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*‘The management of drug resistant seizures
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REVIEW ARTICLE

The management of drug resistant seizures in tuberous sclerosis

Paolo Curatolo¹, Roberta Bombardieri¹, Mariangela Pinci¹, Romina Moavero¹

Abstract: Tuberous Sclerosis Complex (TSC) is a multisystem autosomal dominant genetic disorder resulting from mutations in one of two genes, TSC1 and TSC2. Pathologically TSC is characterized by abnormal cellular differentiation and proliferation, as well as abnormal neuronal migration. The majority of patients with TSC have epilepsy, although the mechanisms underlying epileptogenesis remain unknown. Seizures onset is frequently during the first year of life, and in a sizable proportion of individuals tend to be refractory to antiepileptic drug treatment. This article reviews the progress in understanding drug resistant seizures in TSC, from molecular pathogenesis to the pathophysiological mechanisms of epileptogenesis, and the rationale for appropriate medical and surgical treatment.

Key words: tuberous sclerosis, drug resistant seizures, epilepsy

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Introduction

Tuberous sclerosis complex (TSC) is a dominantly inherited disease of high penetrance, pathologically characterized by the presence of tumour-like lesions (hamartomas) in multiple organ system, caused by mutations in one of the tumor suppressor genes, TSC1 or TSC2, encoding for hamartin and tuberin respectively. The most common neurological manifestations of TSC are epilepsy, mental retardation, challenging behavioural problems, and autism [1,2].

Progress in structural and functional imaging has led to further characterization of the brain lesions, including cortical tubers (CTs), subependymal nodules (SENs), subependymal giant cell tumors (SGCTs), and white matter abnormalities [3,4].

The TSC1/TSC2 complex plays an important role during cortical development and growth control. At distinct stages of central nervous system (CNS) development, including morphogenesis, cell adhesion/migration and cell fate determination, a precise interaction

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of tuberin and hamartin appears critical. Gene mutations in either of the two genes influence neural precursor between weeks 7 and 20 of gestation to result in disrupted cell division, abnormal cell differentiation, dysregulated cell size control and abnormal cellular migration [5]. Fundamental advances in defining the molecular function of hamartin and tuberin have been recently made. These two proteins normally work together to inhibit

the mammalian target of rapamycin (mTOR) and a cascade of other downstream kinases and translational factors that stimulate protein translation, cell growth and proliferation [6]. Thus, mutation in hamartin or tuberin in TSC leads to hyperactivation of the downstream mTOR pathway and the associated kinase signalling cascades and translational factors, resulting in increased cell growth and proliferation. In this article we review the mechanisms underlying epileptogenesis in TSC, from molecular genetics to the pathophysiological mechanisms of epileptogenicity in drug resistant seizures associated with TSC.

Epilepsy in TSC

Epilepsy in TSC often begins during the first year of life and, in most cases, in the very first months. The high incidence of epileptic spasms and hypsarrhythmia has long been emphasized, but it is now clear that infants with TSC are clinically and electroencephalographically different from classical epileptic spasms and hypsarrhythmia of West syndrome [7]. In the same child focal seizures may precede, coexist with, or evolve into epileptic spasms. Subtle focal seizures, such as unilateral tonic or clonic phenomena mainly localized in the face or limbs, and other seizures with subtle lateralizing features, such as tonic eye deviation, head turning, and unilateral grimacing can occur, but may be missed by the parents until the third or fourth month of life when epileptic spasms occur. The EEG at onset usually shows focal or multifocal spike discharges and irregular focal slow activity. Video-EEG monitoring and polygraphic recordings of the epileptic spasms have shown that each spasm consists of a combination of both focal and bilateral manifestations. Although the pathophysiological mechanisms responsible for the coexistence of spasms and focal motor seizures are still uncertain, epileptic spasms associated with TSC may show focal features

at onset, followed by a rapid secondary generalization. The age at seizure onset and the age when epileptiform activity becomes apparent on the EEG is largely dependent on the location of the cortical tubers detected by MRI and may coincide with functional maturation of the cortex, with an earlier expression for temporo-occipital regions than for frontal ones [7].

