenotype correlations so that prognosis regarding clinical course (phenotype) can be predicted on the basis of mutation type (genotype).

Although the disorder being considered fully penetrant, the clinical presentation of TS is highly variable, even among individuals with identical mutations (Kwiatkowska et al. 1999; Dabora et al. 2002). Thereby, the wide variability in the extent and severity of clinical manifestations, even within the same family, suggests that strong correlations between a mutation and its clinical outcome are unlikely. Phenotypic variability of TS has been attributed to the stochastic two-hit mechanism, to mosaicism and to genetic and non-genetic modifiers (Dabora et al. 2002; Au et al. 2008).

Somatic mutation accounts for the majority of lesions that occur in TS following the Knudsen two-hit paradigm for TSC genes (Kwiatkowska et al. 1999). In agreement with this model, the inactivation of both alleles of either TSC1 or TSC2 is needed for tumor development. Thereby, second somatic mutations occur in the cells, giving rise to TS hamartomas. Accordingly, although TS is transmitted in an autosomal dominant manner, mutations in the TSC genes are believed to be recessive at the level of the affected cells (Rosner et al. 2008). Loss of heterozigosity has been consistently observed in the majority of

renal angiomyolipomas, cardiac rhabdomyomas and lymphangiomyomatosis cells, but has rarely been found in cerebral tubers (Henske et al. 1996). This fact has not a well established explanation but indicates that inactivation of either allele is not required to tuber pathogenesis or only a subgroup of cells within a tuber is affected by the second hit (Crino et al. 2006; Yates 2006). It has been reported that genetic and environmental factors may modulate where, when, and how these second hits occur.

Somatic mosaicism has been reported in some patients with TSC1 or TSC2 mutations and is thought to account for a milder clinical phenotype (Kwiatkowska et al. 1999).

The reported association between a high-expression of interferon-gamma (IFN-γ) allele 2 and a lower frequency of renal angiomyolipomas (AMLs) in patients with mutations in TSC2 (Dabora et al. 2002) suggests that modifier genes might have important effects on the phenotype. Better understanding of the nature of genes that modify the function of tuberin and hamartin will help in development of better prognostic information for patients and their families as well as opening up the possibility of new therapeutic targets for patients with TS.

Several independent studies of large cohorts of TS patients have demonstrated that patients with a TSC2 mutation tend to have a more severe phenotype with a higher frequency of mental retardation and seizures, more extensive renal involvement, more retinal abnormalities and more severe facial angiofibromas (Dabora et al. 2001; Sancak et al. 2005; Jansen et al. 2008). The greater severity of TSC2 phenotype could explain the finding in several studies of a more equal ratio between TSC1 and TSC2 mutations in familial as compared to sporadic case, since families with TSC2 mutation are less likely to have offspring. (Dabora et al. 2001; Sancak et al. 2005; Jansen et al. 2008). There are at least two hypotheses to explain why TSC1 disease is less severe than TSC2 disease (Dabora et al. 2001). First, as most hamartomas in TS develop through a two-hit inactivation mechanism (following the Knudson´s model) it is possible that these events occur less often in TSC1 than

in TSC2. Second, it is possible that complete loss of hamartin has different effects in cells, compared with loss of tuberin. Sancak et al. (2005) observed that in familial cases, there was less differences between the clinical features of the groups with a TSC1 or TSC2 mutation compared to the sporadic cases, suggesting that the patients with a sporadic TSC2 mutation have a more severe phenotype than the patients with a familial TSC2 mutation. Consistently with this, a study developed by Jansen et al. (2008) concluded that sporadic TS was associated with an earlier age at seizure onset and a lower cognition index than familial TS.

Patients without defined mutations tend to have milder clinical symptoms of TS than patients in whom mutations are found (Kwiatkowski 2005) and sometimes even milder than those with mutations in TSC1 (Curatolo et al. 2008). It is likely that a proportion of those TS patients are mosaic for a causative mutation, explaining both failure mutation detection and less severity of clinical features (Kwiatkowski 2005). However, it seems that renal involvement represents an exception to this fact.

Despite many biochemical advances, exactly how mutations in TSC1 or TSC2 lead to the clinical manifestations of TS remains unknown and the existence of genotype-phenotype correlations are still datable. Future studies to fully define this relationship are clearly warranted, not only to confirm the diagnosis of TS but also for both clinical management and possible therapeutic or preventive measures.

7. PHYSIOPATHOLOGY

The protein products of the TSC1 and TSC2 genes, hamartin and tuberin, respectively, physically interact and function as a heterodimeric complex (Rosner et al. 2008) that has been linked in several pathways, among which mammalian target of rapamycin (mTOR) signaling has been the most intensely studied. Functions for hamartin and tuberin independent of the complex have been suggested by evidence of their binding to a variety of other proteins, but it

is uncertain whether these interactions have any physiological significance (Kenerson et al. 2002; Yates 2006; Rosner et al. 2008).

Investigators have shown in *Drosophila* that the hamartin/tuberin complex participates in the control of the cell size via the insulin/ribossomal S6 kinase (S6K) pathway (Fig. 1). This complex acts downstream of phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt), and upstream of mTOR and S6K (Kenerson et al. 2002; Tee et al. 2002). Mutations in either of the TSC genes disrupt the function of the complex, which results in enlarged cells because of the defective regulation of cell size (El-Hashemite et al. 2003). The hamartin/tuberin complex formation provides an explanation for the similar disease phenotype in TS patients with mutations in either of the two TSC genes (Rosner et al. 2008).

Under physiological conditions, mTOR is a protein kinase with far-reaching functions in the regulation of cellular protein synthesis, metabolism, differentiation, growth and migration (Kenerson et al. 2002). Constitutive activation of mTOR is associated with abnormal cellular proliferation, like it was demonstrated by Kenerson et al. (2002), who showed that renal AMLs and renal cell carcinoma (RCC) of patients with TS express elevated levels of phosphorylated mTOR and S6K (Fig. 2). Similar dysregulation of mTOR has been found to have a role in the propagation of non-TS human malignancies (Ma et al. 2007).

The Ras homologue enriched in brain (Rheb), which is a guanosine triphosphatase (GTPase) activating protein (GAP), functionally links the hamartin/tuberin complex to the mTOR pathway (Tee et al. 2002). Rheb, like other Ras family members, cycles between an active guanosine triphosphate (GTP)-bound state and an inactive guanosine diphosphate (GDP)-bound state. The hamartin/tuberin complex has also GAP activity and stimulates the conversion of Rheb-GTP to Rheb-GDP, thereby inactivating Rheb. Rheb binds directly to mTOR, and when present as a Rheb-GDP state, inactivates it (Kimball et al. 2008).

Conversely, Rheb-GTP appears to be a major positive regulator of mTOR activity. Activation of mTOR results in increased phosphorylation of two of its downstream targets, ribosomal S6 kinase (S6K) and eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) (Tee et al. 2002), increasing the activity of the first and inhibiting the latter. In both cases, the downstream effect is to promote protein synthesis and cell growth. S6K, in turn, exerts a negative feedback and thereby restrains the insulin/PI3K signaling (Yang and Guan 2007). It has been suggested that the downstream targets of mTOR, S6K and 4E-BP1, could be important therapeutic targets to treat TS. However, this S6K-mediated feedback inhibition may be a potential cause of unfeasibility of these new therapeutics.

