Mechanistic strategies for secondary prevention of developmental and epileptic encephalopathy in children with tuberous sclerosis complex

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Summary

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder caused by pathogenic variants in *TSC1* or *TSC2*, leading to mTOR pathway dysregulation and a spectrum of systemic and neurological manifestations. Tuberous Sclerosis Complex (TSC) is a multisystem genetic disorder frequently associated with early-onset, drug-resistant epilepsy, intellectual disability, and autism spectrum disorder—collectively known as TSC-associated developmental and epileptic encephalopathy (DEE). Advances in prenatal diagnostics and biomarker research now enable presymptomatic identification of high-risk infants. This review aims to synthesize current evidence on biomarker-informed, mechanism-based strategies for secondary prevention of DEE in TSC, offering a framework for personalized early interventions. Biomarkers, such as interictal epileptiform discharges, pathogenic TSC2 variants, and advanced neuroimaging metrics, predict epilepsy risk and neurodevelopmental trajectories. Preventive approaches include early initiation of vigabatrin and mTOR inhibitors, which show potential in reducing epilepsy severity and improving outcomes. Emerging strategies, including gene therapy, multi-omic profiling, and environmental enrichment, offer promise for disease modification. By linking predictive biomarkers to disease-modifying strategies, this review outlines a proactive and personalised approach to prevent or mitigate TSC-associated DEE. These insights help advance clinical decision-making and promote a shift toward precision prevention in paediatric epilepsy.

Keywords: Tuberous sclerosis complex (TSC); mTOR pathway dysregulation; Developmental and epileptic encephalopathy (DEE); Presymptomatic intervention; Biomarkers and precision medicine

Introduction

Genetic Developmental and Epileptic Encephalopathies (DEEs) refer to a diverse group of neurodevelopmental disorders characterised by early-onset, severe epilepsy and developmental encephalopathy with significant phenotypic spectrum.[1] Most DEEs begin early in life, often during infancy, and tend to worsen over time due to persistent seizures, contributing significantly to the global epilepsy burden in children.[2,3] The FDA-approved therapeutic compounds for epilepsy comprise more than 30 anti-seizure medications (ASMs).[4] Few of ASMs are specifically approved for DEEs, such as ganaxolone for cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder (CDD), fenfluramine and cannabidiol for Dravet syndrome (DS) and Lennox Gastaut Syndrome (LGS) and cannabidiol for Tuberous Sclerosis Complex (TSC).[5] Despite the approval of these new compounds many children with DEEs still remain drug-resistant, and no cure is currently available.[6–8] Advancements in understanding the mechanisms underlying some monogenic epilepsies may offer a more proactive approach,[9] including targeted therapy and personalised secondary prevention to minimise multi-morbidities and associated neurodevelopmental disorders.[10,11]

Tuberous Sclerosis Complex (TSC) is a multi-systemic autosomal dominant disorder where early identification of the disease is possible during the pre-clinical stage.[8,12,13] TSC is caused by pathogenic variants

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in the *TSC1* and *TSC2* genes, leading to the overactivation of mTOR pathway and abnormal growth and proliferation of benign tumours in various organs, including heart, brain, kidneys, and skin, with wide phenotypic variability even within the same family.[14] TSC is a syndrome in evolution and manifests progressively from the neonatal period, with age-dependent symptoms.[15] New diagnostic criteria include eleven major (including cortical tubers, subependymal nodules, hypomelanotic macules and cardiac rhabdomyomas) and seven minor features (such as "confetti" skin lesions and intraoral fibromas).[16] There are currently 1429 unique public DNA variants for TSC1 and 4853 for TSC2 reported in the Leiden Open Variation Database (www.lovd.nl/TSC1 and www.lovd.nl/TSC2) but most pathogenic variants are unique to individuals or families, complicating genotype-phenotype correlations.[17,18] Genetic identification of *TSC* pathogenic variants suffices for diagnosis regardless of clinical findings.[16] Between 10 and 15% of patients with TSC meeting clinical criteria show no detectable genetic variants with conventional genetic testing.[16] There is a considerable number of individuals with low level mosaicism for *TSC2* pathogenic variants who are not clinically recognised.[19] Targeted genomic sequencing of *TSC1* and *TSC2* may reveal causal cryptic variants in individual for whom classical genetic testing (Next generation sequencing—NGS—and Multiplex Ligation-dependent Probe Amplification -MLPA) is normal.[20]

