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Genetic Etiologies, Diagnosis, and Treatment of Tuberous Sclerosis Complex

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Abstract

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder that affects multiple organ systems due to an inactivating variant in either *TSC1* or *TSC2*, resulting in the hyperactivation of the mechanistic target of rapamycin (mTOR) pathway. Dysregulated mTOR signaling results in increased cell growth and proliferation. Clinically, TSC patients exhibit great phenotypic variability, but the neurologic and neuropsychiatric manifestations of the disease have the greatest morbidity and mortality. TSC-associated epilepsy occurs in nearly all patients and is often difficult to treat because it is refractory to multiple antiseizure medications. The advent of mTOR inhibitors offers great promise in the treatment of TSC-associated epilepsy and other neurodevelopmental manifestations of the disease; however, the optimal timing of therapeutic intervention is not yet fully understood.

INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder caused by an inactivating variant in either *TSC1* or *TSC2* (22, 29, 42). It affects approximately 1 in 6,000–10,000 individuals, although the variable penetrance and subtle presentations of the disease mean that the incidence may actually be higher (20, 99). Inactivation of one of the *TSC* genes results in hyperactivation of the mechanistic target of rapamycin (mTOR) pathway and the development of benign tumors or hamartomas in multiple organ systems, including the skin, brain, eyes, heart, and kidneys. The brain is often the most severely affected organ system, resulting in developmental delay, epilepsy, and neurobehavioral or neuropsychiatric disorders [e.g., autism spectrum disorder (ASD), hyperactivity, and anxiety] (20, 100). However, the clinical manifestations of TSC demonstrate great phenotypic variability. Even in a family with an inherited form of TSC, the clinical symptoms can vary significantly among individuals.

Many patients with TSC come to medical attention as a newborn or a young child, but some have subtle symptoms and are not diagnosed until adulthood (for diagnostic criteria, see **Table 1**). Seizures or dermatologic manifestations (e.g., hypomelanotic macules, shagreen patch,

Table 1 Diagnostic criteria for tuberous sclerosis

Type of criteria	Description
Definite diagnosis	Two major features OR one major feature with two or more minor features OR a pathogenic variant in *TSC1* or *TSC2*
Possible diagnosis	One major feature OR two or more minor features
Genetic diagnostic criteria	A pathogenic variant in *TSC1* or *TSC2* identified in DNA from normal tissue, where a pathogenic variant (105, 106) is defined as a variant that inactivates the function of TSC1 or TSC2 [i.e., a frameshift (insertion or deletion) or nonsense variant], a variant that prevents protein synthesis (i.e., a large deletion), or a missense variant that has been shown by a functional study to affect the function of TSC1 or TSC2
Clinical diagnostic criteria	Major features: 1. At least three hypomelanotic macules that are at least 5 mm in diameter 2. At least three angiofibromas or fibrous cephalic plaque 3. At least two ungual fibromas 4. Shagreen patch 5. Multiple retinal hamartomas 6. Cortical dysplasias (tubers and cerebral white matter radial migration lines) 7. Subependymal nodules 8. Subependymal giant cell astrocytoma 9. Cardiac rhabdomyoma 10. Lymphangioleiomyomatosis[a] 11. At least two angiomyolipomas[a] Minor features: 1. "Confetti" skin lesions 2. At least three dental enamel pits 3. At least two intraoral fibromas 4. Retinal achromic patch 5. Multiple renal cysts 6. Nonrenal hamartomas

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[a]The combination of these two major clinical features (lymphangioleiomyomatosis and at least two angiomyolipomas) without other features does not meet the criteria for a definite diagnosis.

and/or facial angiofibromas) are often the first overt clinical symptoms that prompt medical evaluation in childhood, but in fact, the development of cardiac rhabdomyomas and cortical tubers—two of the most characteristic manifestations of TSC—occurs during embryogenesis. Cardiac rhabdomyomas can be detected by prenatal ultrasound as early as 20 weeks' gestation. Additionally, both immunohistochemical evidence and prenatal imaging studies suggest the presence of cortical tubers and subependymal nodules during fetal gestation (102, 108). Thus, abnormal neuronal development likely plays a role in the neurologic manifestations of TSC.

The characteristic TSC lesion of the kidneys is the angiomyolipoma, a benign tumor composed of abnormal vasculature and immature smooth-muscle and fat cells (20, 22) that affects 55–75% of patients. Although these tumors are typically asymptomatic, they can result in life-threatening emergencies due to bleeding from the spontaneous rupture of aneurysms when the angiomyolipoma exceeds 3 cm in diameter (20). Additionally, patients with TSC can develop renal epithelial cysts (33%), polycystic kidney disease (5%), and renal cell carcinoma (2–3%) (59; for a review of the clinical characteristics of TSC, see 20, 97). This review highlights the current understanding of both the genetic and molecular bases underlying the diverse nature of TSC, with particular emphasis on the neurologic and neurodevelopmental aspects of the disease.

NEUROLOGIC MANIFESTATIONS

Central nervous system involvement is almost always present and represents a significant cause of morbidity and mortality for individuals afflicted with TSC. Approximately 80–85% of TSC patients have at least one seizure in their lifetime, and nearly all patients develop epilepsy (16). Most patients present with seizures within the first year of life, often by three months of age, but some may not have their first seizure until adolescence or early adulthood. Infantile spasms are the presenting seizure type in approximately 37% of children with TSC, although most children with TSC and infantile spasms go on to develop other seizure types. Within this population, early onset of infantile spasms and/or seizures is often associated with medically refractory epilepsy that does not respond to multiple antiseizure medications (16, 20). Additionally, studies have shown a correlation between the age of seizure onset and intellectual disability, with earlier onset of seizures associated with more significant cognitive deficits (10, 16, 24). Although TSC-associated epilepsy is often difficult to treat, approximately one-third of patients achieve seizure freedom, including 20% of patients who previously had medically refractory epilepsy. Notably, agents that modulate synaptic activity and mTOR signaling—vigabatrin (a GABA transaminase inhibitor) and everolimus (an analog of the mTOR inhibitor rapamycin), respectively—have been most effective in controlling TSC-associated epilepsy (9, 56) (see the section titled Treatment of Tuberous Sclerosis Complex).

TSC results in three characteristic brain malformations or lesions: cortical tubers, subependymal nodules, and subependymal giant cell astrocytomas (SEGAs) (124). The presence of cortical tubers, a subtype of focal cortical dysplasia, within the cerebral cortex and/or subcortical white matter is a hallmark histopathological characteristic of TSC in the brain (82). Cortical tubers are composed of dysplastic neurons and multinucleated giant cells that display a loss of normal cortical lamination that is thought to occur in utero at between 7 and 20 weeks' gestation, during corticogenesis (37). Subependymal nodules, lesions less than 10 mm in diameter that line the lateral and third ventricles, are the most common brain lesions associated with TSC and are observed in 90% of patients (20, 59). Subependymal nodules are often asymptomatic; however, they can enlarge over time and may develop into SEGAs (lesions greater than 10 mm with more than 5 mm of growth). SEGAs are classified as benign, slow-growing grade I astrocytomas that can obstruct cerebrospinal fluid drainage at the level of the foramen of Monro, resulting in obstructive hydrocephalus that can cause worsening neurologic status and even death. SEGAs show a peak in incidence around age 9 and are rare both in newborns and after age 20 (109).