A number of young children with TSC, who present with focal seizures or epileptic spasms at onset, later develop intractable seizures with multifocal EEG abnormalities (Fig. 1) associated with bilateral and more synchronous slow spike-wave complexes and an electroclinical pattern that resembles a Lennox-Gastaut Syndrome (LGS). In patients with TSC, the differential diagnosis between LGS and localization related symptomatic epilepsy originating in the frontal lobe may be extremely difficult and only in few cases long-term video-EEG monitoring can reveal subtle electroclinical manifestations suggestive of a focal seizure onset. In these patients, high time-resolution topographic EEG analysis and dipole localization methods may detect secondary bilateral synchrony (SBS), often originating in frontal regions and corresponding to prominent cortical tubers detected by MRI in the mesial surface of the frontal or the anterior temporal lobes [8].

The natural history of epilepsy in patients with TSC from infancy into childhood tends to be one of increasing seizure frequency and severity, with poor response to antiepileptic drug (AED) treatment. The proportion of children with TSC and epilepsy referred to tuberous sclerosis clinics who achieve prolonged seizure remission is small. Usually seizure remission is associated with mild neurological deficits, and sustained remission is more likely associated with normal intelligence, a greater likelihood of having a normal finding on electroencephalogram



Figure 1. Multifocal electroencephalographic abnormalities with a left fronto-centro-temporal prevalence in a 8 years old boy with intractable epilepsy

at the time of discontinuation, and fewer cortical and subcortical tubers on neuroimaging [9]. Unfavourable prognostic factors include onset earlier than one year of age, presence of multiple seizure types (spasms and focal motor or complex partial seizures, drop attacks and atypical absences), multifocal discharges in the awake state which tend to bilateralise in sleep and/or SBS, and occurrence of new EEG foci during the evolution [10].

Molecular pathogenesis

Present evidence suggests that the CNS lesions of TSC are due to a developmental disorder of neurogenesis and neuronal migration. In TSC two populations of neuroepithelial cells are generated by the germinal matrix. The first consists of normal neuroblasts that form normal neurons and astroglia, which migrate to the cortical plate to form histologically normal cerebral cortex.

The second is an abnormal cell population that forms primitive cells, which often fail to

show clear neuronal and glial differentiation. Some of these cells, named "neuroastrocytes", remain in the germinal matrix zone where they form subependymal nodules and giant cell tumours.

Some neuroastrocytes show partial migration, forming heterotopias in the subcortical white matter. More differentiated cells migrate to the cortical plate where they form aggregates of dysplastic cortex, the cortical tubers. Histologically tubers are characterized by disorganized cortical lamination and by the presence of cells with aberrant morphology. The most prominent abnormal cell types in tubers are large dysplastic neurons and giant cells, as well as bizarrely shaped astrocytes [11]. Tubers are found most commonly in the fronto-parietal cortical regions [12], but in many patients they involve all the brain (Fig. 2). In a report on two cases a disseminated distribution of atypical cells throughout the white matter and cortex of the telencephalon has been found [13]. MRI studies provide excellent in vivo demonstration of the various

pathological lesions. An especially interesting imaging finding is the frequent presence of abnormal wedges of tissue extending from the subependymal zone to the cerebral cortex, and including radial hypomyelinated tracts.

TSC is due to inactivating mutations in either of two genes, *TSC1* (on chromosome 9q34) encoding hamartin, or *TSC2* (on chromosome 16p13.3) encoding tuberin. 393 *TSC1* and 1118 *TSC2* unique allelic variants have been reported (http://chromium.liacs.nl/lovd/index.php?select_db=TSC1 or [_db=TSC2](http://chromium.liacs.nl/lovd/index.php?select_db=TSC2)) [14-18]. These mutations comprise the usual mix of nonsense, missense, insertion and deletion mutations, involving nearly all of the exons of *TSC1* and *TSC2*.

New mutations have been implicated in up to 75% of all cases. Mutational studies of TSC patients have demonstrated that mutations in *TSC2* are about five times more common than mutations in *TSC1* in the sporadic TSC population, whereas the ratio is 1:1 in large families with multiple generations affected. Due to the large number of mutations, phenotype-genotype correlations are complex [2]. *TSC1* disease is milder than *TSC2* disease in multiple respects, which appears to be due to a reduced rate of second hit events [15,17,19].

Fundamental advances in defining the molecular function of hamartin and tuberin have been recently made. Hamartin and tuberin normally work together to inhibit the phosphatidylinositol 3-kinase (PI3K)/insulin activated signaling pathway, involving the mammalian target of rapamycin (mTOR) and a cascade of other downstream kinases and translational factors that stimulate protein translation, cell growth and proliferation [6]. Thus, mutation of hamartin or tuberin in TSC leads to hyperactivation of the downstream mTOR pathway and the associated kinase signaling cascades and translational factors,

resulting in increased cell growth and proliferation.