Epilepsy in TSC often begins in the first months of life, and infants presenting with focal seizures or infantile epileptic spasms at onset frequently develop drug resistant epilepsy with multifocal EEG abnormalities.[21] In TSC, early, frequent, and prolonged seizures can have long-lasting effects on brain function and development.[22] Intellectual abilities in individuals with TSC range from normal to severely impaired, with 57% presenting learning disabilities, 26–45% behavioural issues, including autism spectrum disorder[23] and 16–57% attention deficit hyperactivity disorder.[15,24] Despite recent advances in understanding how mTOR dysregulation affects early brain development, leading to TSC-associated DEE, the rate of drug resistant epilepsy in individuals with TSC remains stable over the years.[25] Current therapeutic options that act through inhibition of mTOR overactivation, including everolimus, rapamycin, cannabidiol, vigabatrin and ketogenic diet, may improve seizure outcomes but do not effectively address developmental symptoms, and no disease-modifying therapies are available to date.[8,26,27]

The aim of this review is to provide a focused and integrative synthesis of predictive biomarkers and mechanism-based strategies for secondary prevention of DEE in TSC. We explore how molecular, electrophysiological, and neuroimaging biomarkers can be used not only for early diagnosis but also for risk stratification and tailored therapeutic interventions. Particular emphasis is placed on evidence from prospective trials assessing pre-symptomatic use of vigabatrin and mTOR inhibitors, as well as non-pharmacological strategies such as environmental enrichment.

Unlike previous reviews, which often separately address epilepsy treatment or biomarker discovery, this article brings together these domains to outline a comprehensive, precision-medicine approach to early prevention in TSC. In doing so, we aim to provide clinicians and researchers with a strategic roadmap to improve outcomes for children at risk of TSC-associated DEE.

Foetal activation of the mTOR pathway

mTOR dysregulation plays a crucial role in a range of epileptogenic developmental disorders known as mTORopathies, including TSC, focal cortical dysplasia (FCD), and hemimegaloencephaly (HME), all of them characterised by early-onset drug resistant epilepsy.[28] mTOR, a serine/threonine protein kinase, is vital in various cellular processes and is crucial in central nervous system (CNS) development, affecting axon guidance, dendritic development, spine morphogenesis, synaptic plasticity, and memory function, though its exact contribution to specific neurological phenotypes is not fully understood.[29,30] In TSC, overactivation of mTOR is linked to abnormal cell differentiation, proliferation, and growth and causes alterations in cell types, axonal and dendritic growth, and oligodendroglial turnover.[31,32] mTOR upregulation also impacts glial cell function, with astrocyte and microglia activation associated with proinflammatory responses and cytokine production.[31,33]

Cortical tubers, the neuropathological basis of the epileptogenic cellular network, consist of cortical dyslamination areas containing different cell types, including dysmorphic neurons, giant cells, and reactive astrocytes. mTORC1 and mTORC2 signalling are activated in foetal tubers.[34,35] Cortical tuber formation is a prolonged, complex process starting with dysmorphic astrocytes by 19–20 gestational weeks, progressing with giant cells by 24 gestational weeks, and maturing with dysmorphic neurons by 36 gestational weeks, leading to the neuropathological substrate of the epileptogenic cellular network.[36] mTOR signalling's role in neuronal development and plasticity suggest it may cause the progressive abnormal axonal sprouting and neurogenesis involved in epileptogenesis and in the co-occurring developmental encephalopathy.[31] Additionally, mTOR dysregulation affects GABAergic system maturation, altering interneuron development and functions, thereby lowering the seizure threshold.[37] The underlying mechanism of epileptogenesis could be due to the disrupted excitatory-inhibitory balance as a consequence of neuronal network alterations.[38] This process is summarized in Fig. 1.