TUBEROUS SCLEROSIS COMPLEX–ASSOCIATED NEUROPSYCHIATRIC DISORDERS

Approximately 90% of patients with TSC are diagnosed with a range of neuropsychiatric conditions and neurobehavioral symptoms termed TSC-associated neuropsychiatric disorders (TAND) (59). The symptoms and disorders that fall under the umbrella of TAND include, but are not limited to, behavior problems, sleep disorders, ASD, attention deficit–hyperactivity disorder (ADHD), intellectual disability, and psychiatric disorders such as anxiety and mood disorder. De Vries et al. (27) recently developed a TAND screening tool and checklist to aid in the assessment and treatment of patients with TAND.

Analysis of the recent TOSCA (Tuberous Sclerosis Registry to Increase Disease Awareness) multicenter, international registry showed that, while some features of TAND—i.e., learning disabilities, intellectual disability, ASD, and ADHD—are relatively well recognized, the diagnosis of these disorders is often made later than it is in patients without TSC. ASD and ADHD are reported in approximately 20% of patients with TSC; however, the mean age of diagnosis of ASD in children with TSC is 7.8 years old (26), whereas the mean age of diagnosis in children without TSC ranges from 3 to 4 years old (68, 89). The TOSCA report also found that, while there is a significant increase in mood disorders—both anxiety and depression—in adults with TSC compared with children, there are still missing data regarding mood disorders and other psychiatric manifestations associated with TSC, likely leading to underdiagnosis (26). These conditions have a deep impact not only on the individuals affected with TSC but also on their family and friends, representing a significant psychosocial burden (26, 27). Taken together, these findings indicate that clinicians must be cognizant of the risk of TAND and continue to not only screen for but also provide treatment for patients with these disorders.

TUBEROUS SCLEROSIS COMPLEX AND mTOR SIGNALING

Although the clinical manifestations of TSC were first described in the late nineteenth century, *TSC2* was not identified as one of the causative genes until the early 1990s (36), which was followed soon thereafter by the identification of *TSC1* (43, 137). *TSC1* is located on chromosome 9q34.13 and encodes the protein hamartin, also known as TSC1, which comprises 1,164 amino acids (130 kDa). *TSC1* is composed of 23 exons, with exons 3–23 encoding the functional protein; exons 1 and 2 make up the 5′ untranslated region (2) and do not affect the encoded protein. *TSC2* is located on chromosome 16p13.3 and encodes tuberin, also known as TSC2, which comprises 1,807 amino acids (200 kDa). *TSC2* is composed of 42 exons, with exons 2–42 encoding the functional protein; exon 1 does not contain coding sequence and is purely part of the 5′ untranslated region (34).

The mTOR pathway mediates cell growth and metabolism in response to growth factors and the energy and nutritional status of the cell (**Figure 1**). TSC1 and TSC2, along with TBC1D7, form a heterotrimeric complex (the TSC protein complex) that functions as a tumor suppressor through its regulation of the mTOR pathway (28, 64). The TSC protein complex inhibits mTOR activation through the action of the GTPase-activating protein (GAP) domain in TSC2. TSC1 binds to TSC2 through both an N-terminal TSC2-interacting core domain and a C-terminal coiled-coil domain (45, 95, 113, 118). TSC1 is required for the formation of the TSC protein complex, and without it, TSC2 is subject to ubiquitination and degradation (45, 64, 113).

The mTOR signaling pathway has been described extensively (for reviews, see 77, 142). Briefly, mTOR is a conserved serine-threonine protein kinase that mediates cell growth, metabolism, and cell survival through the formation of two distinct multimeric complexes: mTOR complex 1 (mTORC1) and mTORC2 (**Figure 1**). mTORC1 is activated by Ras-homolog enriched in the

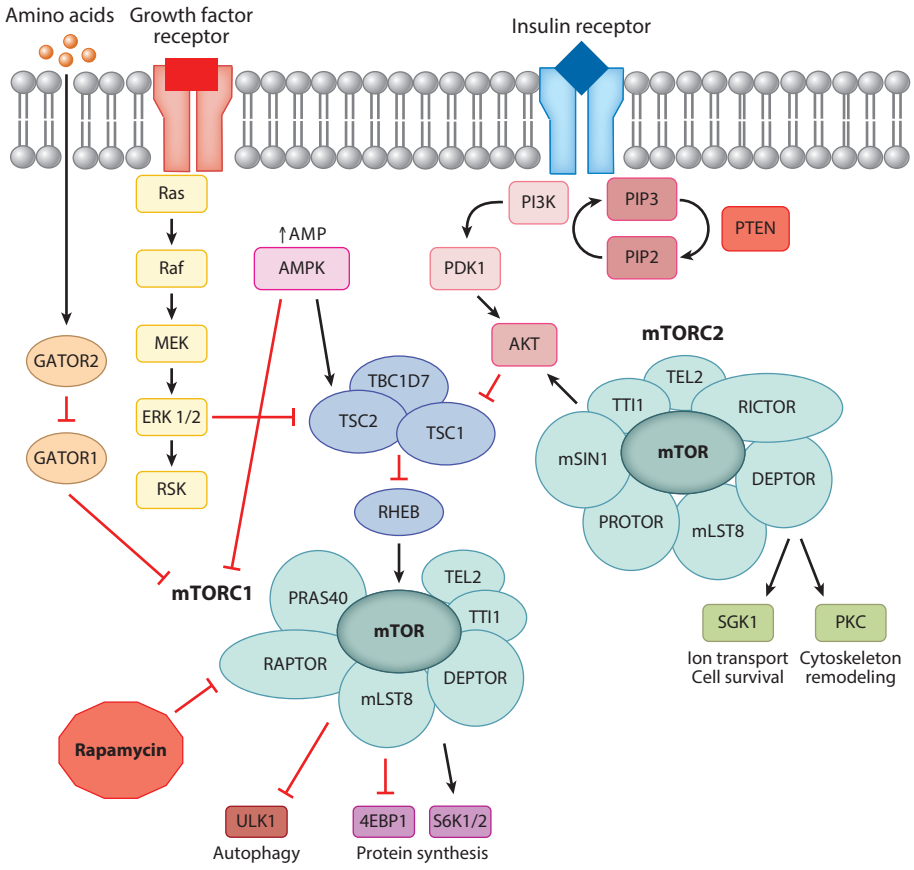

Figure 1

The mTOR signaling pathway. Three major upstream modulators—amino acids, energy (AMP), and growth factors and nutrients—regulate the pathway and its two complexes, mTORC1 and mTORC2. The presence of amino acids stimulates mTORC1 activity to promote cell growth and proliferation, whereas low-energy states activate AMPK, resulting in the inhibition of mTORC1 via the TSC protein complex and inhibition of RAPTOR. Growth factors and nutrients activate the PI3K/AKT and Ras/MEK/ERK pathways, resulting in TSC1/2-mediated disinhibition of mTORC1. The two mTOR complexes share four common components: mLST8, DEPTOR, TTI1, and TEL2. mTORC1 is defined by RAPTOR (a scaffolding protein essential to mTORC1 and sensitive to rapamycin) and PRAS40 (an inhibitor of mTORC1). mTORC2 is formed by mSIN1 (a molecule that is important for mTORC2-mediated activation of AKT), RICTOR (a scaffolding protein that is insensitive to rapamycin), and PROTOR (a scaffolding molecule that mediates activation of SGK1).

brain (RHEB), a small G protein of the Ras family (22). RHEB bound to GTP activates mTORC1 (28, 119). The TSC protein complex stimulates hydrolysis of GTP bound to RHEB and subsequent RHEB inactivation, resulting in inhibition of mTORC1 (8). In the absence of a functional TSC protein complex, RHEB is constitutively active, resulting in the hyperactivation of mTORC1.

mTORC1 integrates several upstream signaling pathways, including inputs from growth factors, amino acids, energy status, and cellular stressors (e.g., hypoxia), to serve as a critical regulator of the homeostasis between cell catabolism and anabolism (115). Under growth-promoting conditions, the canonical mTORC1 pathway involving the phosphoinositide 3-kinase (PI3K)/AKT

pathway is activated. AKT enhances mTORC1 activity through the phosphorylation and inhibition of TSC2 and inhibits the negative regulator PRAS40 (115). During periods of low energy, as measured by increased AMP/ATP ratios, AMP-activated kinase (AMPK) inhibits mTORC1 by positively regulating TSC2 and phosphorylating RAPTOR, causing it to be sequestered and inactivated by 14-3-3 proteins (55). Thus, multiple upstream signaling pathways mediate mTORC1 activity through differential regulation of the TSC protein complex, PRAS40, and RAPTOR.

