

Neurological and neuropsychiatric aspects of tuberous sclerosis complex



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Tuberous sclerosis (also known as tuberous sclerosis complex [TSC]) is a multisystem genetic disorder that affects almost every organ in the body. Mutations in the *TSC1* or *TSC2* genes lead to disruption of the TSC1–TSC2 intracellular protein complex, causing overactivation of the mammalian target of rapamycin (mTOR) protein complex. The surveillance and management guidelines and clinical criteria for tuberous sclerosis were revised in 2012, and mTOR inhibitors are now recommended as treatment options for subependymal giant cell astrocytomas and renal angiomyolipomas—two common features of the disease. However, most morbidity and mortality caused by tuberous sclerosis is associated with neurological and neuropsychiatric manifestations. Treatment of epilepsy associated with tuberous sclerosis remains a major challenge, with more than 60% of patients having ongoing seizures. Tuberous-sclerosis-associated neuropsychiatric disorders (TAND) are multilevel and occur in most individuals with the disorder, but are rarely assessed and treated. Clinical trials of mTOR inhibitors to treat seizures and TAND are underway. Management of the neurological and neuropsychiatric manifestations of the disorder should be coordinated with treatment of other organ systems. In view of the age-related expression of manifestations from infancy to adulthood, continuity of clinical care and ongoing monitoring is paramount, and particular attention is needed to plan transition of patient care from childhood to adult services.

Introduction

Tuberous sclerosis is an autosomal dominant genetic disorder that affects many organs and systems and is characterised by well circumscribed, histologically benign lesions.¹ The term “tuberous sclerosis of the cerebral convolutions” was first used by the French physician Bourneville in 1880 to describe the potato-like appearance of cerebral lesions seen during the autopsy of a girl with intellectual disability who died as a result of refractory seizures.² In clinical practice, the terms tuberous sclerosis complex (TSC) and tuberous sclerosis are used interchangeably. Herein, we will use the latter term, and we will use the abbreviation TSC when referring to the genetic nomenclature of the disorder.

The CNS is affected in more than 90% of individuals with tuberous sclerosis, with the presence of pathological lesions such as cortical or subcortical tubers, subependymal nodules, giant cell astrocytomas, and white matter migration lines (ie, radiologically detectable lines of dysplastic white matter between the periventricular region and the cortical surface).³ These structural CNS lesions are associated with neurological signs and symptoms, such as epilepsy, and neuropsychiatric disorders.¹ The most common non-neurological characteristics of tuberous sclerosis are dermatological, renal, pulmonary, cardiac, and ophthalmological manifestations.

TSC is caused by mutations in either the *TSC1* gene on chromosome 9 or the *TSC2* gene on chromosome 16, which encode the proteins hamartin (TSC1) and tuberin (TSC2), respectively. Hamartin and tuberin form an intracellular protein complex referred to as the TSC1–TSC2 complex.^{4,5} Genetic mutations in either the *TSC1* or *TSC2* genes lead to hyperactivation of the mammalian target of rapamycin (mTOR) pathway

(figure 1),⁶ a downstream signalling pathway involved in various aspects of intracellular functioning, including cell growth and proliferation, protein synthesis, and metabolism. mTOR is a serine/threonine kinase that exerts its activity in response to nutrients and growth factors and is found in two structurally and functionally distinct complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2).⁷ mTORC1 acts as a nutrient and energy sensor and regulates protein synthesis and cell cycle progression, whereas mTORC2 has a role in regulation of the actin cytoskeleton. mTORC1 inhibitors are licensed for the treatment of subependymal giant cell astrocytomas (SEGA) and renal angiomyolipoma in tuberous sclerosis, and several molecular biological studies and clinical trials are underway to expand our understanding of the molecular mechanisms underlying tuberous sclerosis and to identify additional treatments that target those mechanisms. Since marketing authorisation and consensus clinical support only exists for mTORC1 inhibitors at present, we focus only on mTORC1 inhibitors in this Review.

The estimated prevalence of tuberous sclerosis is 6·8–12·4 per 100 000 people, and no sex or ethnic differences have been reported so far.⁸ The estimated incidence of tuberous sclerosis at birth is one in 5800 newborn babies.⁸ No epidemiological studies of tuberous sclerosis have been done since 1999, and therefore no prevalence or incidence studies have been undertaken using the 2012 revised diagnostic criteria.³

The aim of this Review is to provide a clinically oriented and up-to-date overview of tuberous sclerosis, incorporating the revised diagnostic and treatment guidelines for the disorder and recent research progress, with particular emphasis on the neurological and neuropsychiatric aspects of the disorder.

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Clinical features

Tuberous sclerosis is a protean disease with an age-dependent expression of clinical manifestations.¹ Even though neurological and neuropsychiatric manifestations are the major source of morbidity and mortality, tuberous sclerosis can affect almost any organ system, including the skin, kidneys, heart, lungs, liver, and eyes.¹

Neurological manifestations

Brain lesions

The neuropathological findings of tuberous sclerosis include cortical dysplasia (including tubers and white matter migration lines), subependymal nodules, and

SEGA (figure 2).³ Tubers represent focal malformations of cortical development and are characterised by loss of the hexalaminar cortical architecture, presence of an excessive number of astrocytes, and by dysmorphic neurons and giant cells.⁹ Tubers have long been regarded as the main origin of epileptogenic foci, and although this might still be partly true, the perituberal cortex has been shown to have a key role in epileptogenesis, with cellular dysplasia and abnormal mTOR signalling extending beyond the tuber.⁹ Findings from tuber-free animal models suggest that mTOR dysregulation alone seems to be sufficient to cause epileptogenesis.¹⁰ On the basis of their MRI appearance, tubers have been classified into different types, including cyst-like tubers, which are more frequent in patients with *TSC2* mutations than in those with *TSC1* mutations and more often associated with a severe epileptic phenotype than are other tuber types; however, the proposed classification of tubers needs to be confirmed.¹¹

White matter abnormalities are also usually present in tuberous sclerosis, from readily detectable radial migration lines to more subtle, diffuse abnormalities revealed by diffusion tensor imaging in regions of apparently normal-appearing white matter.^{12,13} Although the true association between these findings and the neurological phenotype of tuberous sclerosis is unclear, preliminary data suggest that the amount of white matter involvement might be associated with the severity of neurological impairment.^{12,13}

Subependymal nodules usually arise along the wall of the lateral and third ventricle, occur in about 80% of individuals with tuberous sclerosis, and are often detectable prenatally.³ Subependymal nodules can be asymptomatic, but can grow, degenerate, or calcify.¹⁴ Nodules located near the foramen of Monro that show contrast enhancement on brain MRI and are larger than 5 mm in diameter are at high risk of transforming into SEGA.¹⁵ SEGA are present in at least 5–15% of individuals with tuberous sclerosis.³ They are typically slow growing tumours composed of different cell lineages and are not purely astrocytic in nature.¹⁶ Even though SEGA are histologically benign, their location and tendency to grow can lead to obstructive hydrocephalus and can cause substantial morbidity and mortality.¹⁷ Brain MRI is recommended every 1–3 years in all individuals with tuberous sclerosis up to age 25 years to ensure early detection of SEGA growth, since the risk of SEGA substantially decreases after this age.¹⁸ MRI surveillance is particularly important for individuals with intellectual disability who might not be able to describe early and subtle symptoms associated with raised intracranial pressure, such as headaches, photophobia, diplopia, and changes in seizures or behaviour, or both.