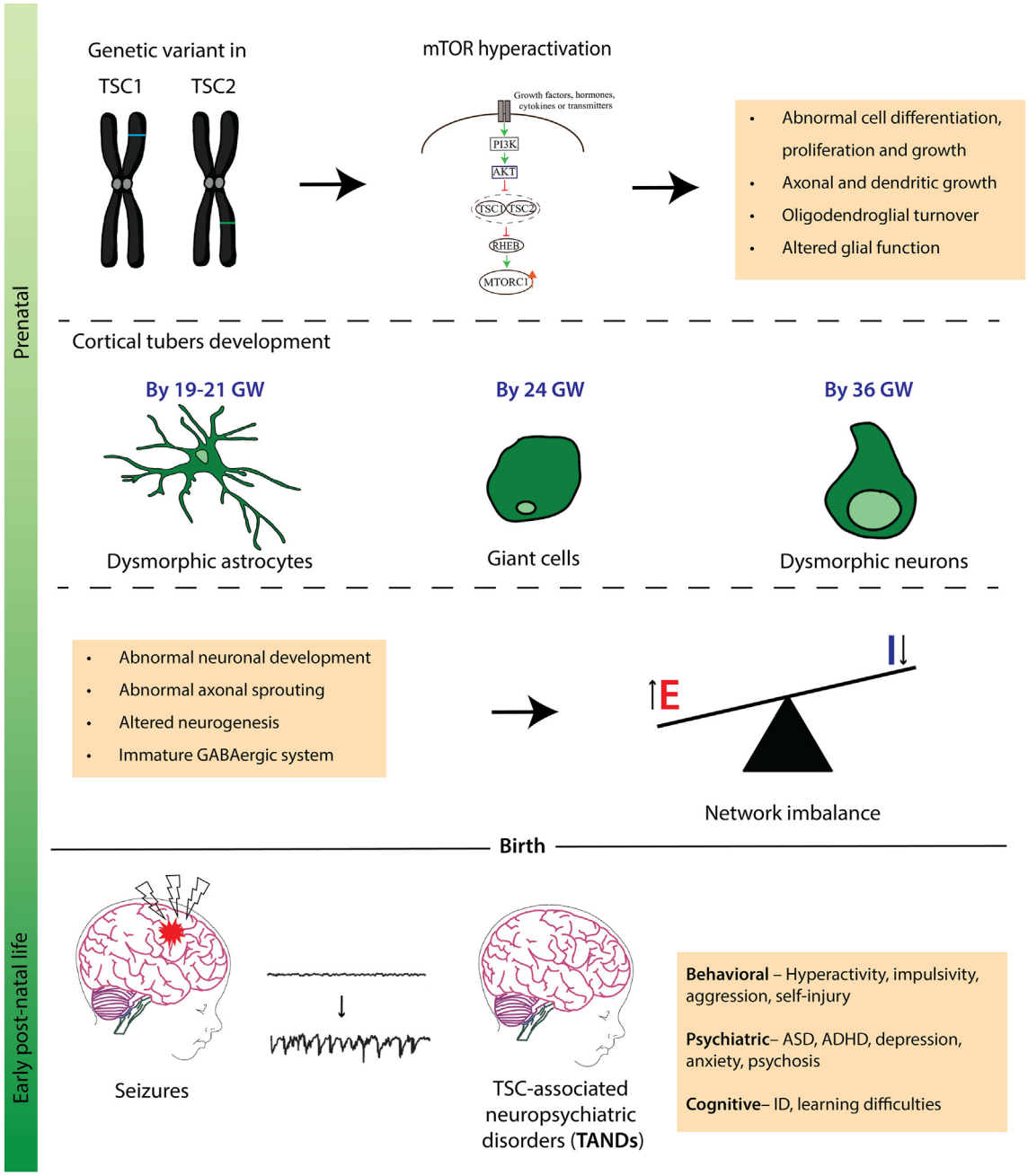

***Fig. 1:* Mechanistic model of prenatal and early postnatal brain development in Tuberous Sclerosis Complex (TSC).** Genetic mutations in the TSC1 or TSC2 genes lead to hyperactivation of the mTOR signalling pathway, resulting in abnormal cellular processes including differentiation, proliferation, axonal and dendritic growth, oligodendroglial turnover, and glial function. During prenatal development, this dysregulation contributes to formation of cortical tubers, characterized by the emergence of dysmorphic astrocytes by 19–21 gestational weeks (GW), giant cells by 24 GW, and dysmorphic neurons by 36 GW. These cellular abnormalities impair neuronal development, axonal sprouting, and neurogenesis, and lead to an immature GABAergic system. This culminates in an imbalance between excitatory (E) and inhibitory (I) neuronal networks, predisposing the brain to seizures and neuropsychiatric comorbidities, including autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), and intellectual disability (ID), observable after birth.

Timing and levels of mTOR overactivation may influence neuropathology and epilepsy severity in mouse models.[39] During brain development, epileptogenesis in TSC involves various convergent and divergent mechanisms progressively establishing a pathological neuronal network capable of generating seizures and co-occurring

neurodevelopmental disorders.[31] In a *TSC1* Knock Out model, hyperactivity of the mTORC1 pathway leading to aberrant neuronal network due to pathological changes (astrogliosis, increased brain size, and neuronal disorganization) was reduced by rapamycin.[40–42] These findings suggest that a therapeutic window for prenatal interventions may exist before or during the critical period of brain lesions formation.[34] Several regulatory factors influencing the development and progressions of TSC associated epilepsy have been identified, such as protein complexes, RNAs and differentially expressed genes (DEGs) that modulate complement and coagulation cascades, glycosaminoglycans, and extracellular matrix-receptor interactions.[43,44]

Presymptomatic diagnosis and its value during the latent period of epileptogenesis