Downstream, mTORC1 promotes protein synthesis and translation through direct phosphorylation of the ribosomal protein S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E–binding protein (4EBP1) (22, 124). Phosphorylation of S6K1 by mTORC1 results in recruitment of S6K1 to the ribosome, where it enhances de novo synthesis of pyrimidines, thereby facilitating protein translation and cell growth (30, 124). mTORC1-mediated phosphorylation of 4EBP1 promotes cell cycle progression, resulting in improved protein translation by stabilizing the mRNA and allowing for efficient initiation and elongation (30, 119). Additionally, mTORC1 promotes de novo lipid synthesis through S6K1-dependent activation of sterol-responsive element–binding protein (SREBP) to provide for the formation of new cell membranes in growing cells.

mTORC1 is subject to allosteric inhibition by rapamycin (a macrolide produced by the *Streptomyces hygroscopicus* bacteria) and multiple derivative compounds. Crystallization of the mTOR kinase revealed that rapamycin forms a complex with FKBP12 that inhibits mTORC1 by binding to mTOR within the catalytic site, thus directly blocking substrate recruitment and mTORC1 activity (144). The mechanism by which rapamycin inhibits mTOR activity has great therapeutic potential in TSC and is discussed below (see the section titled Treatment of Tuberous Sclerosis Complex).

Although the upstream signaling pathways mediating mTORC2 activity are not well defined, there is evidence that growth factor signaling, acting through PI3K, regulates mTORC2 activation (78, 85). Once activated, mTORC2 regulates multiple downstream molecules and pathways, including Rho GTPases, AKT, protein kinase C (PKC), and serum- and glucocorticoid-regulated protein kinase 1 (SGK1) signaling, to affect cell survival, cell cycle progression, and actin cytoskeleton remodeling (66, 78, 125). Specifically, mTORC2 is required for full activation of AKT and is involved in neuronal differentiation (65, 84, 140). Pharmacologically, mTORC2 is insensitive to acute rapamycin treatment, as it does not bind FKBP12. However, prolonged rapamycin treatment is thought to inhibit mTORC2 activity by reducing the amount of unbound mTOR available for mTORC2 assembly (114).

THE CLINICAL GENETICS OF TUBEROUS SCLEROSIS COMPLEX

TSC is an autosomal dominant disorder, where affected individuals have a 50% chance of transmitting the disorder. However, TSC often occurs in the absence of a family history, when de novo mutations have occurred in either *TSC1* or *TSC2*, known as sporadic cases. TSC follows the classic Knudson (71) concept of a tumor suppressor gene, where patients have a germline pathogenic variant in one allele of either *TSC1* (11, 52) or *TSC2* (53). Somatic cells then sustain a second hit, or loss of function of the other allele of the same gene, resulting in cells with complete loss of either TSC1 or TSC2, and these cells display dramatic mTORC1 hyperactivation and form tumors. This two-hit mechanism has been demonstrated most clearly for renal angiomyolipoma, facial angiofibroma, and SEGAs, but there is some evidence supporting it in lymphangioleiomyomatosis and cortical tubers (12, 13, 47, 90, 132). However, it is important to note that some clinical manifestations in TSC are likely due to the loss of one allele, or haploinsufficiency, including in the

brain. Mouse models missing only one copy of *Tsc1* or *Tsc2* exhibit morphological, physiological, and behavioral abnormalities (32, 49, 96, 123).

TSC1 AND *TSC2* PATHOGENIC VARIANTS

We used version 2 of the Leiden Open Variation Database (40) for TSC variants (106, 107) as our reference for a comprehensive review of the pathogenic variants in *TSC1* and *TSC2*. This database is constantly curated and updated by Sue Povey and Rosemary Ekong. It is a wonderful resource for both clinicians and TSC researchers alike and contains 6,388 pathogenic variants in *TSC1* (as of November 15, 2018) and *TSC2* (as of May 7, 2018).

TSC1 and *TSC2* sequence variants include both small variants, including single-nucleotide variants and insertions or deletions (indels) of less than 50 nucleotides, and large genomic variants of more than 50 nucleotides, usually deletions, which affect one or more exons of *TSC1* or *TSC2* and often extend to include portions of neighboring genes (74).

ASSESSMENT OF PATHOGENICITY

Both *TSC1* and *TSC2* are large genes with thousands of observed and possible variants. Although some variants clearly have functional consequences (e.g., nonsense variants, out-of-frame indels, and variants affecting canonical splice nucleotides), others, especially missense variants, are frequently of uncertain pathogenic effect. We used the following (fairly standard) criteria to assess the functional consequences of each variant: (*a*) Sequence variants occurring at conserved splice nucleotide positions (for the 5′ splice site, these are nucleotide positions −1, −2, and +1 through +5; for the 3′ splice site, these are nucleotide positions −15, −5 through −1, +1, and +2) were considered pathogenic unless noted otherwise in the database; (*b*) other intronic variants were considered pathogenic only if there were supporting functional data from a cell-based assay or evidence of de novo occurrence; (*c*) in-frame indels were considered pathogenic unless there was evidence to the contrary in the database; and (*d*) missense variants were considered pathogenic if they occurred de novo, segregated with disease in a family with several affected individuals, and/or were shown to disrupt protein function in a cell-based in vitro assay (18, 60, 61, 63, 94). Of the 6,388 pathogenic variants, *TSC1* variants constitute 26.4% (1,686 of 6,388), while *TSC2* variants account for 73.6% (4,702 of 6,388). Large genomic variants account for 2.8% of all *TSC1* variants and 6.4% of all *TSC2* variants. Therefore, here we focus on small pathogenic variants in these genes.

TSC1 PATHOGENIC VARIANTS

Of the 1,638 small pathogenic variants in *TSC1*, 617 are unique, and 27.1% of all small pathogenic variants are accounted for by 444 variants occurring at one of nine relative hot spots (**Figure 2**). Indels and nonsense variants together constitute the majority of *TSC1* small disease-causing variants (44.8% and 38.7%, respectively), while splice and missense pathogenic variants occur at a much lower frequency (12.0% and 4.5%, respectively) (**Figure 2**). The distribution of pathogenic variants in *TSC1* is highly nonuniform (**Figure 2*a***); 22.9% are in exon 15, while exon 8 has the highest density of variants per nucleotide (**Figure 2*b***). The distribution of pathogenic variants in *TSC1* is a result of both inherent mutability and whether the sequence change has an important effect. All recurrent nonsense variants in *TSC1* are C>T transitions in a CpG sequence context, which are known to occur through a molecular mechanism of deamination of a methylated C residue. These variants account for 20.0% of all *TSC1* small pathogenic variants. *TSC1*