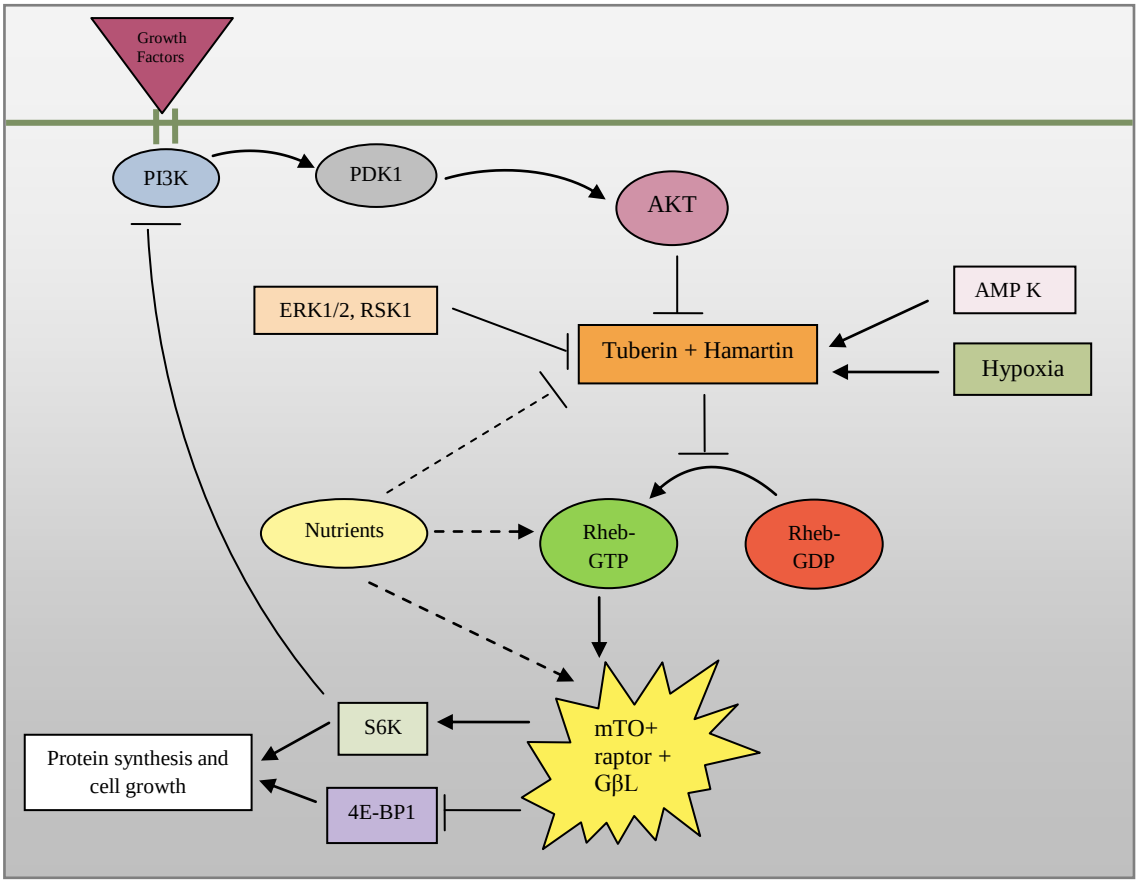

Figure 1: The hamartin/tuberin complex: major inhibitor of the mTOR signaling pathway
Abbreviations: PI3K: phosphatidylinositol 3-kinase; PDK1: 3-phosphoinositide dependent protein kinase-1; Akt: protein kinase B; AMPK: adenosine-monophosphate-activated protein kinase; ERK1/2: extracellular-related kinase 1/2; RSK1: p90 ribosomal S6 kinase 1; Rheb: ras homolog enriched in brain; GDP: guanosine diphosphate; GTP: guanosine triphosphate; mTOR: mammalian target of rapamycin; Raptor: regulatory associated protein of mTOR; GβL: G-protein β-subunit-like protein; S6K: ribossomal S6 kinase; 4E-BP1: eukaryotic translation initiation factor 4E binding protein 1. Solid arrows represent activation, bars represent inhibition and dashed arrows represent possible mechanisms. *Adapted from Tee et al. (2002); Yates (2006); Krueger and Franz (2008); Wang et al. (2008).*

Binding of extracellular growth factors including insulin to their cell membrane receptors activates PI3K signaling, leading to a negative regulation of tuberin through its phosphorykation by Akt (Tee et al. 2002). This reduces the inhibitory action that the hamartin/tuberin complex exerts on mTOR. So, Akt stimulates mTOR signaling by inhibiting the function of tuberin and thereby activating Rheb. Amino acids may also act through Rheb to activate mTOR (Kimball et al. 2008; Wang et al. 2008). Recently, it has been reported the activation of mTOR signaling by inputs from amino acid independent of the Rheb (Yang and Guan 2007). However these mechanisms are not well established. In contrast, catabolic hormones such as glucocorticoids, pro-inflammatory cytokines, hypoxia and conditions that reduce energy levels repress mTOR activity (Kimball et al. 2008; Krueger and Franz 2008; Wang et al. 2008). Multiple other kinases phosphorylate and inactivate TSC2 and thereby activate Rheb and mTOR, like the mitogen-activated protein kinases: extracellular-related kinase 1/2 (ERK1/2) and p90 ribosomal S6 kinase 1 (RSK1) (Yates 2006; Krueger and Franz 2008). The inhibition of these kinases signaling pathways could be future therapeutic targets and studies based on this hypothesis have being developed (Mi et al. 2009).

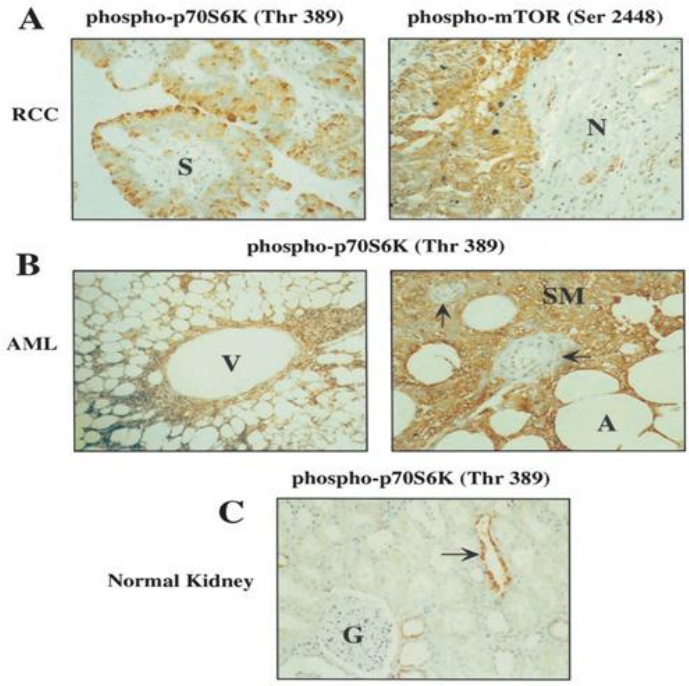

Figure 2: Expression of mTOR and p70S6K in human TS tumors and normal kidney
A. Papillary RCC from a patient with tuberous sclerosis stained with antibodies for p70S6K (Thr389) and mTOR (Ser2448). Note negatively stained stromal cells (S) and adjacent normal kidney tissue (N) compared with brown-stained tumor cells. **B.** TS-related AMLs stained with phospho-p70S6K antibody. Left panel shows uniform staining in all three cellular components, including the central vessel (V). Right panel shows negative-staining vessels (arrows); A: adipocyte; SM: smooth muscle. **C.** Normal human kidney, discrete expression of phospho-p70S6K in the distal tubule (arrow); proximal tubules and glomerulus (G) without immunoreactivity. Abbreviations: RCC: renal cell carcinoma; p70S6K: ribosomal S6 kinase; mTOR: mammalian target of rapamycin; AMLs: angiomyolipomas. *Adapted from Kenerson et al. (2002).*

In summary, mutation of hamartin or tuberin in TS leads to hyperactivation of the downstream mTOR pathway and the associated kinase signaling cascades and translational factors, resulting in increased cell growth and proliferation.