Hamartin and tuberin form together a GTPase activating protein (GAP) complex that inhibits rheb, the GTPase that activates the mTOR [20,21]. Mutations to either *TSC1* or *TSC2* disrupt the function of the complex, explaining why mutations to either gene cause the same disease.

In the brain, *TSC1* and *TSC2* mRNA and protein have been detected in cerebral cortex, hippocampus, cerebellum, brainstem, choroid plexus epithelium, and spinal cord of the immature and mature brain [22]. Mutations of TSC genes, via downstream effects on neuronal and synaptic structures or neurotransmission, have the potential to induce fundamental alterations in network properties, as well as an imbalance in excitation and inhibition, producing epilepsy, mental retardation, and autism.

Several independent studies of large cohorts of TSC patients have demonstrated that patients with a *TSC2* mutation are more often mentally retarded than patients with a *TSC1* mutation [14,15,17,19]. However, in most of these studies, cognitive ability was estimated clinically, or through indirect methods, such as level of schooling [23], rather than with standardized measures, such as intelligence scales.

Patients with a *TSC2* mutation as a group had earlier age at seizure onset, lower cognition index, more tubers, and a greater tuber brain proportion (the proportion of the total brain volume occupied by tubers) than those with a *TSC1* mutation, but the ranges overlapped considerably; familial cases were older at seizure onset and had a higher cognition index than nonfamilial cases [24]. A more severe phenotype is to be expected in TSC patients with mutations inactivating the tuberin

GTPase activating protein (GAP) domain, with a greater number of tubers and a greater tuber brain proportion than those with an intact GAP domain [24].

Different mutations in TSC2 gene have been described in association with severe clinical and epileptic phenotypes, including infantile spasms and Lennox-Gastaut syndrome [25], West syndrome and cardiac rhabdomyoma [26], and autism and mood and anxiety disorders [27]. By contrast, certain TSC2 missense mutations are associated with a milder form of TSC, which in many patients does not meet the standard diagnostic criteria. The TSC2 R905Q mutation is associated with unusually mild disease, characterized by hypomelanotic macules and focal seizures that remitted spontaneously, or were easily controlled with medications [26]. Similarly, a very mild phenotype, including brain imaging, has been reported associated with G1556S TSC2 mutation [28].

The phenotypes of TSC1 and TSC2 mutations may overlap considerably, and although some clinical phenotypes have been associated with specific mutations, prediction of the neurological and cognitive phenotypes should not be only based on the particular TSC1/TSC2 mutation.

The over-representation of cognitive impairments in patients with a TSC2 mutation might be due to a higher frequency of somatic, inactivating mutations at the TSC2 locus. An alternative possibility is that patients with TSC1 mutations may still retain some rheb GAP activity since the tuberin GAP domain is still present. Identification of a mutation is important in confirming a diagnosis of TSC. Yet, neither the onset of seizures nor the degree of cognitive impairment can be predicted on the basis of the mutation type. Current molecular diagnostic methods allow identification of mutations in either TSC1 or TSC2 in 75-85%

of TSC patients. TSC individuals with no mutation identified have milder brain features than TSC1 and TSC2 mutations [17].

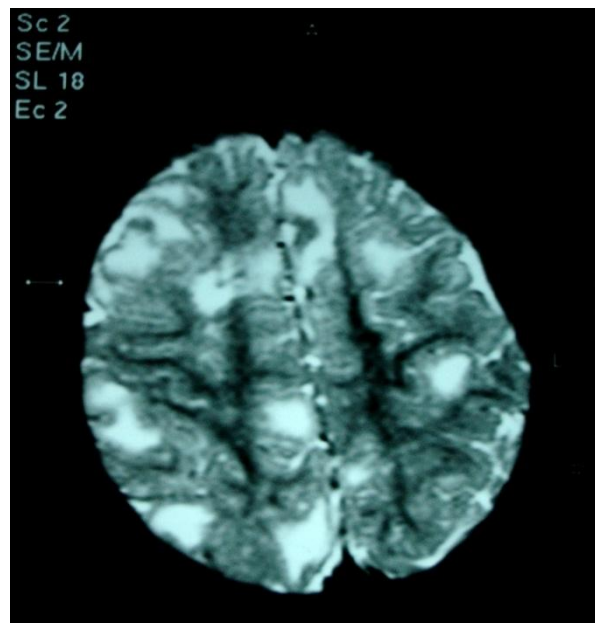


Figure 2. Axial MRI image showing multiple cortical tubers in a patient with autism and drug resistant epilepsy.