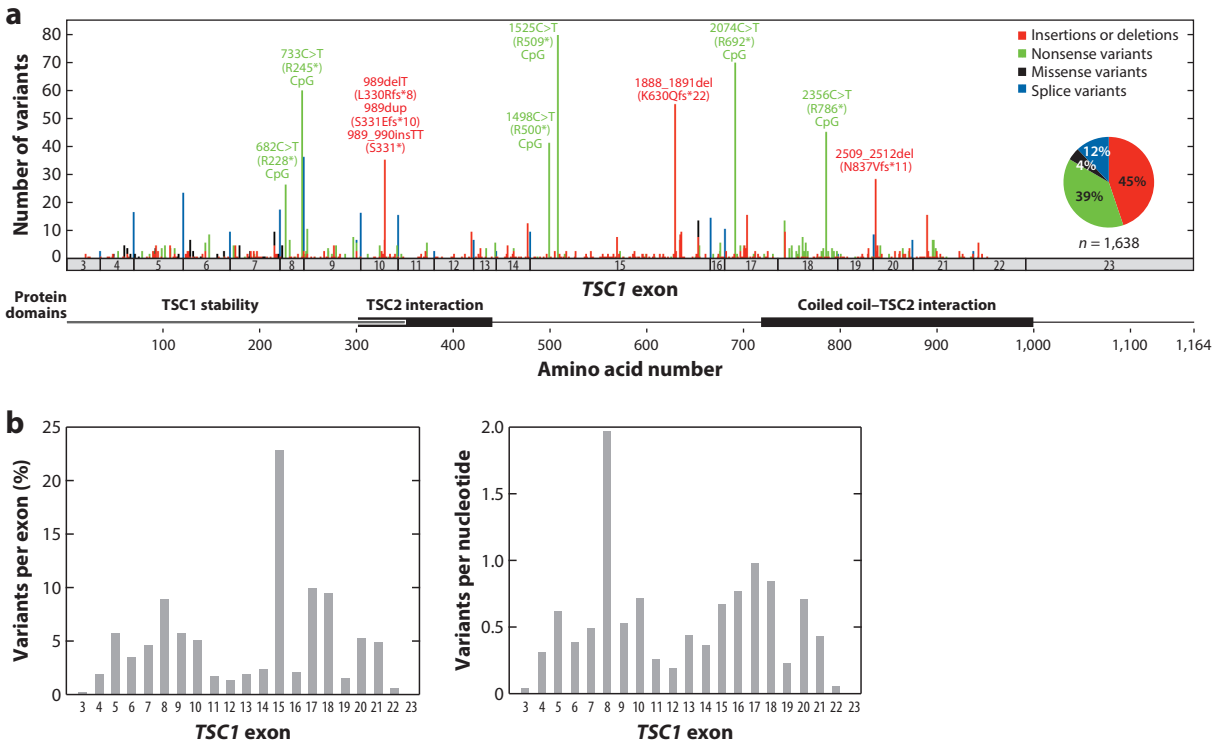

Figure 2

(*a*) Map of all pathogenic variants in *TSC1*, showing the location and number of variants at each nucleotide position. Variants are shown as vertical lines, with the color of the line indicating the type of variant, as shown in the legend. Hot spots with variants seen at least 25 times are labeled with the nucleotide and amino acid. When a given nucleotide position has more than one variant type, the variant types are stacked in one line. The locations of recurrent C>T transitions are labeled "CpG." Splice mutations are summed and shown as a single bar at each exon–exon junction. The amino acid numbering is according to *TSC1* transcript sequence NM_000368.4. The locations of the protein domains labeled "TSC2 interaction" and "coiled coil–TSC2 interaction" were determined based on Reference 138 and the UniProt database (134), respectively. In the protein domain track, the location of the region labeled "TSC1 stability" was determined based on Reference 62. (*b*) Bar plots showing the density of *TSC1* variants per exon (i.e., the fraction of all observed pathogenic variants in each exon) and per nucleotide (i.e., for each exon, the number of variants divided by the exon length).

missense variants are rare overall and largely (27 of 28 unique variants) occur within exons 3–10, which is important for TSC1 protein stability (62).

TSC2 PATHOGENIC VARIANTS

Small pathogenic variants account for 4,402 of the 4,702 variants identified in *TSC2*. Of these small pathogenic variants, 1,595 are unique, and 26.7% of all small pathogenic variants are accounted for by 1,174 variants occurring at one of 16 relative hot spots (**Figure 3**). Indels are the most frequently observed variant (32.6%), while missense, nonsense, and splice variants occur at somewhat lower frequencies (26.0%, 21.5%, and 19.9%, respectively) (**Figure 3*a***). As in *TSC1*, the distribution of pathogenic variants in *TSC2* is highly nonuniform (**Figure 3*b***). Exons 16, 23, 33, and 40 are the most frequently mutated and constitute more than a quarter of all observed *TSC2* pathogenic variants. Exons 16, 23, and 40 also have the highest density of pathogenic variants per nucleotide. Two exons, 25 and 31, in *TSC2* are alternatively spliced and therefore are not present