TSC often presents with cardiac rhabdomyomas, which can be diagnosed from 20 weeks' gestation by foetal ultrasound. Early TSC diagnosis is possible in 40% of foetuses initially presenting with a single cardiac rhabdomyoma and 80% with multiple rhabdomyomas.[45] Non-invasive prenatal diagnosis using cell-free DNA is feasible for TSC at an average and minimum gestational age of 17 and 8 weeks, respectively.[46] Advances in foetal imaging led to more than half of TSC diagnoses confirmed prenatally in children born after 2012.[25] In Sweden, TSC diagnosis is established within a month after birth in about 70% of children born between 2010 and 2020 presenting with cardiac rhabdomyomas in the neonatal period, with preventive EEG performed in 46% of individual with a neonatal TSC diagnosis prior to clinical seizures.[47] Foetal brain MRI at the end of gestation is supporting TSC diagnosis in children with cardiac rhabdomyoma. If prenatal genetic screening does not confirm TSC, newborns' skin should be examined immediately after birth, as hypomelanotic macula may appear within the first months. Neonatal brain MRI and sequencing of both TSC genes are suggested. Before clinical criteria are met, a genetic diagnosis of TSC should lead to strict surveillance for early identification of TSC clinical manifestations.[16]

Diagnosing TSC before seizure onset allows for close EEG monitoring during the first months of life, enabling early seizure detection and treatment.[48] However, in some countries universal EEG screening is not done or is variably performed.[49,50] Moreover, the current international guidelines recommend screening EEGs, but do not go as far as recommending prophylactic VGB for all patients. EEG monitoring can detect which infants with TSC are at the greatest risk of developing seizures, often several months before clinical symptoms appear. In fact, early epileptiform activity predicted seizure onset on average approximately 3.5 months in advance.[51] Early identification of autism spectrum disorder (ASD) in children with profound intellectual disability and behavioural difficulties is challenging.[52] Recently we gained deeper understanding of earlier developmental deviations in children with TSC who will develop ASD also.[22] In the EPISTOP trial infants with TSC who will later manifest ASD begin to show developmental divergence at 6 months of age.[53]

Identification of early predictive biomarkers of disease severity and progression

Simple characterization of different genetic, EEG and MRI features can be combined to predict the risk of epilepsy.[54]

Developing biomarkers for disease progression is crucial to predict the neurological outcome in presymptomatic infants at risk of developing epileptic seizures, ASD and developmental delay. *TSC2* pathogenic variants are associated with more severe neurological phenotypes than mosaic *TSC1* or *TSC2* variants,[55] although milder cases exist,[17] making individual predictions challenging.[56]

Pathogenic variants in critical regions, especially in the catalytic *TSC2* subunit, are associated with increased risk of disease severity, variants in less crucial exons and in non-coding regions may lead to milder phenotypes. Functional studies have demonstrated the importance of coding regions in the N-terminal domain, specifically exons 4–9, as well as exon 18 in the case of *TSC1*, while in case of *TSC2* approximately half of reported pathogenic missense variants encode the C-terminal domain. Understanding this relationship is crucial for effective clinical management.[57]

Biomarkers for drug resistant epilepsy

The occurrence of interictal epileptiform discharges has a 77% positive predictive value for developing seizures by the age of 24 months.[51,58] In the EPISTOP study the presence of early interictal multifocal EEG discharges was a predictor of early epilepsy onset and developmental delay especially in patients carrying *TSC2* pathogenic variants.[59] This creates an opportunity to identify infants at high risk of seizures and initiate potential antiepileptogenic treatment before clinical seizures manifest.[27] *TSC1* pathogenic variants are associated with later epilepsy onset compared to *TSC2* and NMI (No Mutation Identified).[54] *TSC2* variants, expected to result in non-protein product, are associated with higher risk of developing drug-resistant epilepsy. Some TSC2 variants may be associated with a milder phenotype: c.2714G > A (R905Q) genetic variant determined an unusual mild phenotype with hypomelanotic macules or focal seizures that remitted spontaneously or easily controlled with medication.[60] In a family the same genetic variant was responsible for both a severe and a very mild phenotype in different members.[61] Lastly

c.4255_4256delCA genetic variant has been associated with the presence of few minor features non-specific to TSC and no major diagnostic criteria.[56]

Abnormal EEG connectivity precedes infantile epileptic spasms onset in infants with TSC.[62] Furthermore, in a prospective observational cohort abnormal connectivity between tuber location and the bilateral globus pallidus was a stronger prediction of infantile epileptic spasms.[63] Moreover, in a recent neuroimaging study a high posterior dominance of cortical tubers, reduced fractional anisotropy, and increased mean diffusivity and radial diffusivity in subependymal nodules were significantly linked to increased risk of epileptogenicity.[64] A comprehensive multiomic analysis of 93 infants followed from birth up to the age of 2 years identified multiple predictors of epilepsy development in the EPISTOP prospective clinical trial of early intervention with Vigabatrin for presymptomatic epilepsy treatment.[43]