A recent study (Habib 2009), added on important new considerations about physiopathology of TS. The authors concluded that the deficiency in tuberin expression is associated with a decreased protein and mRNA expression of 8-oxoG-DNA glycosylase (OGG1), a DNA repair enzyme, in renal lesions from TS patients. Deficiency of this enzyme leads to the accumulation of 8-Oxo-deoxyguanine (8-oxo-dG), a major form of oxidative DNA, suggesting that tuberin plays a significant role in protecting cells from oxidative DNA damage.

8. CLINICAL FEATURES

TS has an extensive clinical variability even among individuals with identical mutations. There is a wide range of severity of TS manifestations, with some individuals so severely affected they are unable to care to themselves, while other are only mildly affected and may not be aware of their diagnosis until adulthood.

TS is considered to be a tumor-forming syndrome and is characterized by the occurrence of multiple tumor-like proliferations in a variety of locations. Kidney, lung, brain, heart and skin are the most commonly involved organs, but sporadic lesions can occur in virtually every organ system. The following sections review the clinical manifestations of TS with an emphasis on the renal findings.

8.1 Renal Manifestations

Renal involvement in TS is common and potentially serious, with estimated rates of involvement ranging from 48 to 80% (Rakowski et al. 2006). Patients with TS can develop a

number of renal lesions, the most common being AMLs, cysts and RCC (O´Callaghan et al. 2004). Renal pathology is a leading cause of mortality, second only to neurologic disease (Shepherd et al. 1991). It has been reported that TS causes end-stage renal disease in approximately 1 to 3% of patients (Schilinger and Montagnac 1996; Harabayashi et al. 2004). Because of these reasons, imaging surveillance is performed in patients with TS to identify and monitor the progression of such lesions. The tables I and II represent a resume of the contents discussed in the current section.

Table I: Renal manifestations associated with TS

Renal lesion	Prevalence	Comparison to sporadic forms	Main features
AMLs	55-80%	Larger, bilateral, multifocal Younger age More tendency to grow	Frequency and number increase with age Higher incidence and severity in TSC2 patients HMB-45 immunoreactivity Complications: rupture, hypertension and CRF
Cysts	20-30%	No significative differences	Frequency and number increase with age Higher incidence and severity in TSC2 patients More associated with hypertension and CRF than AMLs When associated with ADPKD: larger, bilateral and earlier end-renal stage
RCC	2-3%	Same frequency Younger age More frequently bilateral	HMB-45 negativity Pathological heterogeneity

Abbreviations: AMLs: angiomiolypomas; RCC: renal cell carcinoma; HMB-45: melanosome-associated protein; CRF: chronic renal failure; ADPKD: autosomal dominant polycystic kidney disease

Table II: Effects of genotype, gender and age on TS-associated AMLs

Variable	Effects on frequency and behavior
Genotype	Higher frequency and severity in TSC2 group compared to TSC1 group Higher frequency and severity in NIM group compared to TSC1 group Lower frequency in TSC2 group with IFN-γ allele 2 Higher frequency in TSC2 group with some variations of OGG1 gene
Gender	Higher frequency in females than in males (ratio 2 to 4: 1) Higher severity and tendency to grow in females
Age	Higher frequency and number with increasing age

Abbreviations: NIM: non-identified mutation; IFN-γ: interferon-gamma; OGG1: 8-oxoG-DNA glycosylase

8.1.1 Angiomyolipomas

The most common and characteristic renal lesions are AMLs, composed of abnormal vessels, immature smooth muscle and fat cells (O´Hagan et al. 1996). The histological characteristics of renal AMLs were initially described by Fischer in 1911 but it was not until 1951 when Morgan coined the term "angiomyolipoma" (Lendvay and Marshall 2003; Inci et al. 2006).

The estimated incidence of AMLs in TS ranges from 55 to 80% (O´Callaghan et al. 2004; Rakowski et al. 2006) but 80% of all patients with AMLs do not have TS (Lendvay and Marshall 2003). There are demographic and phenotypic differences in TS and sporadic AMLs. In TS, they tend to be larger, bilateral, multifocal, present at a younger age and have greater tendency to grow compared with sporadic forms (O´Hagan et al. 1996; Lendvay and Marshall 2003). However, histologically both types cannot be differentiated from each other (Inci et al. 2006). The macroscopic appearance of typical AML is usually a lobulated tumor with a yellowish to grey color due to the high fat content (Frago et al. 2006).

The incidence and behavior of the AMLs have been correlated to genotype, gender and age (Table II).

As it was already reported above, patients with TSC2 mutations exhibited a higher incidence and severity of AMLs compared to patients with mutated TSC1 gene (Dabora et al. 2001; Sancak et al. 2005; Rakowski et al. 2006). In addition, Rakowski et al. (2006) noted that patients with TSC2 mutations and renal manifestations were younger on average than patients with TSC1 mutations, suggesting that it is likely that those patients turn to a medical attention earlier. Dabora et al. (2001) reported a higher AML involvement in the group with no identified mutation (NIM) compared to the TSC1 group and no difference between the NIM group and the TSC2 group. Afterward, a study developed by Sancak et al. (2005) became to confirm these evidences. Therefore, oppositely of other TS manifestations, non

identified mutations cases are not associated with a milder renal phenotype and perhaps renal manifestations of NIM patients more closely resemble those of TSC2 patients. Dabora et al. (2002) found an association between IFN-γ allele 2 (which has been shown to be associated with higher levels of IFN-γ production in vitro) and the absence of AMLs in TSC2 patients, suggesting that IFN-γ allele 2 may be a genetic modifier that reduces AML development or growth. Habib et al. (2008) demonstrated that some particular mutations in OGG1 gene are associated with a risk for developing AMLs among TS patients. This study also suggests that genetic modifiers may modulate the renal involvement in TS disease.

In some series, AMLs manifest with equal frequency in both genders (Martignoni et al. 2003; Aydin et al. 2009), though most studies report a higher proportion of women than men with a ratio of 2 to 4:1 (Lendvay and Marshall 2003; Leung and Robson 2007). Other studies have suggested that AMLs are more common, more numerous and may show a greater propensity for growth in female patients (Dabora et al. 2002; Rakowski et al. 2006). Furthermore, in one series, all progesterone receptor positive AMLs were from women younger than 50 years and none from men, suggesting the involvement of hormonal factors in the severity and frequency of renal disease. More studies are needed to determine whether hormonal modulation accounts for the difference in AML manifestations between female and male patients (Lendvay and Marshall 2003).

The incidence and number of AMLs increase with age (O´Hagan et al. 1996; Rakowski et al. 2006), which demonstrates the importance of following patients with TS for renal involvement throughout their adult lives.

- Histological features

In TS, AMLs are mostly of classic triphasic histology and contain all three of its namesake components (Fig. 3A). However, other variants, including epithelioid and oncocytic

can be seen (Aydin et al. 2009). The smooth muscle-like cell has been putatively considered to be derived from a perivascular epithelioid cell (PEC) and PEC-derived tumors lesions have been categorized as PEComas, in which we found AMLs (Martignoni et al. 2003; Aydin et al. 2009). Aydin et al. (2009) developed a clinicopathological study to investigate the histological features of AMLs in TS patients and they concluded that the amount of epithelioid component, epithelial cysts and microscopic AML foci are strongly associated with TS and the presence of all these three features should raise strong suspicion for this disease. As it was already reported the majority of AMLs are sporadic and therefore do not represent TS. Recognition of the histological features associated with TS can therefore benefit patients for early management and family genetic counseling.

It has been shown that AMLs are associated with melanosome-associated protein (HMB-45) immunoreactivity, and that the HMB-45 expression is useful in differential diagnosis between AMLs and RCC (Bjornsson et al. 1996; Aydin et al. 2009).