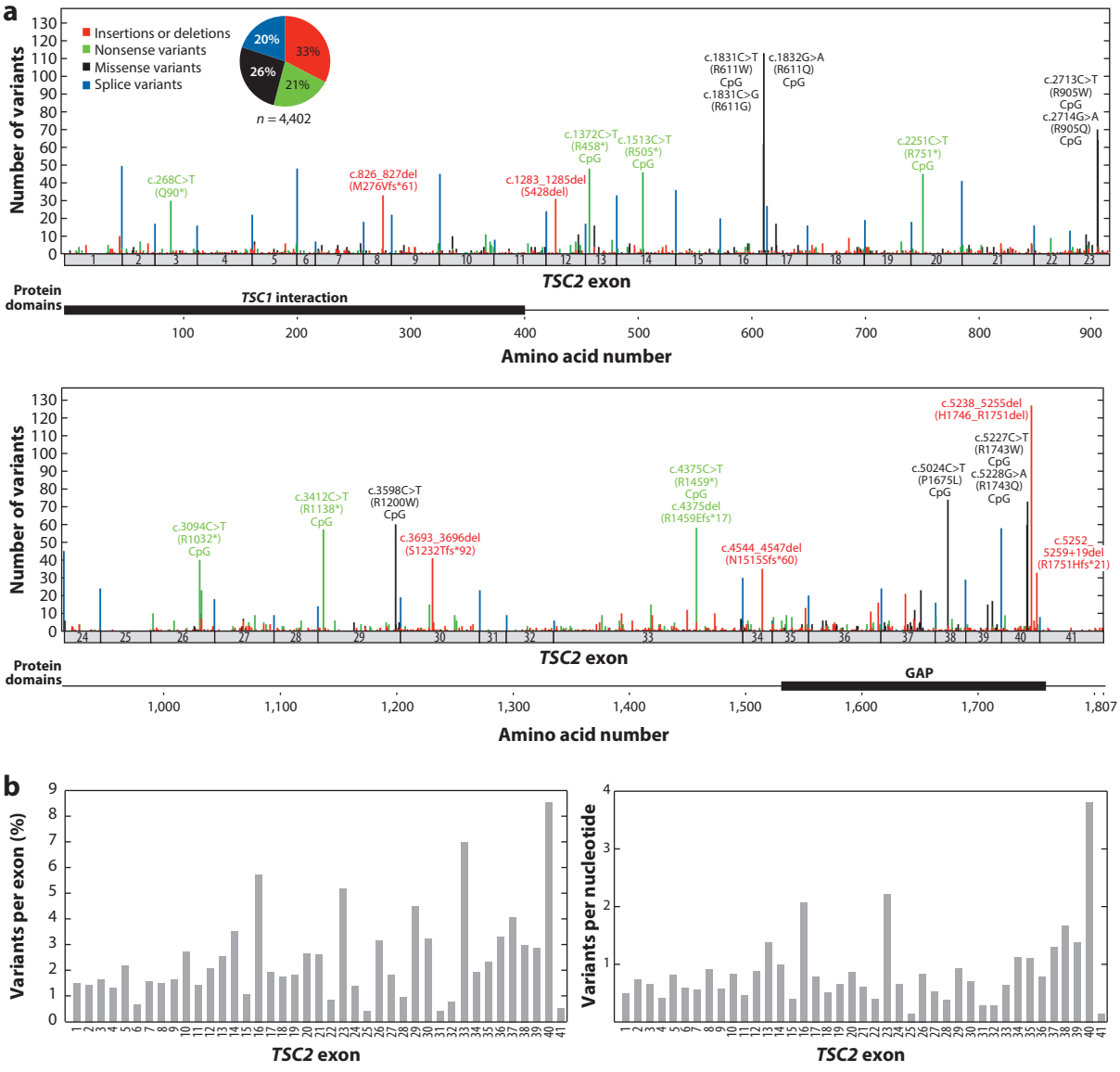

Figure 3

(*a*) Map of all pathogenic variants in *TSC2*, showing the location and number of variants at each nucleotide position. Variants are shown as vertical lines, with the color of the lines indicating the type of the variant, as shown in the legend. Hot spots with variants seen at least 30 times are labeled with the nucleotide and amino acid. When a given nucleotide position has more than one variant type, all variant types are stacked in one line. The locations of the recurrent C>T transitions are labeled "CpG." Splice variants are summed and shown as a single bar at each exon–exon junction. The amino acid numbering is according to *TSC2* transcript sequence NM_000548.5. The locations of the protein domains labeled "TSC1 interaction" and "GAP" were determined based on the UniProt database (134). Note that there is an additional upstream noncoding exon that is often labeled as exon 1, so all exon numbers shown here are therefore increased by 1. (*b*) Bar plots showing the density of *TSC2* variants per exon (i.e., the fraction of all observed pathogenic variants in each exon) and per nucleotide (i.e., for each exon, the number of variants divided by the exon length).

in all *TSC2* mRNA, and these exons sustain nonsense variants without causing TSC (34). Rare disease-causing splice variants have been reported in the database for these exons. As in *TSC1*, all recurrent nonsense and missense variants in *TSC2* occur as C>T transitions at CpG sites. Recurrent indel variants ($n = 304$) are also relative hot spots. Small pathogenic *TSC2* variants appear to be enriched in the 227-amino-acid/681-nucleotide C-terminal GAP domain (**Figure 3*a***). Finally, although hot spots have multiple recurrent variants, it is notable that there are many ($n = 916$) pathogenic small variants in *TSC2* that have been seen only once (**Figure 3**).

LARGE DELETIONS IN *TSC1* AND *TSC2*

Large deletions in *TSC1* and *TSC2* are relatively rare, accounting for 2.8% of all *TSC1* disease-causing variants and 6.4% of all *TSC2* disease-causing variants. Large deletions often extend into adjacent genes. Deletions at the 3′ end of *TSC2* often extend into the closely adjacent *PKD1* gene and cause a unique clinical phenotype of accelerated polycystic kidney disease. Such individuals may have multiple renal cysts identified at birth and tend to progress to renal failure by the teenage years.

TUBEROUS SCLEROSIS COMPLEX MOSAICISM

Mosaicism is the condition in which different cells in a multicellular organism have different genetic constitutions. In TSC, mosaicism occurs when a pathogenic variant arises due to a mutation in a single cell during early embryogenesis and is then inherited by all progeny cells derived from that cell during development. Often mosaicism is generalized, meaning that all tissues in the child or adult have some cells containing the *TSC1* or *TSC2* variant. However, the variant is often not distributed evenly across all tissues and cell types. For example, unilateral facial angiofibromas have been described, suggesting that there was an asymmetric distribution of cells with a *TSC2* pathogenic variant in the facial skin (7). Although mosaicism has been known in TSC for many years (111, 139), recent studies using newer sequencing methods (massively parallel sequencing) have highlighted its frequency and clinical implications (131). It is closely related to the situation of TSC individuals in whom clinical testing has resulted in no mutation identified (NMI).

NO MUTATION IDENTIFIED

Previously, conventional mutation analysis led to identification of a causative, pathologic heterozygous variant in either *TSC1* or *TSC2* in approximately 85% of TSC patients (25, 69), with the remaining 15% of patients categorized as NMI. Although several hypotheses were proposed to explain this occurrence, it is now clear that the vast majority are due to mosaic, usually low-frequency pathogenic variants, mainly in *TSC2*. In the largest study reported to date, a pathogenic variant in either *TSC1* or *TSC2* was identified in 45 of 53 TSC NMI patients (85%). The majority of these variants (26 of 45, 58%) occurred as mosaic variants, with 17 of 45 (38%) present at an allele frequency of less than 5%, 5 of 45 (11%) present at an allele frequency of less than 1%, and 2 of 45 (4%) present only in skin tumor biopsies (not in blood or saliva). The remaining 8 individuals (18%) in this study, in whom no mutation was identified despite extensive testing, were classified as persistent NMI. The clinical features of TSC were milder in the mosaic individuals in this cohort, in comparison with those with heterozygote TSC variants, while the persistent NMI individuals had the mildest clinical features.

PHENOTYPE AND GENOTYPE IN TUBEROUS SCLEROSIS COMPLEX

As stated above, TSC exhibits great phenotypic diversity, including high variability in the clinical manifestations of the disease among patients with the same pathogenic variant. In general, pathogenic variants in *TSC2* are associated with a more severe phenotype of TSC than pathogenic variants in *TSC1* (3, 24, 25, 59, 112). With regard to the neurologic manifestations of TSC, *TSC2* variants are associated with earlier onset of seizures, and the seizures are often more refractory and harder to treat than those of patients with *TSC1* variants. Additionally, patients with *TSC2* variants represent a higher percentage of patients diagnosed with infantile spasms compared with *TSC1* or NMI patients (16, 24). Interestingly, one study has shown that missense variants in exons 23–33 of *TSC2* actually decrease the risk for development of infantile spasms (136), but this has not been confirmed in other populations. *TSC2* variants are also associated with a higher rate of ASD, as well as intellectual disability. The differences in phenotype between *TSC1* and *TSC2* variants seem to result from two main effects. First, just as germline pathogenic variants are less common in *TSC1* than *TSC2*, the frequencies of second-hit events that are crucial for the development of tumors and cortical tubers in TSC are less common in *TSC1* than *TSC2*. Indeed, tumor counts in multiple organs and cortical tuber counts are lower in *TSC1* disease than they are in *TSC2* disease (25, 69). Second, it appears that loss of a single allele of *TSC1* has less effect on the functional activity of the TSC protein complex in the cell than does loss of an allele of *TSC2* (146). Although there is a difference in phenotype in population cohorts, it is not a major distinction, such that one cannot guarantee that a *TSC1* phenotype will be milder than that of *TSC2*. Hence, consensus guidelines for surveillance of developmental issues and tumor development in TSC do not depend on the gene or variant identified or level of mosaicism (97, 103).

EFFECTS OF mTOR DYSREGULATION IN THE NERVOUS SYSTEM

Hamartomatous lesions that form the basis of many of the characteristic lesions associated with TSC are the result of abnormal cell proliferation and differentiation, as well as aberrant migration. The clinical manifestations of TSC are phenotypically variable, and although variants in *TSC2* are purported to be associated with more severe disease phenotypes, no clear genotype–phenotype correlations can be made between TSC genes and phenotype (26, 88). Numerous in vitro, animal, and stem cell models have been developed to recapitulate many of the neurologic and neuropsychiatric features associated with TSC, which have identified molecular and genetic mechanisms by which pathogenic variants in *TSC1* or *TSC2* result in the clinical manifestations of TSC.