Biomarkers for intellectual disability (ID) and autism spectrum disorder (ASD)

Intellectual disability is associated with a history of infantile epileptic spams, cortical tubers burden and the presence of cortical tubers in specific brain areas such the parietal and occipital regions.[65] A slower posterior dominant rhythm on EEG at 24 months was significantly associated with lower cognitive and motor developmental quotients, while a higher fraction of EEGs without epileptiform discharges was associated with higher cognitive, language, and motor developmental quotients at 24 months.[59] Also, controlling focal seizures, and not just infantile epileptic spasms may reduce the impact of intellectual outcome.[21] Sleep disruption may impact on synaptogenesis, and it may provide insights on developmental encephalopathy susceptibility.[66] In children with TSC and drug resistant epilepsy, disruption of sleep architecture and fragmented sleep may contribute to the severity of cognitive impairment.[67,68] Pathogenic missense and in-frame *TSC2* variants are associated with more frequent occurrence of intellectual disability than frameshift and splice site variants,[69] and patients with TSC2 genetic variants are at higher risk of ASD.[23] The detection of brain lesions at foetal MRI correlates with the occurrence of abnormal neurodevelopment and the presence of ASD at the age of two years.[70] The TOSCA registry (2216 individuals) found that early seizure onset and higher seizure burden correlate with worse developmental outcomes, but prompt seizure control lowers the rate of intellectual disability.[21] Moreover, a recent study highlighted how accumulated seizures burden (including infantile epileptic spasms and focal seizures) rather than age at seizure onset, predict neurodevelopmental outcomes at 36 months of age in children with TSC.[71] Early refractory seizures may disrupt cortical connectivity, including the fusiform gyrus, contributing to the presence of autistic behaviours.[26]

Longitudinal analysis of diffusion tensor imaging (DTI) showed that fractional anisotropy in corpus callosum and mean diffusivity of the right inferior cerebellar peduncle were related to positive ADOS findings at 36 months in TACERN study.[72]

Finally, circulating miRNAs and isomiRs have been analysed to support standard clinical testing in the early risk assessment of ASD and ID development in patients with TSC.[73] Predictive biomarkers for high-risk groups are summarized in Table 1.

Mechanistic strategies for secondary prevention of TSC-associated DEE

Prenatal or early post-natal TSC diagnosis allows for presymptomatic treatment, enabling secondary prevention and personalized treatment strategies to reduce disease impact.

An early combined targeted treatment could mitigate the severity of neurological outcomes.

Targeting the GABA type a receptor downregulation with vigabatrin

Vigabatrin is prescribed for focal seizures and infantile epileptic spasms in infancy and early childhood.[21] It inhibits GABA transaminase, increasing GABA concentration in the brain, effectively reducing seizure frequency in children with TSC.[79] Vigabatrin also inhibits glial cell proliferation, reduces astrocyte numbers and mildly inhibits mTOR pathway, contributing to its disease specific efficacy.[80] The EPISTOP and PREVENT studies revealed that the use of pre-symptomatic Vigabatrin reduces the risk of infantile epileptic spasms.[27,81] Both trials failed to show a benefit on the neurodevelopmental outcome; however, the overall outcome at the age of 24 months was significantly better in both the studies than in any previously reported cohort.[22] Larger studies may be needed to confirm findings with regard to neurodevelopment in the EPISTOP and PREVENT trials.

Targeting the mTOR overactivation with mTOR inhibitors

The final analysis of EXIST 3 study demonstrated sustained efficacy of Everolimus as adjunctive therapy in children with TSC and drug resistant epilepsy, with tolerable safety profile.[82]

In a randomized double-blind placebo-controlled trial on 32 children with TSC, the use of Everolimus failed to show an improvement in cognitive functions.[83] In a trial with 249 children aged more than 2 years with TSC associated with drug resistant epilepsy, Everolimus significantly reduced seizures (28% for low exposure, 40% for high exposure) if compared to placebo,