Epithelioid AML (EAML) is an entity that has been differentiated from classic AML and has been associated in more than one half of cases with TS (Frago et al. 2006), the incidence of which is higher than that of classic AML in TS patients. EAML is characterized by polygonal cells with clear to eosinophilic cytoplasm and round to oval nuclei that may show varying degree of nuclear atypia. In contrast to the classic AML, which is benign, EAMLs are potentially malignant with metastasis to the lymph nodes, lungs and vertebrae reported in one-third of cases in the literature (Frago et al. 2006; Kato et al. 2009). No parameters have been strictly correlated to the prognosis but Aydin et al. (2009) proposed that the amount of epithelioid component, necrosis, nuclear atypia and mitotic activity might be useful to predict biological behavior of EAMLs. Nevertheless, additional studies with larger number of cases are needed to address the full spectrum of the biological behavior of EAML.

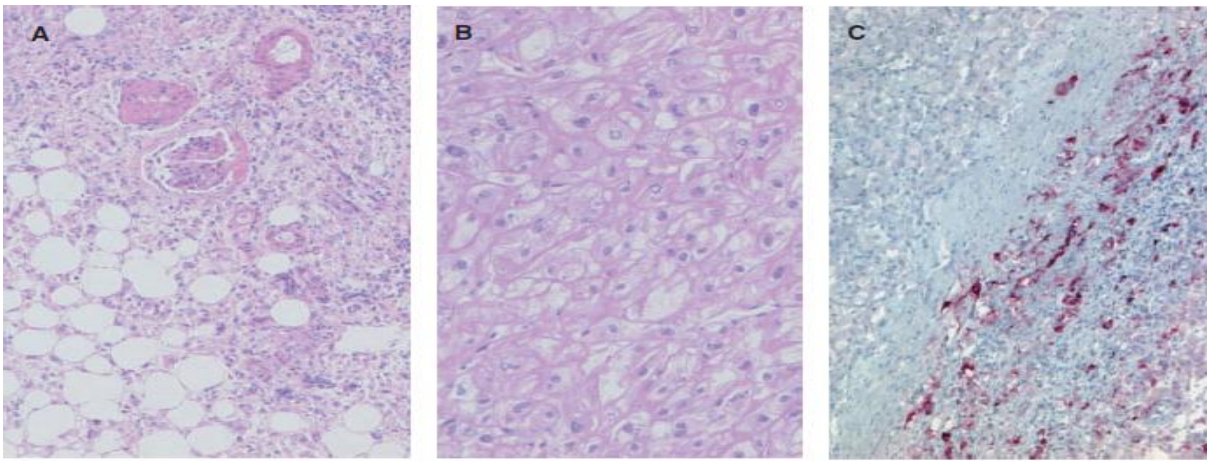

Figure 3: Histology of renal AML and RCC
A. The typical histomorphological characteristics of AML with smooth muscle and vessels. **B.** Histological presentation of the RCC: epithelioid tumour cells with granular cytoplasm (chromophobe-like). **C.** HMB-45 expression of the AML (on the right) and lack of expression in the RCC (on the left). Abbreviations: AML: angiomyolipoma; RCC: renal cell carcinoma; HMB-45: melanosome-associated protein *Adapted from Corsenca et al. (2007).*

Because of its epithelioid architecture, EAML can be erroneously diagnosed as RCC, particularly when other components of AMLs are obscure and atypia is proeminent (Aydin et al. 2009; Kato et al. 2009). The HMB-45 antibody represents a useful marker for this differential diagnosis since RCC is negative for melanoma markers (Fig. 3C).

- Symptoms and complications

In almost all cases they are asymptomatic and less frequently they present with flank pain, a palpable tender mass or gross hematuria (Granata et al. 2009). AML per se can cause fever in TS patients by an unknown mechanism (Kunzi et al. 2005).

Renal AMLs are more likely to grow than not, but their growth rate is highly variable. They do not disappear (Józwiak et al. 2000). Although their tendency to grow slowly, follow-up renal imaging may reveal dramatic progression of tumor size (3-4cm every two years) in adolescents (Curatolo et al. 2008). There is a positive correlation between the tumor size and the severity of symptoms. As AMLs increase in size, they typically become more vascular and may develop multiple tortuous vessels and aneurysms that are prone to rupture. The risk of hemorrhage from AMLs is unknown. However, published series suggest that hemorrhage

occurs in half of patients. AMLs larger than 4cm are more likely to become symptomatic and complicated by secondary bleeding (Lendvay and Marshall 2003; Curatolo et al. 2008; Granata et al. 2009), called Wunderlich syndrome, which may potentially cause life-threatening hemorrhage. Spontaneous retroperitoneal hematoma and acute abdominal bleeding in association with hypovolemic shock should bring AML rupture in mind. The risk factors for bleeding are size, multiple localization and vascular anomalies (Inci et al. 2006). The renal vein invasion is the major reason for spontaneous rupture. Patients with multiple small AMLs rarely experience significant hemorrhage (Krueger and Franz 2008). The onset of TS with spontaneous retroperitoneal hemorrhage episode is rare (Granata et al. 2009).

Even without hemorrhage, large AMLs or multiple smaller lesions may gradually replace normal renal parenchyma resulting in hypertension and chronic renal failure (Casper et al. 2002; Rakowski et al. 2006; Krueger and Franz 2008).

8.1.2 Renal cysts

The second most frequent renal manifestation is the renal cyst, with an incidence estimated to be 20-30% (O´Hagan et al. 1996; O´Callaghan et al. 2004). They may be single or multiple, microscopic or macroscopic (O´Hagan et al. 1996). The histopathology of these cysts shows a lining of hyperplasic eosinophilic cells that may aggregate to form intracystic masses (Robertson et al. 1996). A combination of renal cysts and AMLs is characteristic of TS. Renal cysts are identified in children younger than those who present with an AML (Roach and Sparagana 2004; Leung and Robson 2007) and it has also been reported an association between increasing age and the incidence and number of cysts (Rakowski et al. 2006).

In radiologic and macroscopic appearances, cysts are indistinguishable from ADPKD in the absence of AMLs (O´Hagan et al. 1996; Sampson et al. 1997) and the reported mutations

of either TSC2 and PKD1 genes may be misdiagnosed as an isolated ADPKD (Sampson et al. 1997). This co-existence should be suspected in children with TS and bilateral large renal cysts at birth, especially if there is no family history of ADPKD (Lendvay and Marshall 2003).

- Symptoms and complications

Cysts are usually asymptomatic and the frequency of symptoms also enhances with age, since they tend to grow with the past of years (O´Hagan et al. 1996). However, in opposition to AMLs, it has been reported that individual renal cysts can disappear (Roach and Sparagana 2004). Compared to AMLs, cysts are more commonly the cause of hypertension and progression to renal failure (Nimr et al. 1987; O´Hagan et al. 1996; O´Callaghan et al. 2004). The existence of mutations in both TSC2 and PDK1 genes carries a poor prognosis, given that the cysts are larger and more numerous, with earlier onset of severe renal cystic disease and end-stage renal failure by early adult life (Lendvay and Marshall 2003).

8.1.3 Renal Cell Carcinoma

The occurrence of RCC is a rare but well-known complication of TS that occurs in 2 to 3% of patients (Lendvay and Marshall 2003; Crino et al. 2006). However, some of the previously reported RCC in TS were later diagnosed as EAML, suggesting that the incidence of TS-associated RCC is lower than some have suggested (Corsenca et al. 2007).

The overall incidence of RCC in patients with TS is similar to that in the general population; however, the cancer is diagnosed at a younger age in patients with TS. In one study, the average age in TS-associated RCC was 28 years, compared to 55 years for sporadic RCC (Bjornsson et al. 1996). The tumors may be single or multiple and they are more frequently bilateral than sporadic RCC (Bjornsson et al. 1996; Robertson et al. 1996). As it

was already reported, RCC is negative for melanoma markers, like the HMB-45 antibody (Fig. 3C). An unusual feature of RCC associated with TS is its pathological heterogeneity. Clear-cell, papillary and chromophobe carcinoma subtypes (Fig. 3B), as well as oncocytomas, have all been reported in patients with TS (Bjornsson et al. 1996; Crino et al. 2006). According to the literature, in sporadic cases as well as in the setting of TS, the most common renal is clear-cell RCC (Corsenca et al. 2007). In one series, four of six patients died from metastatic disease of RCC (Bjornsson et al. 1996). However, the impact of RCC on mortality for patients with TS is uncertain, leading to the requirement of new long-term prospective studies.