The formation of the central nervous system is a tightly regulated process that begins at three weeks' gestation. Neurogenesis is the process by which a neural progenitor cell (NPC) integrates multiple signals to determine whether to self-renew or differentiate into a neural cell line (i.e., neurons, oligodendrocytes, or astrocytes), migrate to the cortex, and form functional neuronal circuits. mTOR signaling regulates neural development at many levels, including neuronal stem cell differentiation, neuronal migration, synapse formation, and synaptic plasticity (19, 87). Aberrant mTOR signaling has been implicated in many disease states, including cancer, ASD, and related neurodevelopmental disabilities (for a review of mTOR and ASD, see 142).

TUBEROUS SCLEROSIS COMPLEX, mTOR, AND NEUROGENESIS

For neurogenesis to occur, a population of NPCs with the ability to self-renew and differentiate into terminal cell fates (i.e., neurons or glia) must be maintained. Both extrinsic and intrinsic

signals tightly regulate and maintain this pool of NPCs throughout development. The mTOR pathway is a major intrinsic signaling node that regulates NPC proliferation and differentiation throughout development, including in embryonic, fetal, postnatal, and aging brains (1, 87, 101). Initial studies aimed at identifying the role of mTOR signaling in neurogenesis were unsuccessful because germline deletion of *Tsc1*, *Tsc2*, *Mtor*, or mTOR-associated genes such as *Raptor* or *Rictor* was embryonic lethal (46, 54, 72, 73, 76). The advent of conditional gene expression and more advanced transgenic techniques that target cell-specific expression of genes at specific developmental time points has provided insight into the molecular mechanisms underlying mTOR-mediated neurogenesis.

Dysregulation of mTOR signaling during neural development alters the morphology of the brain both macroscopically and at the cellular level. Disruption of mTORC1 activity during embryogenesis via a conditional knockout of *Raptor* in cortical progenitor cells results in microcephaly beginning at embryonic day 17.5 due to decreased cortical thickness secondary to increased apoptosis, as well as decreased cell size due to alterations in cell cycle progression (17). Mouse models in which *Akt3* (31) or *Mtor* (70) has been deleted also exhibit decreased brain size and cell number, further supporting the role of mTORC1-mediated regulation of cell size and proliferation. Interestingly, Thomanetz et al. (125) showed that alteration of mTORC2 activity through the deletion of *Rictor* also results in decreased brain size but does so in an mTORC1-independent manner, affecting AKT and PKC signaling pathways to alter neuronal cell size and morphology. Conversely, upregulation of mTORC1 activity, as seen by increased S6K1 activity, through the loss of TSC1 (88) or TSC2 (141) results in megalencephaly with increased cortical thickness and dilation of the lateral ventricles, as well as early onset of seizures and early death, presumably due to status epilepticus. In the study by Magri et al. (88), postnatal administration of rapamycin in *Tsc1* conditional knockout mice resulted in not only the resolution of seizure activity but also the normalization of cortical thickness of the brain as well as decreased S6K1 activity.

Given that neurogenesis is predicated on maintaining a pool of NPCs with the ability to self-replicate and/or differentiate at specific developmental time points, it is not surprising that the mTOR signaling pathway plays a pivotal role in the cell cycle progression regulating these processes. AKT activity—particularly AKT1 and AKT3, the two major isoforms in the central nervous system—increases mTORC1 activity and regulates cell cycle progression, thereby influencing the proliferation or differentiation of NPCs (81). mTORC1 activation influences cell cycle progression by enhancing 4EBP-mediated translation of mRNAs controlling cell cycle transitions (30, 33). mTORC2 enhances mTORC1-mediated mechanisms of cell proliferation and differentiation via its action in the AKT pathway (84). Meanwhile, DEPTOR, an inhibitory protein common to both mTORC1 and mTORC2, influences the ability of a cell to self-renew by inhibiting mTORC1 (1). Conversely, mTORC1 hyperactivation results in a loss of stemness and a subsequent differentiation of progenitors into highly proliferative transient amplifying cells through 4EBP2-mediated cap-dependent translation (37, 57).

On a cellular level, the terminal differentiation of progenitors into neurons or glia is also influenced by alterations in mTOR signaling. Under physiologic conditions, differentiation of NPCs into neurons results in the upregulation of negative regulators of mTOR (TSC1, TSC2, and PTEN) (19). Studies in both mice and human embryonic stem cells suggest that increased mTOR activity due to loss of TSC1 (87) or TSC2 (19, 51) results in decreased neuronal differentiation and increased glial differentiation. However, while loss of mTORC1 activity due to deletion of *Raptor* results in deficits in gliogenesis, particularly oligodendrocyte differentiation, it does not affect neuronal differentiation (6, 17, 133).

Alterations in myelination are another neuropathologic characteristic of TSC. Myelination of neurons occurs when oligodendrocytes wrap and insulate the length of an axon. Normal

myelination requires not only the interaction of the oligodendrocytes but also radial glial cells and functional mTOR signaling. Deletion of *Tsc1* in the neurons of mice results in a hypomyelination due to the disruption of oligodendrocyte maturation through increased neuronal expression of connective tissue growth factor (CTGF) (35, 92). Although both mTORC1 and mTORC2 influence myelination, conditional deletion of *Raptor*, *Rictor*, and/or *Tsc1* in oligodendrocyte precursors showed that mTORC1 is the critical regulator affecting AKT signaling and lipid biosynthesis, while mTORC2 plays a more subtle role in oligodendrocyte function (6, 79). Additionally, continued, functional mTORC1 signaling in oligodendrocytes is required for the maintenance of myelinated fibers (79).

NEURONAL MIGRATION

The majority of neurogenesis and brain development is completed by the end of gestation; however, maturation, refinement and pruning of synapses, and myelination continue into late adolescence. Neuron formation begins at embryonic day 42 and is often completed by midgestation, at embryonic day 108 (116). During neurogenesis, NPCs residing in the subventricular zone asymmetrically divide, giving rise to neurons that migrate along radial cells to the cortical plate, where they differentiate into excitatory glutamatergic neurons to form the neocortex. However, in humans the majority of the radial glial cells are formed in the outer subventricular zone and are known as outer radial glial (oRG) cells, distinguishing them from the ventricular radial glial cells that make up of the majority of mouse radial cells. oRG cells allow for the formation and growth of a more complex neocortex and are thought to underlie the majority of human neurogenesis (105). The patterning and formation of the brain is the result of many genetic and cellular signaling pathways occurring in a particular spatiotemporal pattern to form the six-layer inside-out cortex, with the earliest neurons residing in the area closest to the subventricular zone and neurons produced later in development residing in the more superficial layers (81). Furthermore, postnatal neurogenesis persists in a subset of NPCs within the subventricular zone that differentiate into transient amplifying cells, which are highly proliferative and give rise to neuroblasts that can migrate tangentially from the subventricular zone via the rostral migratory stream to differentiate into mature inhibitory neurons within the olfactory bulb. While developing mouse brains have a small number of oRG-like cells, the massive expansion of oRG cells during human development makes this population of cells a tantalizing potential contributor to human-specific pathologies, including tuber formation in TSC.