Epilepsy

72–85% of individuals with tuberous sclerosis have a history of seizures, and in more than 80% of cases

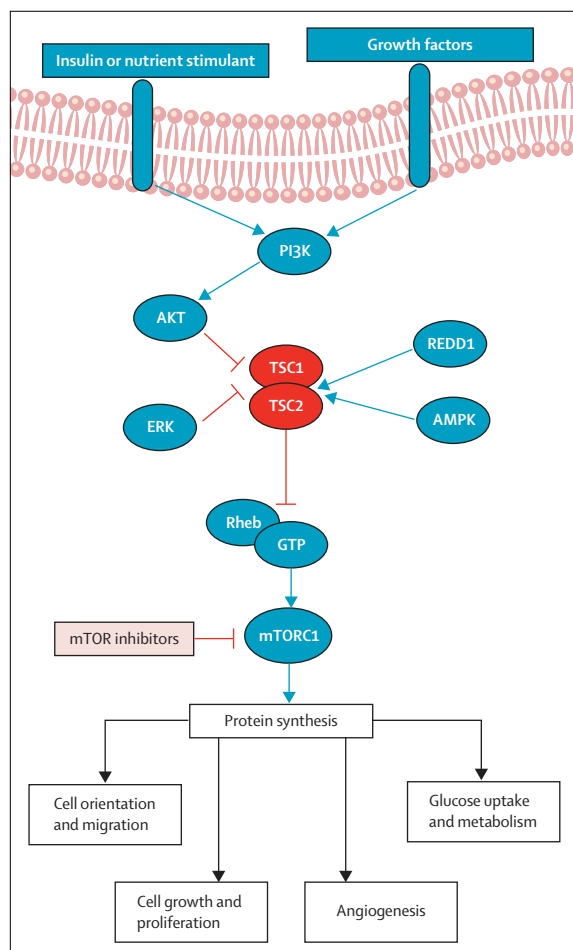


Figure 1: Pathophysiology of tuberous sclerosis

The figure shows the central part played by the TSC1–TSC2 complex and mTOR signalling pathway in several cellular processes relevant to the pathophysiology of tuberous sclerosis. The specific target of mTORC1 inhibition is also shown. Blue arrows show activating phosphorylation sites; red flat surfaces show inhibitory phosphorylation sites. Since marketing authorisation and consensus clinical support only exists for mTORC1 inhibitors at present, we show only mTORC1 signalling in this figure. AKT=protein kinase B. AMPK=AMP-activated protein kinase. ERK=extracellular signal-regulated protein kinase. mTOR=mammalian target of rapamycin. mTORC1=mTOR complex 1. PI3K=phosphatidylinositol-3 kinase. REDD1=regulated in development and DNA damage responses. Rheb= Ras homologue enriched in brain. TSC=tuberous sclerosis complex.

epilepsy begins in the first 3 years of life.^{19,20} Early-onset epilepsy typically presents with focal seizures or infantile spasms.¹⁹ Infantile spasms, which are a severe form of epilepsy, are often followed by other seizure types, leading to refractory epilepsy in up to 75% of cases.¹⁹ Individuals with tuberous sclerosis can present with almost any seizure type—such as tonic, atonic, or tonic-clonic seizures—with about two-thirds having focal-onset refractory epilepsy.^{19,20} Since presymptomatic diagnosis of tuberous sclerosis is increasingly being made, either prenatally or in the early postnatal period, close clinical and electroencephalogram (EEG) monitoring of affected infants—who are all at high risk of seizures—is possible (figure 3).²¹

Tuberous-sclerosis-associated neuropsychiatric disorders

Tuberous sclerosis is associated with a range of neuropsychiatric manifestations across various levels of investigation, such as behavioural, intellectual, and psychosocial, termed tuberous-sclerosis-associated neuropsychiatric disorders (TAND; figure 4).^{22,23} Almost all individuals with tuberous sclerosis will present with some of these neuropsychiatric manifestations during their lifetime. At the behavioural level, aggression, tantrums, anxiety, depressed mood, self-injury, and attention, social, and sleep difficulties have been reported.^{24,25} At the level of psychiatric diagnosis using formal diagnostic criteria, neurodevelopmental disorders such as autism spectrum disorder (40–50%) and attention deficit hyperactivity disorder (30–50%) are common.^{24,26,27} Tuberous sclerosis is one of the medical disorders most strongly associated with autism.²⁶ Anxiety and depressive disorders are often identified from early in adolescence and into adulthood.²⁴ The prevalence of psychotic disorders does not seem to be over-represented in tuberous sclerosis and is the same as occurs in the general population (about 1%).²⁴ About 50% of individuals with tuberous sclerosis have normal intellectual ability, and the remainder have varying levels of intellectual disability. Epidemiological data suggest an over-representation of severe and profound intellectual disability in tuberous sclerosis.^{28,29} At least 30% of school-aged children with tuberous sclerosis are at risk of academic difficulties with reading, writing, spelling, and mathematics, even when they have normal intellectual ability.³⁰ At the neuropsychological level, a range of specific deficits have been identified in tuberous sclerosis, even in individuals with normal and above average intellectual abilities. These include attentional deficits, particularly in dual-task performance; memory deficits, particularly in recall memory; and executive deficits, particularly in cognitive flexibility and complex spatial working memory tasks.^{30–32} At the psychosocial level, there is increasing evidence of the effect of tuberous sclerosis on self-esteem, family functioning, parental stress, and peer relationships.²²

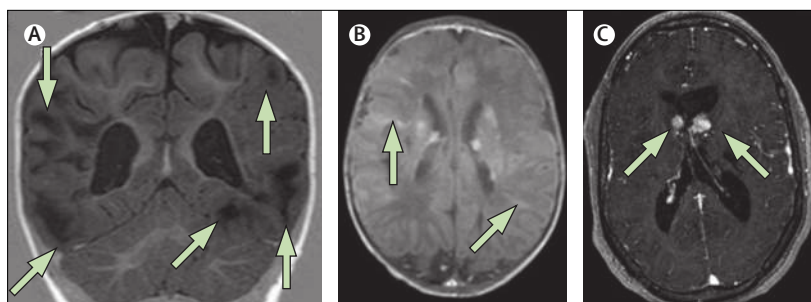


Figure 2: Typical neuroimaging findings of tuberous sclerosis

Brain MRIs showing (A) large T1 hypointense cortical-subcortical tubers (arrows), (B) typical white matter migration lines (arrows), and (C) bilateral subependymal lesions with contrast enhancement located near the foramen of Monro (arrows); the left lesion caused an initial ventricular dilatation.