8.2 Neurologic Manifestations

When present, neurologic complications are the most common causes of morbidity and the most likely to affect the quality of life. Furthermore, they represent the main cause of death in TS disease (Shepherd et al. 1991).

The major cerebral lesions are cortical tubers, subependymal nodules, subependymal giant-cell astrocytomas and white matter abnormalities (Roach and Sparagana 2004).

Cortical tubers constitute the hallmark of the disease and are pathognomonic of cerebral TS (Curatolo et al. 2002). More than 80% of patients with TS have cortical tubers (Lendvay and Marshall 2003), which are characterized by proliferation of glial and neuronal cells, and loss of the six-layered structure of the cortex.

Subependymal nodules are hamartomas typically seen in the subependymal wall of the lateral ventricles (Roach and Sparagana 2004). They are present in most TS patients, mostly developing during fetal life (Curatolo et al. 2008). They are usually asymptomatic and remain dormant throughout life, but they have the potential to increase in size and protrude into the ventricular lumen and to be transformed gradually into a subependymal giant-cell

astrocytoma (Curatolo et al. 2002). Subependymal giant-cell astrocytomas are the most common brain tumors in patients with TS, occurring in 6 to 15% of patients, with the peak incidence in later childhood and adolescence (Curatolo et al. 2002; Roach and Sparagana 2004; Krueger and Franz 2008). They are slow growing tumors that, in approximately 10% of patients with TS, may cause obstruction of cerebrospinal fluid flow, hydrocephalus, increased intracranial pressure and even death (Crino et al. 2006).

Cerebral anatomic distortion creates an epileptogenic environment. In TS, seizures represent the most common symptom and the most common medical problem (Napolioni et al. 2009), occurring in 80 to 98% of patients with TS with their seizure onset during the first year of life (Napolioni et al. 2009). The most common types of seizures are infantile spasms, partial motor seizures and generalized tonic clonic seizures (Curatolo et al. 2002; Krueger and Franz 2008). In the majority of cases, cortical tubers are believed to be the epileptogenic foci. The number and location of cortical tubers appear to have some relationship to the severity of the neurologic manifestations and more difficulty with seizure control (Roach and Sparagana 2004). About 50% of patients with TS have some degree of cognitive impairment (Curatolo et al. 2002; Napolioni et al. 2009). Almost all mentally retarded children with TS have seizures (Roach and Sparagana 2004). The rate of mental impairment in individuals with TS who also have infantile spasms is widely reported to be high but there are studies who demonstrated a range of normal intellectual development of 36% (Goh et al. 2005). So, this highlights that other many variables play role in determining ability level (Joinson et al. 2003).

TS has a striking variability of neurocognitive manifestations and psychopathological features. In the same family, some individuals can be impaired and have severe autism and challenging behaviors, whereas others lead normal lives. Behavior problems affect 40% of patients with TS (Krueger and Franz 2008), of which autism, attention deficit, hyperactivity and sleep problems are the most frequent (Napolioni et al. 2009).

8.3 Pulmonary Manifestations

The classic pulmonary lesion of TS is lymphangioleiomyomatosis (LAM), which is characterized by the proliferation of abnormal smooth muscle cells and cystic degeneration of the lung (Vicente et al. 2004). LAM almost exclusively affects women, usually during their reproductive years (Costello et al. 2000; Hancock et al. 2002). At least 1% of patients with TS develop symptomatic pulmonary dysfunction and many others probably have asymptomatic lung lesions on diagnostic studies later in life(Roach and Sparagana 2004). Computed tomography (CT) scanning of the chest of patients with TS but no respiratory symptoms revealed a frequency of LAM up to 40% (Costello et al. 2000; Moss et al. 2001; Krueger and Franz 2008). Clinical, physiologic, radiologic and histopathological features of LAM in patients with TS are nearly identical to those seen in patients with sporadic LAM (Costello et al. 2000), probably even milder (Krueger and Franz 2008). Typical manifestations are pneumothorax, dyspnea, cough and hemoptysis (Hancock et al. 2002; Krueger and Franz 2008). It can lead to cyanosis, respiratory failure and cor pulmonale (Hancock et al. 2002). LAM is usually generalized and progressive, extremely difficult to treat, with a poor prognosis (Curatolo et al. 2008).

Mutations at both TSC gene loci have been detected. However, most TS-associated cases of LAM are caused by mutations in TSC2 gene (Curatolo et al. 2008). A loss of heterozigosity has been shown to promote lesion development (Lendvay and Marshall 2003).

Molecular genetic studies have shown that LAM cells share identical somatic TSC2 mutations and loss of heterozigosity pattern with AMLs cells from the same patient (Karbownicsek et al. 2003). This clonal origin of multisystem disease suggests a metastatic spread of AMLs cells in the etiology of LAM.

8.4 Dermatologic Manifestations

Several types of skin lesion can occur in TS. Hypomelanotic macules are one of the most common, occurring in about 90% of TS patients (Roach and Sparagana 2004), and usually represent its earliest visible sign, since they are usually present at birth (Roach and Sparagana 2004; Schwartz et al. 2007). They are distributed especially over the trunk and limbs but are often difficult to see in the newborn without an ultraviolet light. Although they are found in most of patients with TS, they are not pathognomonic for this disease. The presence of fewer than three hypomelanotic macules on asymptomatic individual without a familiar history of TS does not imply an extensive evaluation to rule it out. Confetti-like lesions are white spots typically distributed over the extremities (Schwartz et al. 2007). Facial angiofibromas are seen during the preschool years as the form of multiple flesh colored or red papules (Roach and Sparagana 2004). As these lesions are not related to sebaceous glands, the traditional term "adenoma sebaceum" is inappropriate (Yates 2006). Shagreen patches are connective tissue naevi located on the lombosacral area with a yellowish red or pink color and a rough texture resembling an orange peel (Schwartz et al. 2007). They are rare during infancy and tend to increase in size and number with age. Fibrous plaques can develop in the forehead. Ungueal fibromas are nodular or fleshy lesions that arise adjacent to or underneath the nails (Roach and Sparagana 2004). Gengival fibromas and dental pitting can also be found.

8.5 Ocular Manifestations

Retinal hamartomas are present in about 40-50% of TS patients (Curatolo et al. 2008). Unless these lesions affect the macula or optic nerve, they are typically asymptomatic (Schwartz et al. 2007). Sometimes, an achromic patch is seen on the retina, similar to the hypopigmented macules on the skin. They seldom affect vision but are a useful diagnostic sign (Yates 2006).

8.6 Cardiac Manifestations

Cardiac rhabdomyomas are intracavitary or intramural tumors that are present 50 to 70% (Crino et al. 2006) of infants with TS but few of these lesions are clinically important (Roach and Sparagana 2004). They might be the earliest diagnostic finding in some patients with TS, detected on prenatal ultrasonography. They most commonly remain asymptomatic and unlike other lesions seen in TS, cardiac rhabdomyomas often disappear spontaneously in later life (Schwartz et al. 2007). Nonetheless, they can manifest prenatally as arrhythmia, non-immune hydrops or death (Curatolo et al. 2008). Heart failure might occur soon after birth, usually owing to obstruction of blood flow by an intraluminal tumor (Roach and Sparagana 2004).