Biomarkers	Reference	Study design and/or objectives	ASD/ID	Impending DRE epilepsy
Clinical	Nabbout, 2019[21]	Report of a large international cohort (TOSCA Study)	Early seizure onset and higher seizure burden correlated with worse developmental outcomes	–
	Moavero, 2019[74]	Evaluation of early clinical markers of ASD or DD in infants with an early diagnosis (EPISTOP Study)	Total score of ADOS test at 12 months differentiated children with a future diagnosis of ASD from children without	–
	Moavero, 2020[53]	Evaluation of the relationship between age at seizure onset and neurodevelopmental outcome at 24 months	Children with epilepsy (63.8%) had a higher risk of ASD	–
	Ihnen, 2024[71]	Evaluation of age at seizure onset and accumulated seizure burden contribution to neurodevelopmental outcomes through daily seizure diaries, serial neurodevelopmental testing and brain magnetic resonance imaging	Accumulated seizures burden rather than age at seizure onset predicted neurodevelopmental outcome at 36 months	–
EEG	Wu, 2019[51]	Scalp EEG on 40 pts with no previous history of seizures	–	IEDs had a 77.3% positive predictive value for developing seizures
	Davis, 2019[62]	Scalp EEG recordings collected prospectively in infants with TSC in the first year of life, comparing the earliest recorded EEG from infants prior to ES onset (n = 16) and from infants who did not develop ES (n = 28)	–	Infants who later developed ES had EEG connectivity in ↑ sleep, higher global efficiency and average clustering coefficients, shorter characteristic path lengths, and lower modularity across most frequency bands adjusted for age
	Cook, 2020[75]	Comparison of EEG measures during stage II sleep in TSC children who either did (ASD+) or did not (ASD-) over 36 months follow up	Relative alpha band power significantly elevated in the ASD + group at 24 months of age with smaller differences at younger ages, suggesting this may arise from differences in brain development	–
	De Ridder, 2021[76]	Association between timing and characteristics of the first EEG with IEDs and epilepsy and neurodevelopment at 24 months in a conventionally followed group (initiation of VGB after seizure onset) and a preventive group (initiation of VGB before seizures) (EPISTOP Study)	Lower age at first EEG with IEDs was associated with lower cognitive level in patients with *TSC2* genetic variant	FS on first EEG with IEDs was predictive of earlier seizure onset in the conventional group. Earlier recording of IEDs was associated with higher risk of DRE in all patients
MRI	Prohl, 2019[77]	Identification of white matter microstructural deficits associated with ASD in TSC	Subjects with TSC and ASD exhibited reduced fractional anisotropy in 9 of 17 white matter regions	–
	Hulshof, 2021[70]	Correlation of foetal brain MRI findings with epilepsy characteristics and neurodevelopment at 2 y	Low lesion sum score correlates with better cognitive and motor development and less often an ASD diagnosis	–
	Scherrer, 2020[78]	Evaluation of anatomical connectivity patterns of the fusiform gyrus for the risk of developing ASD	Fusiform gyrus connectivity captures the risk of developing autism as early as 1 y of age	–
	Cohen, 2021[63]	Segmented tubers from 123 children with (n = 74) and without (n = 49) ES from a prospective observational cohort	–	>95% of tuber locations was associated with ES (functionally connected to the globi pallidi and cerebellar vermis)
	Srivastava, 2024[72]	Analysis of longitudinal, repeated diffusion tensor imaging metrics to select white matter tracts in relation to positive ADOS test (TACERN Study)	Fractional anisotropy corpus callosum and mean diffusivity of the right inferior cerebellar peduncle were related to positive ADOS findings at 36 months	–
	Li, 2024[64]	Multimodal MRI biomarkers of epilepsy to develop a prediction model of epileptogenicity. Patients with TSC, 32 with and 16 without epilepsy underwent MRI.	–	Higher PD of cortical tubers and decreased FA, increased MD, and increased RD of subependymal nodules were associated with epileptogenicity in both the centre and perilesional tissue, independent of *TSC* gene variation.
Genetics	Ogórek, 2020[55]	Genotype-phenotype correlations in a cohort of 94 infants (EPISTOP Study)	–	Eight of 24 clinical features assessed at age 2 y were significantly less frequent in those with TSC1 versus TSC2 variants, including developmental delay drug-resistant epilepsy and median seizure-free survival
	Farach, 2024[54]	Genotype-phenotype correlations for epilepsy severity and onset in a comprehensively cohort followed from infancy (PREVeNT trial)	–	Presence of a *TSC2* pathogenic variant was significantly associated with DRE

(Table 1 continues on next page)

Biomarkers	Reference	Study design and/or objectives	ASD/ID	Impending DRE epilepsy
(Continued from previous page)				
Molecular	Scheper, 2022[73]	RNA sequencing analysis to define the miRNA and isoform (isomiR) expression patterns in serum, as part of the EPISTOP project	Circulating miRNAs and isomiRs have the potential to aid standard clinical testing in the early risk assessment of ASD and ID in TSC patients	–
	Huschner, 2023[43]	Multi-omic analysis of the EPISTOP trial, with 93 infants with TSC followed from birth to age 2 years, seeking of associations between analyte levels and risk of seizure development, development of drug-resistant epilepsy, and ongoing seizures at age 2 years	–	Significant differences in proteins, metabolites, and genes between age-matched control and TSC infants; difference in expression between control, TSC without epilepsy, and TSC with epilepsy groups. A multivariate approach identified multiple 3-variable predictors of epilepsy, with the best having a positive predictive value of 0.987