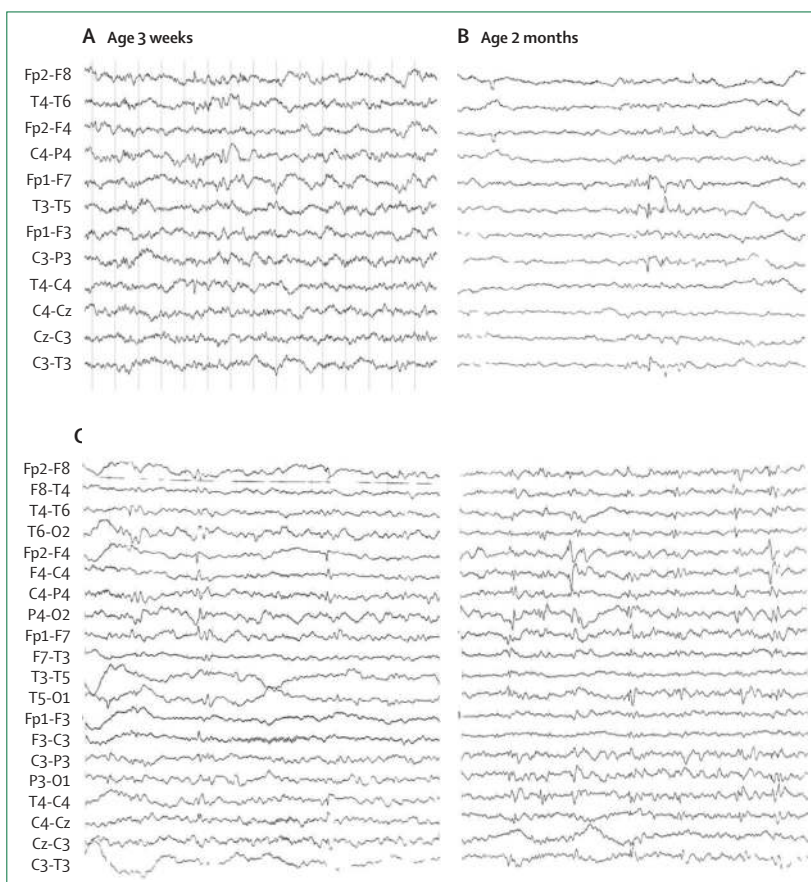


Figure 3: Prospective electroencephalogram monitoring of an infant with tuberous sclerosis

Monitoring showed a progressive increase of interictal abnormalities preceding epilepsy onset. (A) The first EEG at 3 weeks of age showed a good background organisation with no clear epileptiform abnormalities. (B) By age 2 months, sleep and awake EEGs continued to show a good background organisation. Rare and isolated spikes appeared only during sleep in the left temporal region (T3). (C) By age 5 months, the EEG pattern has started to change. Epileptiform abnormalities have become sustained and multifocal, and are starting to become generalised. (D) At 6 months, substantial evolution of the EEG pattern shows recurrent and sustained epileptiform abnormalities with spikes and waves, and a marked tendency towards generalisation. EEG=electroencephalogram.

Non-neurological manifestations

Dermatological manifestations often lead to the first suspicion of tuberous sclerosis, and include hypomelanotic macules (90%), angiofibromas (75%), fibrous

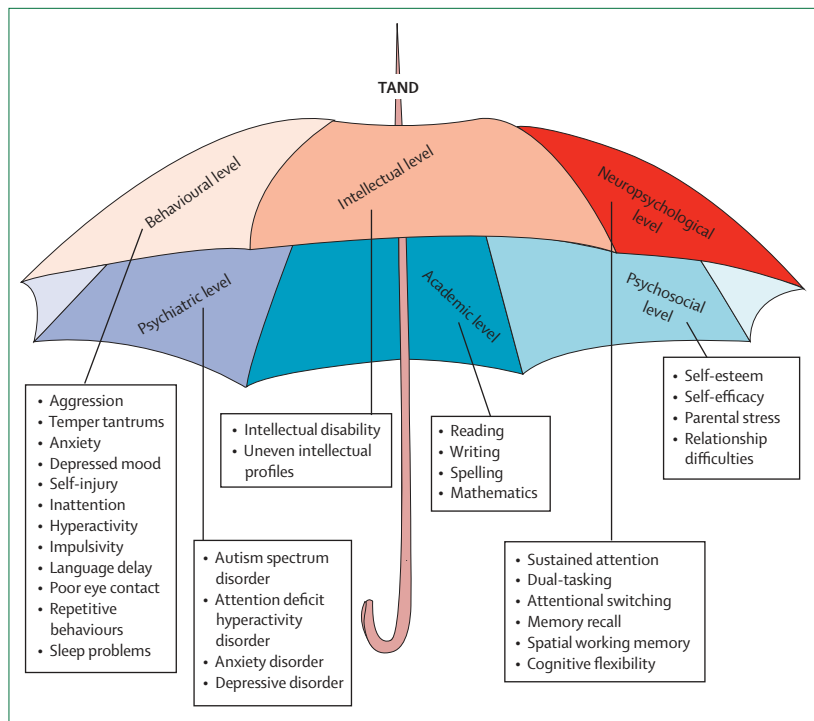


Figure 4: TAND

TAND is used as an umbrella term to capture the range of neuropsychiatric disorders associated with tuberous sclerosis across different levels of investigation. TAND=tuberous-sclerosis-associated neuropsychiatric disorders.

cephalic plaques (25%), unguinal fibromas (20%), shagreen patches, and confetti skin lesions (figure 5).³ In the updated diagnostic criteria, at least three hypomelanotic macules, each with diameters of at least 5 mm, are regarded as a major criterion.³ Dermatological manifestations have a clear age-related expression and, with the exception of hypomelanotic macules, are not likely to be identified in the first few years of life.¹

Cardiac rhabdomyomas, which occur in about 60% of individuals with tuberous sclerosis, are often the first identifiable manifestations of the disorder, and therefore can have a key role in the diagnosis of tuberous sclerosis, and sometimes enable prenatal diagnosis. Rhabdomyomas can usually be detected on routine antenatal ultrasound after 20 gestational weeks, but have been reported as early as 17 gestational weeks.³³ Although they are mostly asymptomatic, rhabdomyomas can lead to cardiac arrhythmias and Wolff-Parkinson-White syndrome or can cause outflow obstruction. Therefore, a cardiology follow-up is recommended, even in asymptomatic individuals.¹⁸ By contrast with the other hamartomatous lesions that occur in tuberous sclerosis, cardiac lesions typically spontaneously regress during childhood.³⁴

Renal manifestations represent a substantial source of morbidity and mortality in tuberous sclerosis.^{17,35} Most individuals with tuberous sclerosis will have renal angiomyolipomas (80%) and about 50% will have renal

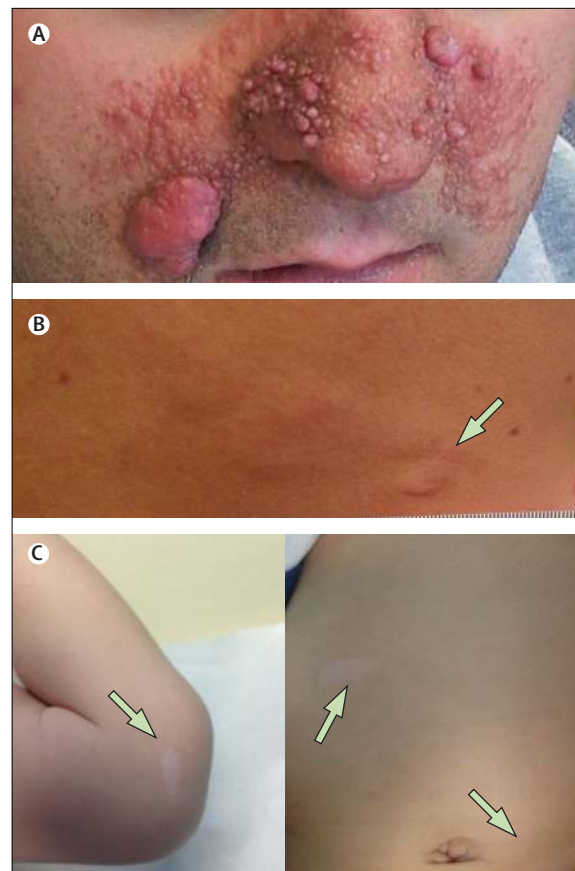


Figure 5: Dermatological manifestations of tuberous sclerosis

(A) Facial angiofibroma, (B) dorsal shagreen patch, and (C) hypomelanotic macules. The detection of any of these dermatological manifestations in a patient referred with epilepsy should always raise the clinical suspicion of tuberous sclerosis.