8.7 Other Manifestations

Hamartomas also occur in the liver, spleen and other organs, although evidently less often than in the kidney. Liver hamartomas occur in about 25% of TS patients (Lendvay and Marshall 2003; Lenci et al. 2008), being more frequent in adults and in women (Lenci et al. 2008). Hamartomatous polyps in the gastrointestinal tract, especially in the rectum, are common but asymptomatic (Józwiak et al. 2000). Symptomatic bone disease is rare but bone cysts in the phalanges of hands and feet, sclerotic lesions and periosteal new bone formation can be found (Curatolo et al. 2002; Lendvay and Marshall 2003).

9. DIAGNOSTIC APPROACH

9.1 Clinical Diagnosis

In 1998, a panel of international experts revised the diagnostic criteria for TS at the Tuberous Sclerosis Complex Consensus Conference in Annapolis (Roach et al. 1998) (Table III). The aim of this conference was the re-evaluation of the clinical diagnostic criteria of TS

given that some clinical features, once regarded as pathognomonic, were found as isolated findings in individuals with no other clinical or genetic evidence of TS and, thereby, were considered less specific for TS. Accordingly, the clinical and radiographic features were simplified into two main categories, major and minor, based on the diagnostic importance and the degree of specificity for TS of each feature. The definite diagnosis of TS is established if two major features or one major plus two minor features are demonstrated.

Because of its striking variability of clinical expression and severity, the diagnosis of TS can be difficult. The fact that many findings, traditionally considered as the most specific for TS, become apparent in late childhood or adulthood limits their usefulness for early diagnosis (Józwiak et al. 2000). For these reasons, the diagnosis of TS can be challenging.

9.2 Molecular Diagnosis

In the majority of patients, a good clinical work-up will be sufficient to make a definite diagnosis of TS. However, molecular genetic testing of the TSC1 and TSC2 genes is currently viewed as corroborative and is potentially useful in several settings (Curatolo et al. 2008). First, it can be helpful in confirming a suspicious case of TS, especially in young patients in whom many clinical features have not developed yet. Second, in the non-familial cases of TS, genetic testing can provide reassurance to parents and other family members that they do not have a mutation. Nevertheless, as it was already reported, about 2% unaffected parents might have germline mosaicism, bearing the risk of the next child with TS. Third, DNA testing is useful for prenatal diagnosis and can be performed in families with either a child or a parent with a known mutation.

Table III: Revised Diagnostic Criteria for Tuberous Sclerosis Complex.
Adapted from Roach et al. (1998)

Major Features	Minor Features
Facial angiofibromas or forehead plaque	Multiple randomly distributed pits in dental enamel
Nontraumatic ungual or periungual fibroma	Hamartomatous rectal polyps[c]
Hypomelanotic macules (three or more)	Bone cysts
Shagreen patch	Cerebral white matter radial migration lines[a, d]
Multiple retinal nodular hamartomas	Gingival fibromas
Cortical tuber[a]	Nonrenal hamartoma
Subependymal nodule	Retinal achromic patch
Subependymal giant cell astrocytoma	Confetti skin lesions
Cardiac rhabdomyoma, single or multiple	Multiple renal cysts
Lymphangiomyomatosis[b]	
Renal angiomyolipoma[b]	

Definite Tuberous Sclerosis:
Either two major features or one major feature plus two minor features

Probable Tuberous Sclerosis:
One major plus one minor feature

Possible Tuberous Sclerosis:
Either one major feature or two or more minor features

[a] *When cerebral cortical dysplasia and cerebral white matter migration tracts occur together, they should be counted as one rather than two features of tuberous sclerosis*
[b] *When both lymphangiomyomatosis and renal angiomyolipomas are present, other features of tuberous sclerosis should be present before a definite diagnosis is assigned.*
[c] *Histologic confirmation is suggested*
[d] *Radiographic confirmation is sufficient*

10. CURRENT MANAGEMENT

The management of TS is divided in two important stages: the first represents the time of the diagnosis, in which complementary studies are performed to clarify the cause of symptoms, to confirm the presence of the disease and to survey its extension; the second stage is characterized by the follow-up of TS patients and their surveillance testing towards lesions that are frequent, on grounds that if identified early, they can be treated, obviating possible

dysfunction or even death (Table IV). Patients with TS should be evaluated and managed with a multidisciplinary approach, involving dermatologists, neurologists, nephrologists, urologists, pediatricians and geneticists.

The management of TS patients begins with the appropriate diagnosis by identifying major and minor features.

For initial evaluation, a careful skin examination, including use of Woood´s lamp, of individuals at risk for TS is essential because many of the major features are cutaneous, and these lesions often herald the diagnosis (Leung and Robson 2007; Schwartz et al. 2007).

Prenatal ultrasonography may reveal cardiac failure and show rhabdomyomas in most of newborns with TS. Clinical examination of the newborn can lead to the suspicion of cardiac abnormalities and, therefore, investigations must be done. Echocardiography and electrocardiogram (ECG) must be performed at the time of diagnosis (Yates 2006).

When TS is suspected, brain magnetic resonance imaging (MRI) or CT should be done to identify cortical tubers or subependymal giant-cell tumors (Curatolo et al. 2002). Electroencephalogram (EEG) is useful when the initial presentation includes seizures to define background cerebral activity, characterized patterns and identify epilepsy foci (Crino et al. 2006). Children or adolescents who have never had seizures generally do not need an EEG.

Renal ultrasonography should be performed at the time of diagnosis to identify AMLs and the possible coexistence of ADPKD, which is associated with a poor prognosis. An initial normal ultrasound does not rule out their future development.

Fundoscopic examination to identify retinal hamartomas is necessary at the time of diagnosis (Yates 2006). Eye examination can help establish the diagnosis.

The second important stage in the management of TS is the long-term follow-up, including the monitoring of lesion growth and the recognition of new ones, to perform the earliest and the most effective treatment.

It has been recommended the renal ultrasound scanning at 1-3 yearly intervals, depending on the level of concern, to monitor the development, progression or changes in appearance of renal AMLs, particularly in a perspective of elective treatment of larger AMLs (Yates 2006; Krueger and Franz 2008). Renal CT or MRI might be needed to detect complications, such as bleeding and rupture in large lesions (Curatolo et al. 2008). A persistent problem in the diagnosis of kidney masses in TS is the difficulty in distinguishing fat-poor AML from RCC on imaging. Biopsy is often discouraged, as it can lead highly vascular AMLs to hemorrhage and can scatter malignant carcinoma cells (Rakowski et al. 2006). The decision to progress surveillance or to start intervention depends on the clinician. Clear guidelines for screening and follow-up of AMLs in patients with TS are needed. These guidelines should include the appropriate frequency of surveillance for patients in different age groups and at different stages of AML development. Patients should be routinely screened and treated for hypertension, which can aggravate underlying renal dysfunction (Krueger and Franz 2008). Renal function tests must be performed in the cases of ADPKD or in adults with extensive renal involvement.

Annual MRI or CT of the brain is suggested in patients until they are at least 21 years old, and then imaging studies should be performed every 2 to 3 years both to diagnose and to monitor subependymal giant-cell astrocytomas (Crino et al. 2006). Careful clinical surveillance during childhood and adolescence, and close monitoring with MRI of the brain in the presence of changing clinical symptoms or rapidly growing lesions, are strongly recommended to facilitate early intervention against subependymal giant-cell astrocytomas (Curatolo et al. 2008).

Children diagnosed with TS need age-appropriate screening for developmental and behavioral impairments including autism and attention deficit hyperactivity disorder

(Curatolo et al. 2002). Those affected need retesting as part of their ongoing management, particularly at preschool age to determine their educational needs and at times of transition.