ADOS: Autism Diagnostic Observation Schedule; ASD: Autism Spectrum Disorder; DD: Developmental Delay; DRE: Drug Resistant Epilepsy; ES: epileptic spasm; FS: focal slowing; ID: Intellectual Disability; IEDs: interictal epileptiform discharges; mo: months; MRI: Magnetic Imaging Resonance; pz: patients; VGB: Vigabatrin; y: years; PD: proton-density mapping; FA: fractional anisotropy; MD: mean diffusivity; RD: radial diffusivity.

Table 1: **Biomarkers of prediction of autism spectrum disorders, intellectual disability and drug resistant epilepsy.**

especially in children under the age of 6 years.[84] A recent study showed that in individuals with TSC diagnosed prenatally, early intervention soon after birth with Sirolimus or Vigabatrin reduced the incidence of epilepsy, the percentage of patients with drug resistant epilepsy, and improved developmental outcomes.[85] mTORC1 inhibitors (e.g., Rapamycin) have shown therapeutic effects on cognitive impairment, synaptic plasticity, astrogliosis, and myelination deficits in TSC mouse models.[40,86,87] Th early use of mTORC1 inhibitors may improve white matter abnormalities.[88] Mouse models suggest that postnatal (days 13–15) Rapamycin treatment may reduce astroglia abnormalities and myelination defects, even if it seems not to act on cortical lamination.[89] Safety and efficacy of mTOR inhibitors in children under the age of 2 years have been reported.[90] Optimal timing for mTORC1 inhibitor treatment remains unclear. Everolimus is increasingly used in infants at high risk of cardiac rhabdomyomas. Anecdotal cases revealed clinical efficacy in prenatally detected cardiac rhabdomyomas treated with maternal Sirolimus, with appropriate short-term development.[85,91–93] More studies are required to assess the long-term potential toxicity of Sirolimus and to determine whether early mTOR inhibition may sometimes be too late to reverse pathological abnormalities and clinical symptoms. Cannabidiol and ketogenic diet may also reduce seizure frequency and could play a role in the regulation of the mTOR pathway.[94] Two randomized double blind controlled clinical trials (ViRap study, NCT04987463; TSC-STEPS trial, NCT05104983) have begun enrolling infants with TSC to evaluate the efficacy and safety of Vigabatrin versus Rapamycin as a preventative treatment and to evaluate the safety and efficacy of early Sirolimus treatment to prevent or delay seizure onset in infants with TSC. Results are expected at the end of 2026.

Targeting epileptogenic tuber with early surgery

Early delineation of the epileptogenic zone among multiple dysplastic lesions is a challenging step in presurgical evaluations of children with TSC.[95] Surgical interventions in children under 3 years of age positively impact on developmental trajectories particularly among patients affected by severe drug-resistant epilepsy and multiple EEG epileptiform abnormalities. While the presence of multiple lesions makes children with TSC poor candidates for surgery, resective surgery can always be considered if epileptogenic zone is confined to one or two lesions.[96] In such cases tuberectomy results in seizure freedom in about 60% of patients.[97] High number of cortical tubers, seizure onset before 12 months and multifocal EEG discharges may predict seizure recurrence after surgery in a cohort of 35 children with a mean follow up of 7.5 years.[96] The extent of seizure onset zone is another predictor of seizure freedom after epilepsy surgery.[98]

Focal tuber epileptogenic zone organization, in which seizures are confined to the dominant tuber was reported to be a predictor of better post-operative outcome.[99]

Identifying the epileptogenic zone remains challenging and requires comprehensive pre-surgical evaluation recommended after the failure of two antiseizure medications, evaluated through multimodal functional and structural imaging.[100] A hybrid approach of open surgery associated with minimally invasive surgery may have promise in the next future being effective and safe.[101] Optimal surgery timing is crucial to prevent infantile epileptic spasms syndrome (IESS), and a short duration of epilepsy and early surgery are associated with an improvement in postoperative language development.[102]