Pathophysiological mechanisms of epileptogenicity

Although mechanisms of epileptogenesis remain unknown, tubers represent the epileptic foci. Cortical tubers are characterized by a proliferation of both glial and neuronal cells and by a loss of the normal six-layered structure of the cortex. Tubers are variable in size and multiple in number, and may be detected by fetal MRI as early as 26 weeks of gestation [29,30].

A spectrum of abnormal cell types, including large **dysplastic neurons (DNs)**, **giant cells (GCs)**, and **bizarrely shaped astrocytes**, has been identified in tubers, based on morphological characteristics, markers of neuronal and glial differentiation, mTOR pathway activation, and ionotropic glutamate receptor (iGluR) subunit expression [31]. Dysplastic cells in cortical tubers expressing

high S6 levels demonstrate unique iGluR expression patterns consistent with a hyperexcitable state and reminiscent of those present on immature neurons and astrocytes in normal human developing cortex[32]. Differences in iGluR expression profiles between dysplastic and nondysplastic epileptic tissue suggest that, in human cortical tubers, *TSC1/TSC2* dysfunction may alter the developmental regulation of iGluRs and enhance glutamatergic function. DNAs exhibit disrupted radial orientation in cortex and abnormal dendritic arborization, showing GABA transporter defect and reduced GABAergic inhibition [33].

Epileptogenesis in TSC arise by an imbalance of decreased inhibition secondary to molecular changes of γ -aminobutyric acid (GABA) receptors in giant cells and dysplastic neurons, and increased excitation secondary to molecular changes of glutamate receptors in dysplastic neurons[34]. The deficiency of GABAergic interneurons may explain the early onset and severity of seizures in TSC [35-36]. By acting as a sensory gate, inhibitory GABAergic interneurons regulate the degree of glutaminergic excitation in the neocortex, filtering the input, and coordinating the output of multiple projection neurons. The importance of GABAergic inhibitory system in TSC has been confirmed by the particular efficacy of Vigabatrin (VGB), an inhibitor of GABA transaminase, able to completely stop spasms in 95% of TSC infants [37-39]. Prompt seizure control is crucial and could prevent the development of an epileptic encephalopathy [40].

One of the critical factors regulating neuronal excitability is expression and function of synaptic receptors for the main excitatory neurotransmitter glutamate [41]. GluRs expression is developmentally regulated, and during early cortical development, certain

receptor subtypes are over expressed resulting in physiological hyperexcitability during the critical periods of enhanced synaptic plasticity[42]. Also expression of metabotropic glutamate receptor (mGluR) subtypes is developmentally regulated, and several studies suggest an involvement of mGluR mediated glutamate signaling in the regulation of proliferation and survival of neural stem-progenitor cells, as well as in the control of tumor growth [43]. Recent findings expand our knowledge concerning the cellular phenotype in cortical tubers and in SGCTs, and highlight the role of group I mGluRs as important mediators of glutamate signaling in TSC brain lesions.

Individual mGluR subtypes may thus represent potential pharmacological targets for the treatment of the neurological manifestations associated with TSC brain lesions [44].

Increasing experimental data suggest a direct effect of the *TSC1* or *TSC2* mutations on brain development and excitability. Loss of *TSC1* or *TSC2* in mature postmitotic hippocampal neurons in vitro causes enlarged somas, abnormal dendritic spines, and enhancement of glutamatergic neurotransmission [45]. Astrocytes are specifically involved in TSC pathology. Recent evidence suggests that astrocyte changes may result from mTOR cascade activation in astrocytes, secondary to a mutation of *TSC1/TSC2* genes [46]. Astrocyte deficiency in potassium and glutamate uptake may combine with neuronal hyperexcitation to contribute to epileptogenesis in TSC individuals [47].

Early rational treatment for epilepsy

The treatment of seizures in TSC is often difficult and frustrating with a limited response to the conventional AEDs. The introduction of new anticonvulsant has improved this otherwise bleak scenario.