cysts, both of which tend to increase in number and size with age.³⁵ For this reason, an abdominal MRI is recommended every 1–3 years, and renal function and blood pressure should be monitored annually.¹⁸ Renal cysts are sometimes detectable very early in life, particularly in individuals with genetic deletions in *TSC2* and the adjacent *PKD1* gene, which is implicated in polycystic kidney disease.³⁵

Pulmonary lymphangioleiomyomatosis represents another source of tuberous-sclerosis-related morbidity.³⁶ Lymphangioleiomyomatosis affects women almost exclusively, although less severe forms of the disease have been described in men.^{37–39} 47% of women with tuberous sclerosis will develop lymphangioleiomyomatosis, which can be completely asymptomatic.^{37–39} Symptomatic lymphangioleiomyomatosis is characterised by dyspnoea, spontaneous pneumothorax, chylous effusion, and haemoptysis.^{36,40} Lymphangioleiomyomatosis can be associated with progressive cystic lung transformation and can be disabling, with a risk of rapid progression from minimal to severe lung disease and an abrupt reduction in lung function.⁴⁰ For this reason, all asymptomatic women

with tuberous sclerosis and lung cysts are advised to have periodic functional and radiological assessments.

Other systemic manifestations of tuberous sclerosis include dental enamel pits, intraoral fibromas, liver angiomyolipomas, retinal hamartomas, and retinal achromic patches.^{1,3}

Genetics and neurobiology

At present, disease-associated mutations are identified in about 85–90% of individuals with tuberous sclerosis.³ So far, about 700 unique DNA variants have been described on the *TSC1* gene⁴¹ and almost 2000 on *TSC2*,⁴² with no obvious mutation hotspots. A mutation in either the *TSC1* or *TSC2* gene is sufficient to disrupt the TSC1–TSC2 complex, thus explaining why both genes are associated with the same disease phenotype.⁷ In the CNS, several interacting neurobiological processes are associated with mTOR signalling—haploinsufficiency of *TSC1* or *TSC2* is sufficient to dysregulate mTOR activity and disrupt a range of mTOR-related physiological processes in the CNS.⁷ mTOR and other proteins in the mTOR signalling pathway have crucial roles during neurodevelopment, and dysregulated mTOR signalling is associated with aberrant brain development, although the exact mechanisms are still unclear.^{43,44} The mTOR signalling pathway is involved in neuronal migration and cortical lamination and is essential for the regulation of arborisation of dendrites in the early postnatal cerebral cortex and in determining neuronal polarity.⁴⁵

mTOR dysregulation is also involved in tuberous-sclerosis-related epileptogenesis through a range of potential mechanisms, including alteration of neuroblast migration, cortical lamination, cell body size, and dendritic arborisation, and by altering neuronal excitability through modulation of the expression of voltage-gated potassium channels.^{43,46–48} Epileptogenesis in tuberous sclerosis derives, at least in part, from an imbalance between excitation and inhibition, which has been suggested to result from molecular alterations of glutamate and GABA receptors within tubers.⁴⁹

Evidence from animal models suggests that some of the neuropsychiatric disorders associated with tuberous sclerosis, such as autism spectrum disorder, intellectual disability, and specific neuropsychological deficits, might also be directly attributable to molecular aberration in mTOR signalling (figure 6).^{50–52} The hypothesis of a direct role of mTOR in determining the behavioural phenotype of tuberous sclerosis is supported by preclinical evidence in adult mouse models of tuberous sclerosis that brief treatment with sirolimus (rapamycin) resulted in improved synaptic plasticity, reversal of spatial learning deficits,⁵³ and reversal of social behavioural deficits.^{54,55} Evidence from combinatorial or additive models also suggests that a mutation in one of the TSC genes might lead directly to some specific social deficits, and that seizures might

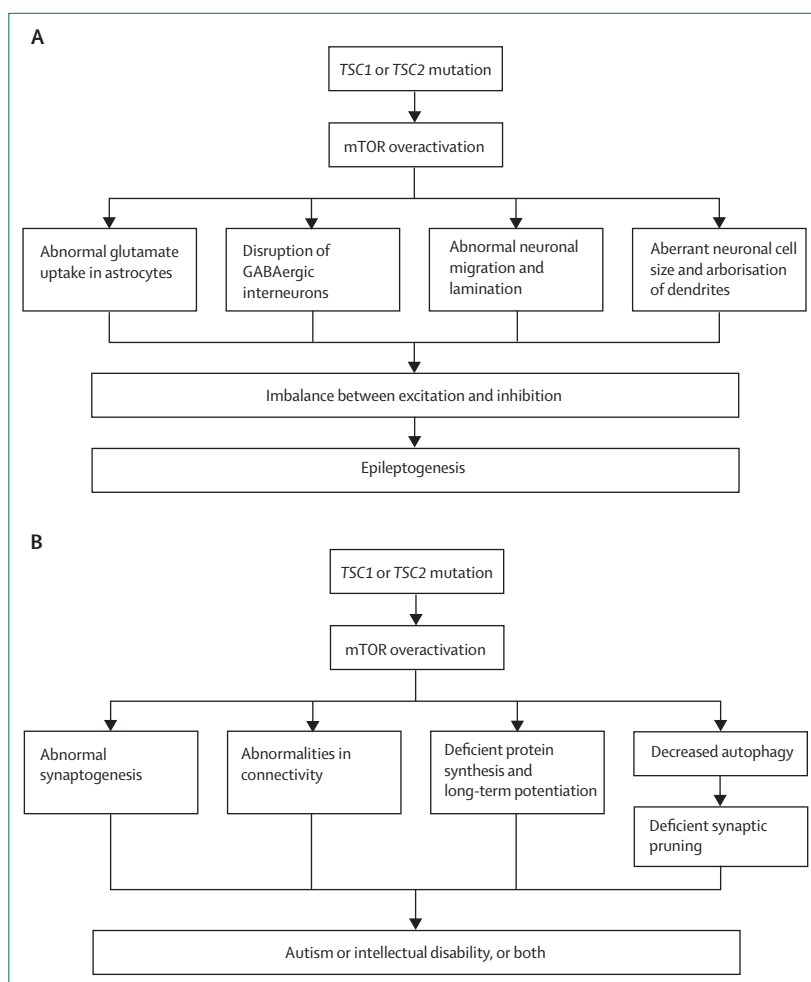


Figure 6: Schematic representation of the potential roles of mTOR overactivation in determining the neurological and neuropsychiatric manifestations of tuberous sclerosis