Aberrant mTOR signaling during neuronal differentiation and migration underlies the characteristic neuropathologic features of TSC: cortical tubers, subependymal nodules, radial migration lines, and SEGAs. Dysregulated mTOR-mediated signaling during corticogenesis results in the formation of large, dysmorphic neurons with prominent, multinucleated soma that undergo abnormal migration and cortical lamination patterns that are characteristic of cortical tubers. Indeed, deletion of *Tsc1* in embryonic NPCs has resulted in the formation of tuber-like dysplasias and periventricular nodules in mouse models (38, 39, 149). Hyperactivation of mTORC1 activity through the loss of *TSC2* or upregulation of 4EBP-mediated cap-dependent translation during corticogenesis also results in the aberrant migration and soma enlargement of cortical neurons (83, 141). Interestingly, a recent study has shown that oRG cells have increased expression of many regulatory proteins of the mTOR pathway (98); however, further investigation must be performed to understand how variants in *TSC1* or *TSC2* affect oRG neurogenesis and migration. Lin et al. (83) showed that the neurons with aberrant migration also undergo a change in their molecular identity, acquiring the characteristics of the cortical layer they ultimately resided in rather than their initial fate, thereby altering the synaptic network. The pathologic changes within cortical tubers

and other characteristic features of TSC occur predominantly in a cell-autonomous manner—that is, only cells harboring the inactivating variant in either *TSC1* or *TSC2* are affected and undergo altered neurogenesis and abnormal neuronal migration (50, 92). However, some studies have also reported non-cell-autonomous migration defects following in utero electroporation with a *Tsc2*-knockdown construct (129).

AXON AND DENDRITE FORMATION

Neurite formation—that is, the formation of axons and dendrites—is essential to forming functional neuronal circuits. During normal development, neurons typically form a single axon and multiple dendrites to facilitate the propagation of electrochemical information within a neuronal circuit. Newly differentiated neurons begin to undergo axon–dendrite polarization as soon as they exit the cell cycle and begin to migrate to the cortex (4). Many signaling pathways, including mTOR activity, mediate neuronal polarity and neurite formation (for a full review, see 4); here, we discuss not only the role of mTOR signaling in neurite formation in TSC but also how these changes at the cellular level may affect neuronal circuit formation, accounting for the phenotypic variability in TSC.

Interestingly, while TSC1 and TSC2 are expressed throughout the growth cones of cultured hippocampal cells, the phosphorylated, inactivated form of TSC2 is enriched only in axonal processes. Inhibition of mTOR activity through the overexpression of TSC1 or TSC2 or administration of rapamycin (14), as well as manipulation of the downstream targets of mTORC1 with constitutive activation of the translational repressor 4EBP1 or knockdown of S6K (80, 93), prevents axon formation in cultured hippocampal neurons and decreases axonal length in vivo (48). Conversely, in vitro modeling of mTORC1 hyperactivation through the conditional deletion of *Tsc1* or *Tsc2* (14) or overexpression of activated S6K (93) resulted in the formation of multiple ectopic axons. The increase in mTORC1 activity within growing axons corresponded with increased expression of the neuronal polarity protein SAD. Knockdown of SAD kinases in cultured hippocampal neurons in which *TSC2* was conditionally deleted decreased the formation of multiple axonal processes, further supporting the role of mTORC1-mediated axonogenesis (14). However, a recent in vivo mouse model that utilized in utero electroporation to induce constitutively active RHEB into the anterior cingulate cortex suggested that mTORC1 activity mediates the length of the axon rather than the number of axons (48). Furthermore, the authors showed that the effect of mTORC1 on axon length was mediated by GSK3β signaling and that inhibition of GSK3β activity prevented increased axonal growth in affected neurons without affecting physiological axonal growth. Although there is some controversy regarding the exact mechanisms by which mTORC1 regulates axonogenesis, it is clear that dysregulated mTORC1 activity results in altered neuronal connectivity.

Dendritic differentiation is a highly regulated process determined by external signals and activity-dependent afferent inputs from adjacent neurons, culminating in the formation of mature synaptic networks (143). Dendritic arborization—that is, the extension and branching of dendrites, the formation of dendritic spines to enhance synaptic transmission, and the pruning of both dendrites and spines into a mature network—is highly influenced by mTOR signaling (81, 143). Activation of the PI3K/AKT/mTORC1 pathway through the constitutive activation of upstream modulators of mTORC1 (67, 75) or the conditional deletion of *Pten* (15) promotes dendrite extension and branching in an mTORC1-sensitive manner, as these increases in dendritic arborization are blocked by rapamycin and/or overexpression of 4EBP1 (67). Additional evidence has shown that mTORC2-mediated activation of the AKT pathway is necessary for ensuring proper

dendritic growth in hippocampal neurons (135), as knockdown of either RAPTOR or RICTOR results in simplified dendritic arbors. Interestingly, Jaworski et al. (67) reported that there is a critical period for dendritic differentiation and arborization after which modulation of mTOR-mediated signaling no longer affects dendritic properties. Thus, tightly controlled mTORC1 and mTORC2 signaling is essential for proper dendritic arborization during development. However, recent data have also suggested the presence of mTOR-independent mechanisms of dendritogenesis in mice with *Tsc1* deletion, which have increased filamin A expression that is regulated by the MEK-ERK pathway (148), suggesting a potential new mechanism for therapeutic intervention in TSC.

Dendritic spines are highly specialized, actin-rich membranous protrusions that serve as the primary site of excitatory neurotransmission within the central nervous system (145). Mature spines are often mushroom shaped; they form synaptic connections with adjacent neurons and serve as the site of localized protein translation and synthesis within synaptic networks, thereby playing an important role in synaptogenesis and the regulation of neuronal circuits (145). While mTOR signaling is critical to the formation of both axons and dendrites, the role of mTOR in spine formation is not well understood. In fact, in vivo and in vitro studies have shown conflicting results regarding the role of mTOR on spine formation, with *TSC1* or *TSC2* loss resulting in decreased, unchanged, or increased spine density along dendrites (5, 91, 123, 127). Live cell imaging studies have recently shown that increased mTORC1 expression alone does not induce changes in spine morphology, but when paired with chemically induced LTP, it led to a significant increase in spine width (58). Additionally, there is evidence that loss of functional TSC protein complexes results in a decrease in the degradation of syntenin, a molecule that mediates cytoskeletal formation within spine heads, thereby resulting in impaired spine morphology and an increase in shaft synapses (117). Thus, the variation in the mTOR-mediated effects on dendritic spine formation is complex and is likely secondary to multiple factors, including the spatiotemporal alterations in TSC1 and TSC2 expression in conjunction with not only neuronal subtype but also changes in synaptic activity.

NEURONAL CIRCUITS AND EPILEPTOGENESIS

Synapses within the brain are highly dynamic. The strengthening or weakening of a synapse in response to neuronal activity, often with subsequent increases in localized protein synthesis at the synapse, is known as synaptic plasticity. Two specific forms of synaptic plasticity—long-term potentiation (LTP) and long-term depression—form the basis of learning and memory and are often affected in neurodevelopmental disorders such as TSC. *N*-methyl-D-aspartate (NMDA) receptor–dependent LTP within the hippocampus is one of the most studied and characterized models of learning and memory. NMDA-mediated LTP is induced by high-frequency stimulation that results in a protein-independent early phase of LTP and a protein-dependent late phase of LTP, leading to long-lasting changes in synaptic strength (122). Given that mTORC1 is a critical regulator of protein synthesis, it is not surprising that stimulation-induced LTP results in the activation of downstream mTORC1 targets, leading to increased protein translation that can be blocked if rapamycin is introduced during LTP induction (122, 130). Thus, mTORC1 plays an integral role in maintaining the changes in synaptic function secondary to synaptic plasticity.