It has been recommended that asymptomatic adult women with TS should be undergo a one-off chest CT, on the grounds that early diagnosis might allow symptomatic treatment to be started early (Costello et al. 2000). No respiratory investigations are indicated in asymptomatic children or adult men. The gold standard to diagnose LAM is lung biopsy demonstrating abnormal infiltration of smooth muscle cells, although the presence of characteristic CT findings, especially in patients with known TS or AML is felt to be sufficient for a clinical diagnosis, thereby obviating the need for biopsy in most cases (Krueger and Franz 2008). Chest CT reveals a striking picture of diffuse cystic changes throughout the lung parenchyma (Costello et al. 2000). It has been recommended that women with symptomatic LAM might be undergo to annual chest CT and pulmonary function testing (Yates 2006). The later shows an obstructive more often than a restrictive pattern (Hancock et al. 2002) and may provide a measure of disease progression.

As cardiac rhabdomyomas tend to regress and disappear with age, it is not performed cardiac imaging in periodic intervals. However, cardiac symptoms, like heart failure, must be routinely discarded and if present, further investigations must be done. Before any surgery, ECG is recommended (Yates 2006).

Periodic dermatologic examination is useful, since facial angiofibromas can cause cosmetic disfiguration (Schwartz et al. 2007).

TS rarely affects vision and routine follow-up ophthalmologic evaluation is not necessary (Yates 2006). Nonetheless, the requirement of reevaluation must be guided by the symptoms progression.

Table IV: Testing recommendations for TS patients

Organ system or clinical problem	Evaluation	Initial testing	Repeated testing
Heart	ECG Echocardiography	At diagnosis	As clinically indicated
Eye	Fundoscopy	At diagnosis	As clinically indicated
Skin	Inspection including Wood´s lamp	At diagnosis	As clinically indicated
Intracranial lesions	Brain MRI or CT	At diagnosis	Until young adults: every year After: 2-3 yearly
Epilepsy	EEG	Evaluation of seizures	As clinically indicated
Development and behavior	Neurodevelopmental testing	At diagnosis	Pre-school age Times of transition
Kidneys	Renal US Renal function tests	At diagnosis	Every 1-3 yearly Concomitant ADPKD Adults with extensive renal involvement
Lungs	Chest CT Lung function tests	Women with asymptomatic LAM, one-off investigation	Women with symptomatic LAM, 6-12 monthly

Abbreviations: ECG: electrocardiogram; MRI: magnetic resonance imaging; EEG: electroencephalogram; US: ultrasonography; CT: computed tomography; LAM, lymphangioleiomyomatosis.

11. TREATMENT OF RENAL MANIFESTATIONS

The life expectancy of TS patients has increased largely due to improved treatments of their neurological lesions. Preservation of renal function has now become an increasing focus of attention. Current treatment options for renal lesions in TS patients include observation and invasive strategies, embolization or surgery, since no pharmacological treatment options are yet available.

11.1 Current treatment strategies

Some prospective studies commenting on sporadic, solitary AMLs have based the criteria for prophylactic treatment on AML tumor size, typically 4 cm (Osterling et al. 1986; Steiner et al. 1993), with the thought that AMLs larger than this size will become symptomatic.

However, the criteria for AMLs in TS has not yet been defined. AMLs in patients with TS should have a different management algorithm since tumors are generally multifocal, bilateral and grow synchronously.

Further studies based on TS-associated AMLs concluded that all tumors larger than 4 cm do not necessarily require intervention and that they can be monitored via imaging due to the slow-growing nature of AMLs (Harabayashi et al. 2004; Hadley et al. 2006). They proposed that the treatment of AMLs in TS patients should be considered in selected, closely followed patients until symptoms (pain refractory to medical management and/or bleeding requiring a transfusion) develop or if there is a strong suspicion of malignancy on imaging studies. Preemptive invasive treatment may unnecessary expose these patients to complications with an unquantified benefit. On contrary, one prospective study based on TS patients proposed that arterial embolization should be performed in all symptomatic AMLs or when they reach the threshold size of 4 cm (Ewalt et al. 2005).

If more aggressive treatment is necessary, arterial embolization is a conservative treatment that should be considered first (Harabayashi et al. 2004; Ewalt et al. 2005; Williams et al. 2005; Hadley et al. 2006). The goal of arterial embolization is complete ablation of blood flow to the AML (Fig. 4) (Lee et al. 2005b). However, like any procedure, embolization is not completely benign and can negatively affect the surrounding parenchyma. In addition, patients may complicate with fat embolization (Hadley et al. 2006) and post-embolization syndrome which is characterized by abdominal or flank pain, nausea, vomiting and fever as a consequence of amount of intra-abdominal necrotic tissue (Osterling et al. 1986; Ewalt et al. 2005). The repeatability of this intervention is an advantage.

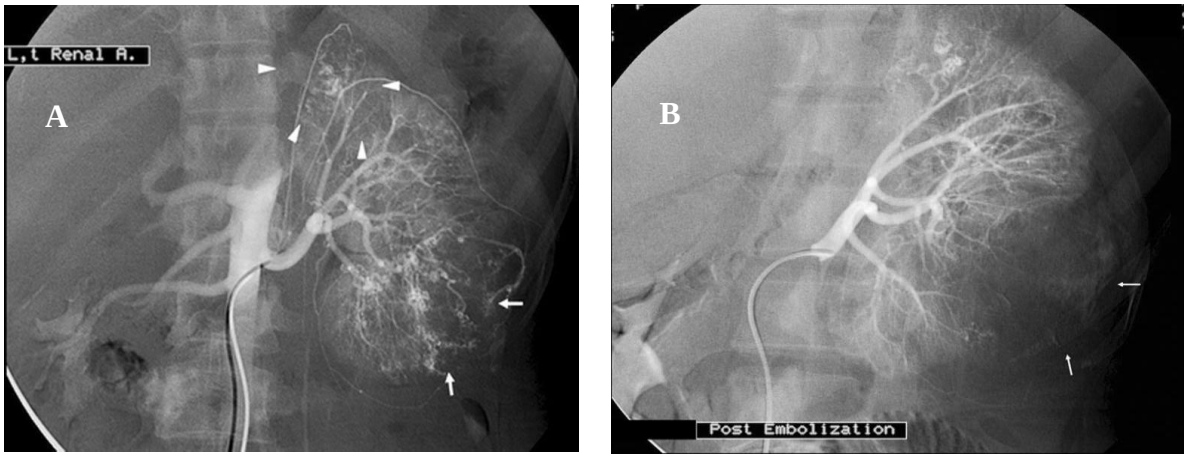

Figure 4: Arterial embolization of renal AML in a patient with TS
A. Pre-embolization angiography shows marked hypervascularity and aneurysmal dilatation of the AML (arrows) located in the lower pole of the left kidney. There are several smaller as at the upper pole (arrowheads). **B.** Left renal arterial angiography after selective embolization of the lower polar angiomyolipoma shows markedly diminished vascularity of this angiomyolipoma (arrows). Abbreviations: AML: angiomyolipoma; TS: tuberous sclerosis. *Adapted from Lee et al. (2005b).*

Partial nephrectomy is also a treatment option though, in order to preserve best residual renal function, it should be limited to selected situations such as severe acute hemorrhage of Wunderlich syndrome, uncontrolled hypertension resulting from a total non-functioning kidney, detrimental bulk effect, polar location which makes partial nephrectomy more technically desirable and a very strong evidence of malignancy (Hadley et al. 2006; Krueger and Franz 2008; Granata et al. 2009). Potential disadvantages of partial nephrectomy include loss of remaining functioning parenchyma if total nephrectomy has to be performed due to complications during the surgery, more difficulty in a subsequent renal surgery comparing to arterial embolization and regrowth of the remaining lesions which may replace the excised lesion (Hadley et al. 2006; Krueger and Franz 2008). Undertaking more conservative forms of management might delay and prevent renal failure (Clarke et al. 1999; Dallos et al. 2006). End-stage renal failure requires dialysis, which is controversy in some patients given their unsuitability for these procedures (Nimr et al. 1987). Renal transplantation may be a good option for these patients regarding life quality and should be considered based on the patient's general condition and the prognosis of the original disease (Dallos et al. 2006). Some authors

recommended binephrectomy after starting dialysis and before transplantation, given the risk of cancer or bleeding related to AMLs (Schilinger and Montagnac 1996).