(A) mTOR overactivation can dysregulate the balance between neuronal excitation and inhibition, leading to epileptogenesis. (B) mTOR overactivation can alter synaptogenesis and synaptic pruning, connectivity, and long-term potentiation, leading to an increased susceptibility to autism or intellectual disability, or both. mTOR=mammalian target of rapamycin.

lead to additional but different social deficits. In an example of a combinatorial model, a TSC gene mutation was needed in the presence of a viral infection in pregnant mice to lead to social deficits in the pups.^{56,57}

Although phenotypes associated with *TSC1* or *TSC2* overlap substantially,⁵⁸ a greater proportion of *TSC2* mutations than *TSC1* mutations are associated with a more severe outcome, in particular with earlier age at seizure onset, more severe intellectual disability, and higher tuber load.^{59–61} However, some *TSC1* mutations have been associated with severe neurological phenotypes, including epileptic encephalopathy;^{60,62} conversely, *TSC2* mutations have been described in individuals with mild clinical expression.^{60,63,64} Therefore, at present, prediction of the phenotype of an individual with tuberous sclerosis on the basis of a specific genetic mutation is not possible or advisable.

Diagnosis

The diagnostic criteria for tuberous sclerosis were revised at the International TSC Consensus Conference in 2012 (panel 1).³ A definite diagnosis is made when two major features, or one major feature with two or more minor features, are detected. A possible diagnosis is made if there is one major feature or two or more minor features. Many subtle changes were made to the diagnostic criteria in the 2012 revision. Two of the most relevant changes compared with the previous criteria⁶⁷ were the elimination of a probable diagnosis of tuberous sclerosis and the inclusion of a genetic criterion in addition to the clinical ones.³ According to the new genetic criterion, a definitive diagnosis of tuberous sclerosis can be made when a pathogenic *TSC1* or *TSC2* mutation has been identified.³

Panel 1: Updated diagnostic criteria for tuberous sclerosis complex

Genetic diagnostic criteria

The identification of either a *TSC1* or *TSC2* pathogenic mutation in DNA from normal tissue is sufficient to make a definite diagnosis of tuberous sclerosis. A pathogenic mutation is defined as a mutation that inactivates the function of the *TSC1* or *TSC2* proteins (eg, frameshift indel or non-sense mutation), prevents protein synthesis (eg, large genomic deletion), or is a missense mutation, of which the effect on protein function has been established by functional assessment.^{41,42,65,66} Other *TSC1* or *TSC2* variants whose effect on function is less certain do not meet these criteria and are not sufficient to make a definite diagnosis of tuberous sclerosis. 10–25% of patients with tuberous sclerosis have no mutation identified by conventional genetic testing, and a normal result does not exclude tuberous sclerosis or have any effect on the use of clinical diagnostic criteria to diagnose tuberous sclerosis.

Clinical diagnostic criteria

Definite diagnosis: two major features or one major feature with ≥ 2 minor features

Possible diagnosis: either one major feature or ≥ 2 minor features

Major features

- 1 Hypomelanotic macules (≥ 3 , at least 5 mm in diameter)
- 2 Angiofibromas (≥ 3) or fibrous cephalic plaque
- 3 Ungual fibromas (≥ 2)
- 4 Shagreen patch
- 5 Retinal hamartomas
- 6 Cortical dysplasias*
- 7 Subependymal nodules
- 8 Subependymal giant cell astrocytoma
- 9 Cardiac rhabdomyoma
- 10 Lymphangioliomyomatosis†
- 11 Angiomyolipomas (≥ 2)†

Minor features

- 1 Confetti skin lesions
- 2 Dental enamel pits (>3)
- 3 Intraoral fibromas (≥ 2)
- 4 Retinal achromic patch
- 5 Several renal cysts
- 6 Non-renal hamartomas

TSC=tuberous sclerosis complex. *Includes tubers and cerebral white matter radial migration lines. †A combination of lymphangioliomyomatosis and angiomyolipomas without other features does not meet criteria for a definite diagnosis. Reproduced from Northrup and colleagues.³

The previous Consensus Conference, held in 1998, took place shortly after identification of the *TSC1* and *TSC2* genes.⁶⁷ The increasing availability of molecular testing and the advent of sophisticated and high-throughput techniques has allowed genetics to become a key contributor to the diagnosis of tuberous sclerosis.³ This new criterion should make diagnosis easier, especially in infants who might not yet fulfil the clinical diagnostic criteria for tuberous sclerosis in view of the age-related expression of clinical manifestations.¹ In countries where mutation analysis is not available, the clinical criteria remain the primary way to diagnose tuberous sclerosis.

The revised diagnostic criteria also group cortical tubers and radial migration lines together as cortical dysplasias.³ Additionally, the term TAND was introduced as an umbrella term to capture all the biopsychosocial difficulties noted in tuberous sclerosis.²² The International Tuberous Sclerosis Complex Consensus Panel recommended annual screening for TAND, in addition to comprehensive multidisciplinary neuropsychiatric assessments at key developmental timepoints: infancy (age 0–3 years); preschool (age 3–6 years); before transition to middle school (age 6–9 years); adolescence (age 12–16 years); early adulthood (age 18–25 years); and thereafter as clinically indicated.¹⁸ A short TAND checklist was developed by the Neuropsychiatry Panel and piloted for key aspects of validity.^{22,23}

Treatment

Neurological manifestations

Epilepsy

Early treatment for epilepsy in patients with tuberous sclerosis is recommended. However, with the exception of infantile spasms, evidence to guide anticonvulsant treatment is limited. Vigabatrin was recommended by the Consensus Panel as first-line treatment of infantile spasms, with adrenocorticotropic hormone as a second-line treatment option,²¹ albeit on the basis of low-level evidence (category 2) from one trial.⁶⁸ Vigabatrin seems to interrupt tuberous-sclerosis-related spasms in up to 95% of cases.^{18,21,68} Vigabatrin is an inhibitor of GABA transaminase, but it also acts as an inhibitor of the mTORC1 pathway, which might explain some of its efficacy in tuberous sclerosis.⁶⁹ Balancing the potential benefits of seizure control with the risk of progressive and irreversible visual field defects, reported in 9–63% (mean 37%) of children treated with vigabatrin,⁷⁰ remains important. Some expert groups also recommend vigabatrin as first-line treatment of early-onset focal seizures,²¹ although this was not expressly stated by the Consensus Panel.¹⁸ In a prospective study, initiation of vigabatrin treatment immediately after the onset of the first focal seizures in children with early-onset epilepsy associated with tuberous sclerosis minimised the risk of subsequent epileptic encephalopathy and intellectual disability.⁷¹ Preventive antiepileptic treatment before the onset of any clinically detectable seizures has also been proposed,⁷² but there is insufficient evidence to recom-

mend a prophylactic treatment approach.²¹ Seizures other than infantile spasms and focal seizures should be treated according to standard treatment guidelines.