Epileptogenesis is the process by which a normal neural network undergoes a functional change secondary to a genetic or environmental insult, increasing the probability of spontaneous recurrent seizures (104). In TSC, seizures are thought to be secondary to alterations in excitability that are

further influenced by the presence of cortical tubers, which display increased mTORC1 activity and decreased mTORC2 activity (44, 110). Functional studies have shown abnormalities in interneurons in *Tsc1* knockout mice, highlighting the role of TSC-mediated inhibitory GABAergic transmission. Enhanced excitatory, glutamatergic neurotransmission has been proposed to contribute to the initiation of seizures in TSC. In fact, cortical tubers have altered electrophysiologic properties due to increased expression of AMPA and NMDA receptors—ionotropic glutamate receptors that mediate fast excitatory neurotransmission in the brain. Specifically, the dysplastic neurons found in cortical tubers have increased expression of GluA1/GluA4-containing AMPA receptors, as well as increased GluN2B and GluN2C expression but decreased GluN2A-containing NMDA receptors (86, 120). Interestingly, the subunit expression pattern of NMDA receptors within cortical tubers mirrors that of immature synapses; early in development, NMDA receptors are predominantly composed of GluN2B-containing subunits and are localized perisynaptically, whereas maturation brings a developmental switch to GluN2A-containing NMDA receptors that are localized to the synapse (126). Thus, the changes in the subunit compositions of AMPA receptors and NMDA receptors, as well as decreased inhibitory neurotransmission within the cortical tubers and likely the surrounding perituberal cortex, may underlie the initiation of seizures in these areas (121).

Many antiseizure medications target inhibitory, GABAergic receptors to modulate the altered excitatory–inhibitory activity and prevent seizure activity. While patients with TSC often have refractory epilepsy even when treated with GABAergic agents, children with infantile spasms often respond well to vigabatrin, consistent with the notion that TSC affects inhibitory neuronal circuits within the brain. Inhibitory neurotransmission is mediated by the $GABA_A$ receptor, a chloride permeable ion channel. During development, the flow of chloride ions across the membrane is mediated by two major transporters: NKCC1 and KCC2 (121). In a mature neuron, the intracellular chloride concentration is low due to the activity of KCC2 transporters; therefore, when $GABA_A$ receptors are activated, there is an influx of chloride ions into the cell, further hyperpolarizing the cell and making it less likely to fire an action potential. Conversely, immature neurons exhibit high expression of the NKCC1 transporter, resulting in an increased intracellular chloride concentration (121). Thus, early in development, activation of $GABA_A$ receptors results in excitation and the generation of an action potential due to the efflux of chloride out of the cell, with subsequent membrane depolarization.

Analysis of cortical tubers in TSC shows impaired inhibitory neurotransmission with alterations in $GABA_A$ receptor expression, as well as dysregulation of NKCC1 and KCC2 expression. In TSC, there is decreased expression of the α_1 subunit of $GABA_A$ receptors, resulting in decreased sensitivity to benzodiazepines, which may account for the refractory nature of TSC-associated epilepsy to many GABAergic medications other than vigabatrin (121). Furthermore, cortical tubers display an immature chloride transporter phenotype with increased KCC2 and decreased NKCC1 expression, possibly secondary to mTORC2 dysfunction, as PKC, a downstream effector of mTORC2, mediates the expression of KCC2 (121). Additionally, conditional deletion of *Tsc1* in GABAergic interneuron progenitor cells results in a reduction in the number of cortical and hippocampal interneurons, while the remaining cells are enlarged and dysmorphic and display increased mTORC1 signaling (44).

Given the persistence of an immature phenotype with alterations in both glutamatergic and GABAergic receptor expression in cortical tubers, one could surmise that the surgical removal of cortical tubers and the perituberal cortex should lead to seizure freedom. However, studies have shown that surgical removal of even highly epileptogenic tubers does not necessarily cure one's epilepsy, suggesting that there may be secondary changes in the neuronal network contributing to the epileptogenicity, as a result of the altered neuronal phenotypes observed in TSC. Thus,

further investigation must be performed to understand the multifactorial mechanisms underlying the refractory epilepsy observed in TSC.

TREATMENT OF TUBEROUS SCLEROSIS COMPLEX

Given that TSC is the result of dysregulation of the mTOR signaling pathway, the advent of mTOR inhibitors such as everolimus, an analog of rapamycin, has provided great therapeutic promise in treating the mTORC1-mediated sequelae of TSC. Multiple studies have recently looked at the efficacy and safety of everolimus for treating TSC-associated sequelae. The EXIST-1 (Efficacy and Safety of Everolimus for Subependymal Giant Cell Astrocytomas Associated with Tuberous Sclerosis Complex) trial showed that everolimus reduces not only the size and progression of SEGAs but also the burden of angiomyolipomas and skin lesions in patients with TSC (41). However, withdrawal of everolimus is associated with regrowth of SEGAs (21). The EXIST-3 (Everolimus as Adjunctive Therapy for Patients with Tuberous Sclerosis Complex and Refractory Partial-Onset Seizures) trial showed that adjunctive treatment with everolimus resulted in a sustained decrease in seizure frequency in children and adolescents, with larger reductions in seizure frequency occurring in children less than six years old (23). Both the EXIST-1 and EXIST-3 trials have provided evidence that, while everolimus treatment is safe, it can have some systemic side effects, including stomatitis and increased risk of infection (23, 41).

Because the onset of seizure activity is an important prognostic indicator of neurodevelopmental outcomes in children with TSC, it is important to be able to accurately identify seizures in children with TSC as soon as possible to allow for early therapeutic intervention. Two ongoing trials are currently focusing on TSC-associated epilepsy. EPISTOP (Long-Term, Prospective Study Evaluating Clinical and Molecular Biomarkers of Epileptogenesis in a Genetic Model of Epilepsy–Tuberous Sclerosis Complex; ClinicalTrials.gov identifier NCT02098759) is a randomized clinical trial comparing vigabatrin with no treatment for EEG abnormalities indicative of epileptogenesis in TSC infants, as well as performing intensive characterization of possible molecular biomarkers of epilepsy. PREVeNT (Preventing Epilepsy Using Vigabatrin in Infants with Tuberous Sclerosis Complex; ClinicalTrials.gov identifier NCT02849457) seeks to determine whether the early identification and treatment of children with TSC-associated seizures improve developmental outcomes. The first-line treatment for children with TSC who present with infantile spasms is vigabatrin, rather than adrenocorticotropic hormone or corticosteroids, as used in children with non-TSC-associated infantile spasms. The mechanism by which vigabatrin treats infantile spasms in TSC is not well understood. It not only inhibits GABA transaminase, thereby preventing the breakdown of GABA within the brain and enhancing inhibitory neurotransmission, but may also decrease mTOR activation (147). Regardless of the mechanism of action, vigabatrin is a highly effective treatment for infantile spasms in TSC, resulting in the cessation of spasms in 95% of patients, often within one or two doses of the medication (56), highlighting the importance of the timing of intervention.

Recent studies have demonstrated that rapamycin-therapy-sensitive periods exist for autistic-like social impairments and behaviors in a mouse model of TSC and ASD in which *Tsc1* was deleted (127, 128). Treating these mice with rapamycin either at postnatal day 7 or at 6 weeks of age rescued not only the social deficits in the mutant mice but also the structural and electrophysiologic changes observed in the cerebellar Purkinje cells lacking *Tsc1*. Treatment with rapamycin at later ages did not rescue the autistic-like features, supporting the hypothesis that the timing of therapeutic intervention is crucial in mediating the neurodevelopmental sequelae of TSC (128). Thus, further research—both with animal models and through translational studies—must be performed to determine whether time-sensitive therapeutic windows exist for other neurodevelopmental aspects of TSC, including epilepsy and intellectual disability.

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