Hancock and Osborne [39] reviewed English language studies on the efficacy of vigabatrin (VGB) in the treatment of epileptic spasms. Of the patients affected by TSC, 73 (95%) had complete cessation of their epileptic spasms, as compared with 169 (54%) of the remaining patients. In the vast majority of patients, the effect was observed within one week, a much quicker response than that observed with steroids, benzodiazepines or sodium valproate, which can take weeks before they show any efficacy. It has been reported that VGB could be effective in reducing frequency of focal seizures in up to 74% of patients with TSC [38]. Localization related effectiveness of VGB shows better results on focal seizures originating from parieto-occipital lobes. Unfortunately, in recent years, there have been several reports on the appearance of alterations of the visual fields of patients treated with VGB [48]. Tong et al. [49] have demonstrated that VGB levels are dose-related, but are significantly higher in the retina than in any other brain tissues.

It has recently become clear that the use of VGB was associated with delayed appearance of concentric narrowing of the visual fields in up to 40-50% of cases. This narrowing can be severe and irreversible and continuation of the drug can be associated with progressive visual field loss [50]. This phenomenon may result from a sequence of events starting from cone cell injury to the more severe disorganization of the photoreceptors layer [51]. Currently, the minimum duration and doses of VGB treatment that can produce this side effect are unknown and the feasibility of using low dosages and short treatment periods (2-3 months) should be investigated.

Topiramate (TPM) is emerging as a more effective drug in TSC affected by focal seizures with or without secondary generalization and in the Lennox-Gastaut syndrome [52]. The mechanisms of action of TPM, apart from state-dependent blockade of

sodium and calcium channels and inhibitory effect on carbonic anhydrase, include the enhancement of GABA activity on GABA-A receptors with elevation of cerebral GABA levels, and antagonism of glutamate receptors.

Non pharmacologic management of seizures

Up to 20% of individuals with TSC develop partial seizures that are refractory to anticonvulsants. For these patients, the ketogenic diet, vagus nerve stimulation, and resective epilepsy surgery are being increasingly utilized.

The ketogenic diet, whereby 80-90% of daily calories are administered as a fat, is one of the oldest treatments for epilepsy [53]. Since carbohydrate depletion serves to inhibit mTOR, this approach may have particular relevance for TSC patients. In one series of TSC patients with refractory epilepsy, 11 of 12 patients experienced >50% reduction in seizures, despite having previously failed to improve with multiple traditional anticonvulsants [54]. The ketogenic diet is often associated with improved cognition, which can relate to improved seizure control and the ability to eliminate concurrent antiepileptic drugs [55,56]. Recent work suggests that carbohydrate restriction alone may produce benefits equal to those of the classical ketogenic diet [57,58]. Vagus nerve stimulation is generally well tolerated and appears to be effective primarily for partial seizures with secondary generalization [59,60]. Vagus nerve stimulation can also be associated with improvements in cognition and behavior; whether this is due primarily to improved seizures control or attributable to the vagus nerve stimulation independently is not clear. Traditionally avoided in patients with tuberous sclerosis, epilepsy surgery has an increasing role in this disorder [61]. Novel techniques such as magnetoencephalography, co-registered magnetic resonance, and positron emission tomography (PET) are

increasingly able to localize epileptogenic tubers or adjacent abnormal cortex [62].

A specific PET tracer, alfa-methyltryptofan, has also been used successfully to identify epileptogenic tubers [63]. However, owing to technical difficulties with its synthesis and a short half-life once formulated, its use has been limited to a few centers. Many patients may have a so-called “dominant” tuber or zone of cortical dysplasia that is responsible for the bulk of their epileptic activity. These patients are particularly suitable for resective surgery. Recent work has also described successful surgical treatment of multifocal partial epilepsy in TSC [61,64]. Sometimes removal of a primary epileptic focus may disrupt an underlying “epileptic network), with resulting improvement despite the presence of multiple areas of epileptic cortex. Another approach used at some centers is to employ a staged technique in which what appears to be the primary focus is resected, cortical grids are replaced, further monitoring is performed, and, if appropriate, additional foci are subsequently removed. In the largest series of surgical epilepsy patients with TSC and partial seizures to date, 37 of 70 patients (53%) were seizure free and another 8 (11%) had significantly reduced seizure frequency [65]. These findings are consistent with earlier reports that found 68-69% of TSC patients seizure free following resection [64,66]. Therefore, epilepsy surgery should be considered for all TSC patients with seizures refractory to medical treatment, as these patients have the potential to have significant improvement following resection.