The prevalence of medically refractory seizures is high in patients with tuberous sclerosis, and other treatment options, such as surgery, a ketogenic diet, vagus nerve stimulation, or mTOR inhibitors, might be needed. In a systematic review of epilepsy surgery in tuberous sclerosis,⁷³ 59% of patients no longer experienced seizures after surgery; this strategy was most effective in individuals with seizure onset after the first year of life, those with unilateral EEG foci, and those who underwent a lobectomy rather than tubectomy.⁷³ In another study in 33 patients who underwent epilepsy surgery,⁷⁴ preoperative factors, such as age at onset of seizures, seizure duration, or seizure frequency, did not affect outcome. Also, outcome was not affected by the type and extension of resection, but concordance between ictal and interictal EEG findings was the most accurate predictor of seizure outcome.⁷⁴ Presurgical assessment is crucial for the correct identification of the best candidates, but this needs expert assessment using an appropriate combination of video EEG, SPECT, PET, magneto-encephalography, diffusion sequences in MRI, and, eventually, invasive recording.^{73,74}

In individuals with medically refractory epilepsy who are not candidates for surgery, vagus nerve stimulation can be considered. Findings from four retrospective studies⁷⁵⁻⁷⁸ that included a total of 49 patients showed a greater than 50% reduction in seizure frequency in about 70% of patients, although seizure freedom was reported in only 4% of cases. Another possible option for treatment of refractory seizures in tuberous sclerosis is the ketogenic diet; the diet's precise mechanism of action is unknown, but seems to involve inhibition of the mTOR pathway.⁷⁹ Few studies exploring the efficacy of a ketogenic diet in tuberous-sclerosis-related epilepsy are available.^{80,81} One study reported that, after 6 months on a ketogenic diet, more than 90% of children with tuberous sclerosis had a greater than 50% reduction in seizure frequency, across several seizure types.⁸⁰ Also, a low glycaemic index diet produced more than a 50% reduction in seizure frequency in 47% of patients.⁸²

Despite present pharmacological and non-pharmacological treatment options, seizures associated with tuberous sclerosis persist in more than 60% of patients,¹⁹ suggesting that there is an urgent need for new treatment options. New hope is provided by findings from preclinical studies that suggest early treatment with sirolimus might reverse neuronal disorganisation and seems to exert an antiepileptogenic effect in a mouse model.¹⁰ The mTORC1 inhibitor everolimus was the first drug to be approved in the USA and Europe for the treatment of tuberous-sclerosis-related SEGA and renal angiomyolipomas.^{83,84} The potential role of everolimus in the treatment of tuberous-sclerosis-related refractory epilepsy is under investigation in the EXIST-3 trial (NCT01713946). After the early antiepileptogenicity results from animal models,

findings from a case report in one patient⁸⁵ and a small clinical series of 34 treated individuals⁸⁶⁻⁸⁸ have shown promising results with mTOR inhibitors. In the clinical series, 61% of participants had a greater than 25% seizure reduction,⁸⁶⁻⁸⁸ measurement of the proportion of patients with a greater than 50% reduction in seizure frequency, which is the usual cutoff used to define responders in this type of study, was not always possible. In the multicentre randomised controlled EXIST-1 study,⁸³ no significant effect of everolimus on seizures was noted, but the primary outcome of the trial was SEGA size, not reduction in seizure frequency. Since baseline seizure rates were low, the study was not sufficiently powered to identify any treatment effects on epilepsy. The EXIST-3 study is investigating the role of everolimus as an adjunctive treatment for focal seizures in individuals with tuberous sclerosis (NCT01713946).

SEGA

Surgery remains the treatment of choice in all acutely symptomatic cases of SEGA. CSF shunt might also be needed in acute cases.^{18,89} The International Tuberous Sclerosis Complex Consensus Panel recommended that either surgical intervention or medical treatment with an mTOR inhibitor could be used for growing but otherwise asymptomatic SEGA.¹⁸ How to make a clinical decision regarding surgical versus medical intervention is one of the key clinical challenges, particularly in view of the absence of good systematic studies of the morbidity and mortality associated with elective surgery. In patients with growing but asymptomatic SEGA, in whom a complete removal of the lesion seems possible without other risk factors or comorbidities, surgery might be preferred to mTOR inhibition; however, for patients in whom multisystem disease or multiple infiltrating SEGA not amenable to total resection are present, mTOR inhibition is a more appropriate option. The Consensus Panel advised that a range of clinical factors should be considered when making a treatment decision, including risks of complications associated with surgery, adverse effects and length of potential pharmacotherapy, and potential effect on other tuberous sclerosis comorbidities.⁹⁰

The efficacy of mTOR inhibitors on tuberous-sclerosis-related SEGA was first described in 2006,⁹¹ and the first randomised double-blind study, EXIST-1, was published in 2013.⁸³ In this phase 3 clinical trial, treatment with everolimus led to shrinkage of SEGA and prevented disease progression in all treated individuals (figure 7).⁸³ Additionally, more than 35% of individuals with SEGA associated with tuberous sclerosis had at least a 50% reduction in SEGA volume after 6 months of treatment with everolimus.⁸³ In an extension study of EXIST-1,⁹² at a mean everolimus treatment duration of 29.3 months, 49% of patients had a greater than 50% reduction in SEGA volume.⁹² However, withdrawal of everolimus is associated with regrowth of lesions, suggesting that continuous treatment might be necessary to keep SEGA under

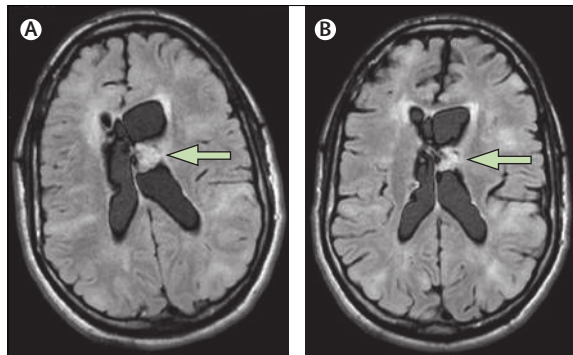


Figure 7: Bilateral subependymal giant cell astrocytomas
Axial flair images of a 25-year-old man with a TSC2 mutation who was given everolimus for bilateral subependymal giant cell astrocytomas. (A) Before and (B) after 1 year of everolimus treatment. A shrinkage of subependymal giant cell astrocytomas (arrow) and an overall reduction of ventricular enlargement is evident.

control.⁹³ Stomatitis and mouth ulcerations were the most frequent treatment-related adverse events reported in EXIST-1; infections were the most commonly reported serious adverse events, occurring in 14% of patients.⁹² Long-term data suggest that adverse effects seem to reduce over time.⁹² These phase 2 and phase 3 results led to the approval of everolimus by the US Food and Drug Administration and the European Medicines Agency as a new therapeutic option for individuals with tuberous sclerosis who present with SEGA but are not amenable to surgery. Although surgery has low rates of perioperative complications in elective cases, it can be associated with increased morbidity and mortality when done in patients with raised intracranial pressure.⁹³ Reported complications include transient memory impairment, hemiparesis, and infections,⁹³ but few rigorous investigations of the long-term neuropsychiatric consequences of SEGA surgery have been done.