11.2 New therapeutics: possible use of sirolimus in tuberous sclerosis

The explosion of molecular-genetic discoveries and elucidation of signaling pathways involved in cell growth lead to the hypothesis that if normal hamartin/tuberin complex tend to reduce mTOR activity then the pharmacologic mTOR inhibitor sirolimus is the logical candidate to control manifestations of TS. With this purpose in mind, several studies have been planned and performed to evaluate the efficacy of sirolimus in controlling progression of renal lesions in TS disease.

Sirolimus (or rapamycin) is a Food and Drug Administration (FDA)-approved for immunosuppression following kidney transplantation (Józwiak et al. 2006). Sirolimus and its analogs [temsirolimus (CCI-779, cellcycle inhibitor-779, rapamycin-42,2,2-bis(hydroxymethyl)-propionic acid), everolimus (SDZ RAD, RAD001, 40-O-(2-hydroxyethyl)-rapamycin) and AP23573] are under investigation in many ongoing studies about therapy of cancers of the breast, prostate, lung and liver (Kenerson et al. 2002; Józwiak et al. 2006; Sampson 2009). Tensirolimus and everolimus are FDA-approved agents for the treatment of advanced RCC (Józwiak et al. 2006).

11.2.1 Animal models

Sirolimus and its analogs have been successfully used to treat TS-associated renal lesions in mouse models and sirolimus is currently being evaluated for its safety and efficacy in treating TS-related lesions in human populations.

Preclinical studies have demonstrated that treatment with sirolimus is effective in reducing the volume of renal tumors and, besides that, it might effectively inhibit tumor

formation (Kenerson et al. 2002; Kenerson et al. 2005). Several studies have demonstrated that sirolimus seems to be more effective than its analog tensirolimus in tumor reduction and improving survival (Messina et al. 2007; Lee et al. 2009).

In recent clinical studies, sirolimus treatment has caused TS-related tumor regression. However, this tumor regression has been incomplete and responses are not durable (Bissler et al. 2008; Davies et al. 2008). Identification of the facts that compromise the therapeutic potential of mTOR inhibitors, and therefore induce drug resistance, is thus critical for the improvement of targeted TS therapies. Multiple feedback loops have been identified in the signaling networks centered on the hamartin/tuberin complex and this suggests that targeting multiple signaling pathways may be a useful strategy for the treatment of TS. As it was already reported, high-expressing allele of IFN-γ is associated with lower frequency of renal AMLs. Thus, it has been postulated that higher concentrations of IFN-γ may partially inhibit tumor proliferation in vivo (Dabora et al. 2002). With this purpose in mind, further studies have been developed to investigate the therapeutic potential of IFN-γ when used as a single agent and in combined therapies with sirolimus or its analogs. It has been concluded that IFN-γ can reduce the severity of AMLs in TS mouse models (El-Hashemite et al. 2004; Lee et al. 2005a; Lee et al. 2009) but there are conflicting results regarding whether treatment with an mTOR inhibitor plus and IFN-γ is more effective in reducing tumor growth than an mTOR inhibitor used as a single agent (Lee et al. 2006; Messina et al. 2007; Lee et al. 2009).

It has had significant interest in identifying novel therapeutic agents to be used either as single agents or in combination with sirolimus and results are being auspicious (Finlay et al. 2009; Lee et al. 2009; Mi et al. 2009; Pollizzi et al. 2009). Preclinical studies are therefore warranted to define the benefits and risks of the application of such new therapies in TS animal models.

11.2.2 Clinical trials

Although the mouse tumors are histopathologically distinct from AMLs in humans, molecular and immunohistochemical analyses point to similar mechanisms of tumorigenesis, thus further validating the translation of preclinical findings to clinical trials in humans.

Two case-reports represent the first clinical evidences that sirolimus shows activity against renal tumors in patients with TS, given that after the administration of this drug, it was observed a significant shrinkage of renal AMLs (Wienecke et al. 2006; Herry et al. 2007) (Fig. 5).

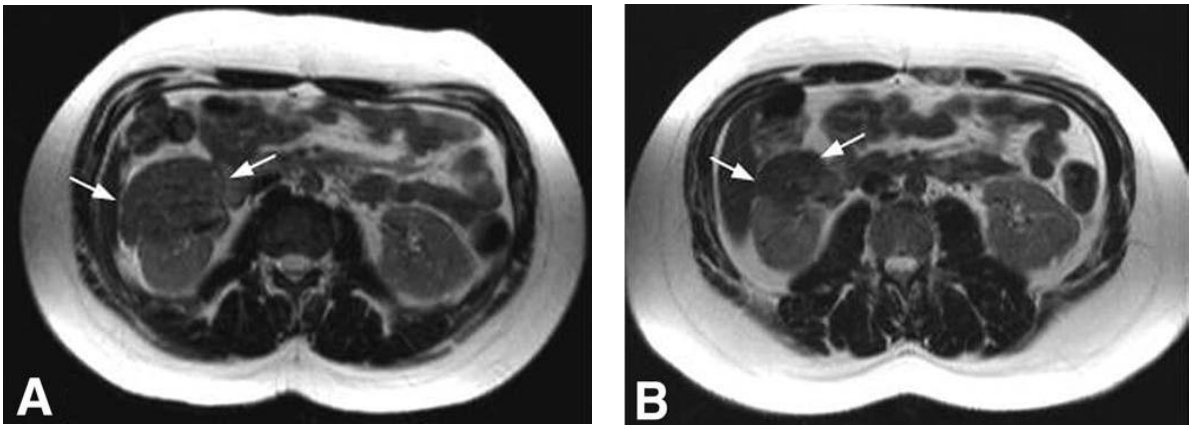

Figure 5: Antitumoral activity of sirolimus in renal AML
A. Pretrial abdominal MRI: large hypointense tumor mass representing a renal AML (arrows). **B.** Follow-up MRI (4 months after the treatment with sirolimus): relevant decrease in tumor size. Abbreviations: AML: angiomyolipoma; MRI: magnetic resonance imaging. *Adapted from Wienecke et al. (2006)*.

A recent study developed by Bissler et al. (2008) consisted of one year on active treatment with sirolimus and one year of observation off study medication. Using doses below or approximated to standard immunosuppressant regimen, AMLs consistently demonstrated reductions in size ranging from 20 to 80%. Once sirolimus was discontinued, while in some patients AMLs returned to baseline, a subset of patients sustained reduction in volume even after one year off therapy. It has been considered that a 30% reduction in the largest diameter of a neoplasm is considered evidence of therapeutic success (Paul and Thiele 2008). However, how this is significant in reducing the hemorrhagic complications from renal AML remains unclear.

The authors of an ongoing prospective study have reported interim data about the efficacy and safety of sirolimus in adults with TS and they observed a reduction in the longest diameter of 10% or more of AMLs in all patients (Davies et al. 2008).

In both of these studies, side effects (mostly aphthous ulcers, diarrhea and upper respiratory infectios) of sirolimus therapy were common and required variable periods off therapy for most patients. However, all side effects were predictable (being the same as those already observed in non-TS transplant patients) and they were mostly low-grade and self-limiting.

There are further ongoing clinical trials for mTOR inhibitors for AMLs in TS patients that have not yet been reported.

12. CONCLUSIONS

The work summarized in this review should help to identify individuals who have TS, to illuminate the recent challenges in its management and to understand the spectrum of renal manifestations, emphasizing the screening of individuals at risk and referral for genetic consultation. This overview places special attention on current treatment options and highlights the promising preclinical and clinical trials with the sirolimus, sustaining the exciting prospect of drug therapy for renal lesions.

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