New therapeutic approaches

Rapamycin is a potent translational modifier in neurons although its effects on gene transcription and neuronal development are poorly understood. Rapamycin mediates protein synthesis stimulated by brain derived neurotrophic factor and has also been shown to have direct neurophysiological effects

[67,68]. The single channel activity of the Ca^{2+} -dependent K^+ channel is modulated by rapamycin through its direct association with the FK506 binding protein-12 (FKBP12)[69]. Rapamycin might also influence mTOR-mediated propagation of inhibitory transmission via the glycine- and GABA-receptor clustering protein gephyrin [70-72]. In one model, rapamycin did not enhance the firing of spontaneous action potentials [73-75], perhaps because unlike other calcineurin inhibitors, cyclosporine A and tacrolimus, rapamycin does not inhibit the Ca^{2+} calmodulin dependent phosphatase calcineurin.

The effect of rapamycin on long term-potential (LTP) is controversial. One study demonstrated that low dose rapamycin leads to NMDA-dependent induction of LTP in CA1 neurons of rat hippocampal slices when paired with weak presynaptic stimulation [76]. In contrast, others have shown that rapamycin impairs LTP by inhibiting mTOR through reduced protein synthesis [77-78]. Finally, a third study demonstrated that rapamycin did not affect LTP at baseline, and did not prevent the inhibition of LTP by H_2O_2 [79]. The effect of rapamycin on cell morphology, gene expression, and seizure-like firing patterns in cultured rat hippocampal neurons at post-natal time periods was demonstrated [80]; rapamycin does not produce neurotoxicity, nor alter dendritic growth and complexity *in vitro*, and does not significantly alter voltage-gated sodium and potassium currents. Rapamycin does affect neuronal gene transcription *in vitro*.

A recent study has tested rapamycin and RAD001 [40-O-(2-hydroxyethyl)-rapamycin], both mTOR inhibitors, as potential therapeutic agents in a mouse neuronal model of TSC, in which *TSC1* is ablated in most neurons during cortical development [81]. Rapamycin/RAD001 were highly effective

therapies for this neuronal model of TSC, with benefit apparently attributable to effects on mTOR and Akt signaling and, consequently, cell size and myelination.

In another recent experiment early treatment with rapamycin prevented the development of epilepsy and premature death observed in vehicle-treated TSC1^{GFAP}CKO mice. Late treatment with rapamycin suppressed seizures and prolonged survival in TSC1^{GFAP}CKO mice that had already developed epilepsy. Rapamycin inhibited the abnormal activation of the mTOR pathway, astrogliosis, and neuronal disorganization, and increased brain size in TSC1^{GFAP}CKO mice [82]. Although caution is appropriate, the results suggest the possibility that rapamycin/RAD001 may have benefit in the treatment of TSC brain disease, including infantile spasms.

Ehninger et al.[83] recently reported about deficits in learning and memory presented by mice with a heterozygous, inactivating mutation in the TSC2 gene (TSC2^{+/-} mice), but without any neuropathology or seizures, thus suggesting that other disease mechanisms were involved. These findings demonstrated a role of mTOR signaling in altering the LTP in a specific hippocampal region leading to deficits in hippocampal-dependent learning, and showed that treatment with mTOR antagonists ameliorated cognitive dysfunctions of these animal models.

Rapamycin is being proposed for use in individuals with TSC. Recent clinical trials using rapamycin have demonstrated to be effective in reducing the volume of renal angiomyolipomas, SGCTs, angiofibromas, as well as an improving pulmonary function in individuals with sporadic lymphangiogliomyomatosis [84-86]. However, when the therapy was stopped, the lesions tended to increase again in volume, even if some improvement in spirometric measurements persisted after treatment [84]. One group

reported rapamycin-induced regression of astrocytomas in five patients with TSC. Four of these patients remained seizure-free during the treatment period [86]. A dramatic reduction in seizure frequency with rapamycin therapy has been recently reported in a 10 years old girl [87].

Although caution is appropriate, the results of these studies will influence future clinical trials and suggest the possibility that rapamycin may have benefit in the treatment of TSC brain diseases, including epilepsy, mental retardation and autism.

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