TAND

Even though most individuals with tuberous sclerosis will have some neuropsychiatric challenges, survey data suggest that, even after the introduction of consensus guidelines to assess these difficulties,⁹⁴ fewer than 20% of patients with tuberous sclerosis ever received appropriate assessment and treatment of their neuropsychiatric problems.²² In real-life, busy clinics, it is understandable that the clinical team might prioritise seizure control, monitoring of potential SEGA, and renal manifestations over neuropsychiatric problems. However, the neuropsychiatric manifestations of tuberous sclerosis represent a substantial burden of disease. Therefore, clinical teams must consider TAND in each individual at each clinic visit. So far, no unique treatments for TAND exist and almost no evidence of the effectiveness of any psychopharmacological or psychological treatment in individuals with tuberous sclerosis is available. The International Tuberous Sclerosis Complex Consensus Panel recommended the use of clinical guidelines and practice parameters as set out for

individual disorders, for instance, early-intervention programmes for autism or psychosocial and pharmacological treatment for attention deficit hyperactivity disorder.¹⁸ All school-aged children with tuberous sclerosis should be considered for an individual educational plan with additional support when needed.¹⁸

There is growing interest in the pathophysiological mechanisms of the neuropsychiatric manifestations of tuberous sclerosis, and models suggesting direct molecular pathways from gene disruption to disorder through mTOR dysregulation are supported by evidence from animal studies.^{50,51,53–55,95} So far, only one early-phase study of the role of mTOR inhibitors in individuals with tuberous sclerosis or sporadic lymphangiomyomatosis has been published.⁹⁶ Five of eight individuals with tuberous sclerosis treated with the mTOR inhibitor sirolimus showed improvement in recall memory and executive skills.⁹⁶ Several phase 2 trials exploring the effect of mTOR inhibition on various levels of TAND are ongoing (see later).

Other manifestations of tuberous sclerosis

Treatment of renal angiomyolipomas has changed substantially in the past 5 years, with mTOR inhibitors becoming the treatment of choice for lesions larger than 3 cm.^{18,84} The efficacy of everolimus was confirmed in the EXIST-2 phase 3 randomised clinical trial,⁸⁴ in which the response rate (defined as a reduction of at least 50% in angiomyolipoma volume) was 42% of participants treated with everolimus versus 0% of participants in the placebo group. Patients treated with everolimus also showed no disease progression or renal haemorrhage.⁸⁴ In patients for whom mTOR inhibition is not available or is contraindicated, embolisation followed by corticosteroid treatment should be considered; this is also the treatment of choice in all acutely bleeding angiomyolipomas or in cases of intralesional aneurysms.¹⁸

Treatment of lymphangiomyomatosis is still being investigated. However, in a double-blind trial,⁹⁷ sirolimus stabilised lung function, reduced symptoms, and improved quality of life in moderate-to-severe cases or in cases with rapid progression.

Facial angiofibroma can lead to severe disfigurement, and can cause discomfort, bleeding, and psychological distress. In both EXIST-1 and EXIST-2,^{83,84} improvement of skin lesions was noted, but so far marketing authorisation for the treatment of skin manifestations has not been sought. Consensus recommendations advocate the use of the intervention most appropriate to the skin lesion and context. These interventions include laser treatment, surgical excision, or topical mTOR preparations.⁹⁸ Further results of trials of topical sirolimus treatment are awaited (NCT01853423; NCT01526356).

Findings from several reports suggest that everolimus, because of its ability to act simultaneously on different organ systems in tuberous sclerosis, might be considered as a potential systemic treatment for this disease.⁹⁹

Ongoing clinical trials

Several clinical trials are investigating the efficacy and safety of mTOR inhibitors across different neurological and neuropsychiatric manifestations of tuberous sclerosis. On April 21, 2015, we identified six active neurological or neuropsychiatric trials on ClinicalTrials.gov: three phase 2 trials, one phase 2/3 trial, one phase 3 trial, and one further interventional trial (table).

Two trials are focused on tuberous-sclerosis-related epilepsy. The EPISTOP randomised trial (NCT02098759) is a Europe-based, long-term, prospective study assessing clinical and molecular biomarkers of epileptogenesis in tuberous sclerosis; a secondary objective of EPISTOP is to compare the effects of standard antiepilepsy treatment in patients diagnosed with epilepsy and overt clinical seizures versus those with EEG evidence of epileptiform discharges without overt clinical seizures. As described

earlier, the EXIST-3 trial (NCT01713946) is an international, multicentre phase 3 trial designed to assess the efficacy and safety of two trough ranges of everolimus given as adjunctive treatment in patients with tuberous sclerosis who have refractory partial-onset seizures. The study consists of three phases for each patient: a baseline phase, a core phase, and an extension phase up to 48 weeks.

Neuropsychiatric aspects, including autism and neuropsychological skills, are the focus of the four other trials. The trial of everolimus and neurocognition in tuberous sclerosis (NCT01289912) is a US-based, two-site phase 2 trial investigating the safety and efficacy of everolimus on behavioural, psychiatric, neuropsychological, and academic difficulties in children and young adults with tuberous sclerosis compared with placebo. The TRON study

	Study type	Intervention	Primary objectives	Secondary objectives	Number of patients (estimated enrolment)	Phase
Tuberous-sclerosis-related epilepsy						
EPISTOP (NCT02098759)	Prospective observational and interventional	Standard epilepsy treatment	To identify clinical and molecular biomarkers of epileptogenesis	To compare the effects of standard antiepilepsy treatment in children aged up to 4 months at enrolment diagnosed with epilepsy after overt clinical seizures vs after epileptiform discharges on EEG but in the absence of clinical seizures	100	NA
EXIST-3 (NCT01713946)	Interventional	Everolimus vs placebo	To assess the efficacy and safety of two trough ranges of everolimus given as adjunctive treatment in patients with refractory partial-onset seizures	To measure a range of additional outcomes, including seizure-free rate, quality of life, adaptive behaviours, and non-verbal intellectual ability	355	Phase 3
Tuberous-sclerosis-associated neuropsychiatric disorders						
Trial of RAD001 and neurocognition in tuberous sclerosis complex (TSC) (NCT01289912)	Interventional	Everolimus vs placebo	To assess the safety and efficacy of everolimus in children and young adults with tuberous sclerosis. Outcome measures included standardised neuropsychological instruments	To identify signs of change in epilepsy, sleep, autism spectrum characteristics, academic skills, and behavioural difficulties	50	Phase 2
TRON (NCT01954693)	Interventional	Everolimus vs placebo	To assess the efficacy of everolimus in the treatment of neuropsychological deficits. Outcome variables include episodic memory, planning, spatial working memory, and dual tasking	To identify additional neuropsychiatric markers of change. Measures include psychiatric questionnaires, quality of life, adaptive behaviours, and autism rating scales	48	Phase 2
RAPT (NCT01929642)	Interventional (single participant design)	Everolimus and sirolimus	To assess the feasibility and safety of mTOR inhibitors in children with tuberous sclerosis and self-injurious behaviour. Outcome measures include compliance, carer burden, and parental stress	To measure behavioural change (including self-injury, repetitive behaviours, and autism characteristics) and EEG and MRI changes	3	Phase 2
RAPIT (NCT01730209)	Interventional	Everolimus vs placebo	To assess the efficacy of everolimus in the treatment of autism and neuropsychological deficits in children with intellectual disability. Primary outcome is intelligence quotient as measured by the Wechsler Preschool and Primary Scale of Intelligence	To assess efficacy on other neuropsychiatric characteristics. Outcome variables of interest include autism, working memory and attention, sleep, sensory profiles, and epilepsy	60	Phase 2/3
EEG=electroencephalogram. mTOR=mammalian target of rapamycin.						
Table: Summary of key interventional neurological and neuropsychiatric clinical trials in tuberous sclerosis registered on ClinicalTrials.gov						

Panel 2: Summary of the principal clinical recommendations for the management and follow-up of tuberous-sclerosis-related manifestations

Genetics

- Genetic testing and counselling should be offered to all patients who have never had genetic testing, particularly to individuals of reproductive age

CNS involvement

Brain lesions

- Brain MRI should be done every 1–3 years up to age 25 years. For cases in which suspicious lesions are identified, the frequency of MRI should be increased to, for instance, every 3–6 months

Epilepsy

- Obtain baseline routine EEG. If abnormal, especially if features of TAND are also present, follow-up with a 24-h video EEG to assess for subclinical seizure activity

TAND

- Screening for TAND using a quick screening instrument, such as the TAND checklist, should be done at least annually. A more comprehensive assessment should be completed at key developmental timepoints: infancy (0–3 years), preschool (3–6 years), before entry to middle school (6–9 years), adolescence (12–16 years), early adulthood (18–25 years), and as clinically indicated thereafter

Systemic involvement

Kidneys

- Abdominal MRI should be done every 1–3 years to assess progression of renal angiomyolipomas or cysts, or both
- Renal function and blood pressure should be assessed at least annually

Lungs

- Clinical interviews to assess for possible LAM symptoms should be done at every clinical assessment
- High-resolution chest CT should be done every 5–10 years in asymptomatic and undiagnosed individuals at risk of LAM. If a previous CT detected cysts, annual pulmonary function testing and CT should be done every 2–3 years

Skin

- Clinical assessment should be done annually

Teeth

- Clinical assessment should be done every 6 months, and panoramic radiographs should be done by age 7 years

Heart

- In asymptomatic patients, an echocardiogram should be taken every 1–3 years until regression of rhabdomyomas is noted. In symptomatic patients, the screening schedule should be modified according to individual needs

Eyes

- An ophthalmological examination should be done annually

Recommendations taken from Krueger and colleagues.¹⁸ TAND=tuberous-sclerosis-associated neuropsychiatric disorders. EEG=electroencephalogram. LAM=lymphangioliomyomatosis.

(NCT01954693) is a UK-based, single-centre, two-arm, individually randomised, phase 2, double-blind, placebo-controlled trial of everolimus versus placebo for the treatment of behavioural, psychiatric, and neuropsychological difficulties in adults with tuberous sclerosis. The RAPT study (NCT01929642) is a small phase 2 trial of the feasibility and safety of sirolimus or

Search strategy and selection criteria

We searched PubMed for peer-reviewed publications between Jan 1, 2008, and March 31, 2015, with the term “tuberous sclerosis” and found 2390 publications. We then searched for “tuberous sclerosis” AND “brain” (650 reports), “epilepsy” (509), “brain lesions” (155), “intellectual disability” (96), “subependymal giant cell astrocytomas” (138), “neurosurgery” (125), “diagnostic criteria” (81), “autism” (177), “ADHD” (14), “neuropsychiatric disorders” (24), “mTOR inhibitors” (271), and “neurobiology” (40). We included only papers written in English. We selected the newest studies and those most relevant to child and adult neurologists. We also included relevant historical references outside the search timeframe.

everolimus in three participants with tuberous sclerosis and self-injurious behaviour; intellectual and behavioural changes will also be measured. The phase 2/3 Dutch RAPIT study (NCT01730209) aims to assess the efficacy of everolimus versus placebo in the treatment of autism and neuropsychological deficits in children with intellectual disability.

Comprehensive care and multidisciplinary management

In view of the multisystem nature of tuberous sclerosis, optimum management of the disorder needs a well coordinated, multidisciplinary approach. Each individual with tuberous sclerosis will have a unique combination of physical and neuropsychiatric features, and these features are likely to change or emerge over time. For this reason, regular monitoring and surveillance is needed for all individuals with the disorder, as recommended by the International Tuberous Sclerosis Complex Consensus Panel (panel 2).¹⁸

Individuals with tuberous sclerosis and their families are often particularly concerned about transitional care. Transitions of care occur between preschool and school-aged services (typical school entry between 4 and 7 years of age), but tend to be most problematic from child and adolescent services to adult health services. A theoretically ideal model would be a lifespan service, in which no transitions are needed. However, health-care systems around the world differ substantially with regard to age cutoffs and flexibility and some countries even have dedicated transitional care services for individuals with tuberous sclerosis. Clinical teams need to consider how to optimise and manage transitional care arrangements and generate a transition plan in partnership with the family and the multidisciplinary team.

Conclusions and future directions

We are witnessing a substantial change in the treatment of tuberous sclerosis, moving from a symptom focused approach to a molecularly targeted disease focused one.

Targeting the mTOR pathway with mTOR inhibitors represents a promising treatment option for many neurological and non-neurological manifestations of the disorder. mTOR inhibition is an exciting area of research, but many questions remain unanswered. To what extent mTOR inhibitors prevent the onset of new manifestations versus reversing or slowing the progression of existing manifestations remains to be established. In neurological and neuropsychiatric manifestations, this differentiation is particularly important. Since a diagnosis of tuberous sclerosis is possible in utero, very early intervention is theoretically possible. However, preliminary data suggest substantial neurodevelopmental or neurotoxic risks. Findings from research undertaken in the next few years will be crucial to clarify how to identify the optimum time to start treatment, optimise the therapeutic regimen, and minimise side-effects. Findings from new clinical trials will clarify whether the intermittent use of mTOR inhibitors or long-term continuous use of low doses can maintain clinical efficacy without clinically significant side-effects. The identification of biological markers will hopefully help clinicians to identify individuals more likely to benefit from early mTOR inhibition.

Other inhibitors of the mTOR signalling pathway are in development, and preclinical studies might identify additional or new molecular treatment targets. In view of the rapid changes and progress in our understanding of tuberous sclerosis, clinicians will need to remain up to date with these developments. We anticipate that the clinical trials in progress will lead to an improved understanding of the place of mTOR inhibitors alongside other interventional strategies in the management of neurological and neuropsychiatric aspects of tuberous sclerosis.

Contributors

PC proposed and designed the manuscript and wrote the first draft of the neurological sections. RM did the first scientific literature search, selected the most relevant articles to be considered for this Review, and prepared figures, the table, and panels. Pjdv wrote the first draft of the neuropsychiatric aspects and comprehensive care sections and contributed to manuscript writing and revisions. All the authors independently reviewed the results of the search of published work, read all selected articles, and revised and approved the final version.

Declaration of interests

PC was on the study steering committee of the EXIST-1 and EXIST-3 trials, funded by Novartis; is on the scientific advisory board of a natural history study of tuberous sclerosis, funded by Novartis; and has received honoraria from Novartis for participation in advisory board meetings. Pjdv was on the study steering committee of the EXIST-1, EXIST-2, and EXIST-3 trials, funded by Novartis; is on the scientific advisory board and working committee of a natural history study of tuberous sclerosis, funded by Novartis; is coprimary investigator on two phase 2 investigator-initiated studies, part-funded by Novartis; and has received honoraria from Novartis for participation in advisory board meetings. RM declares no competing interests.

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