Targeting white matter abnormalities with environmental enrichment

The promise of environmental enrichment (EE) as a preventative strategy against neuropsychiatric disorders is especially high during early post-natal development when the brain is still able to undergo network reorganization.[103] First introduced by Donald Hebb in the late 1940s, the environmental enrichment paradigm illustrates how exposure to rich sensory, social, and cognitive stimuli can influence brain structure and function. Although methods vary across studies, research consistently shows that enriched environments promote positive behavioural and neuroanatomical changes in rodents - such as greater synaptic plasticity, improved motor coordination, and enhanced cognitive abilities, making it a widely used approach in the study of neurodevelopmental disorders, especially autism spectrum disorders.[104,105] EE, as a preventive strategy, has the potential to restore the neurodevelopmental consequences of mTOR overactivation and may improve the disruption of synaptic plasticity resulting in a more efficient transsynaptic communication flow.[106] In a pilot study of 5 children with TSC aged 1–3 years, early play-based and parent-mediated behavioural interventions were shown to improve communication skills.[107]

Gene therapy

We are entering an emerging era of gene-based therapeutics of significant potential particularly for rare genetic disorders. Future efforts should focus on exploring the possibility of applying a disease-modifying treatment to patients with TSC, and gene therapy may be a promising avenue for such a goal. Different gene therapy approaches are under development at a preclinical level. Potential treatment involves the supplementation of hamartin. In mouse models with *TSC1* variants, the delivery of human *TSC1* gene through retroorbital injection of AAV9 was able to improve brain pathology biomarkers, enhance rotarod performance, and increase life span.[108] In another preclinical study, systemic injection of AAV9 encoding a condensed form of tuberin extended survival in a model of TSC2, showing a potential alternative therapy for TSC2 individuals involving gene replacement using an AAV vector.[109] It is likely that the new AAV variants will further enhance brain cell transduction leading to improved symptomatic recovery.

Recently Guo and colleagues used CRISPR-Cas9-mediated homology directed repair in patient -derived induced pluripotent stem cells (iPSCs) to correct a frameshift and missense *TSC2* pathogenic variants.[110]

Implications for clinical practice

A proper diagnosis should be made as early as possible. Following detection of cardiac rhabdomyomas during pregnancy, foetal brain MRI can confirm a definitive diagnosis. Early detection of gene variants should occur as soon as the clinical diagnosis is made, since molecular diagnosis significantly influences clinical management and prognosis. Neonatal EEG monitoring may identify subclinical seizures in patients with large dysplastic lesions, and close EEG monitoring and developmental follow-up of TSC infants can improve neurological outcomes.

Pre-symptomatic treatment with Vigabatrin during the latent period of epileptogenesis should be considered after detecting interictal EEG epileptiform discharges. Developmental surveillance and detection of deviations in developmental trajectories should prompt early intervention with environmental enrichment to stimulate plasticity in social brain circuitry, remodelling brain functional connectivity. Non-pharmacological treatment should be integrated into multidisciplinary care.

Current challenges and future directions

TSC exemplifies the ongoing revolution in the fields of secondary prevention of epileptogenesis and both targeted and disease-modifying therapies. Given that TSC is associated with a broad phenotypic spectrum, including epilepsy, developmental delay, and ASD, early diagnosis is crucial. Early identification of such symptoms is essential for a more effective treatment of this disorder. Our growing understanding of preictal changes allows us to predict seizures occurrence and to initiate treatment earlier, however we are still behind in early treatment of non-seizure symptoms.

The creation of large-scale international databases has been instrumental in bridging gaps in the knowledge of the natural history of TSC and in developing innovative strategies for clinical monitoring and better management. Advances in identifying biomarkers specific to disease progression are driving our clinical practices forward.

We are now in an era where the treatment of epilepsy in TSC is shifting from merely symptomatic to

secondary prevention, with a proactive approach that includes pharmacological intervention targeting mTOR overactivation during a sensitive developmental window. Preventative and precision medicine remain complex, however we learned more about diagnostic and prognostic biomarkers and these advancements have led to new treatment approaches for TSC-associated DEE.

Presymptomatic treatment may give promising results, particularly for children diagnosed with TSC before seizure onset, with EEG serving as a potential biomarker to predict when clinical seizures may appear. The timing of preventative intervention appears crucial in addressing early neonatal epileptogenesis.

The ongoing refinement of TSC prediction models at disease onset holds the potential to significantly improve clinical decision-making and patient outcome predictions. Although various epigenetic factors may influence the natural history of the disease in individual children, advances in preventive medicine have shown that it is possible to reduce the burden of epilepsy associated with severe cognitive impairment. Understanding individual epigenetic profiles could lead to personalised treatment strategies based on specific epigenetic changes.

A progressive understanding of the molecular basis of DEE and discerning the pathogenic mechanisms that trigger both the epileptic and the developmental encephalopathy components may help to find combinatorial treatment strategies to engage different targets involved in the epileptogenic network. The primary step in unravelling the mechanisms of epileptogenesis involves gaining insights into gene expression and function, identifying specific brain regions where they are expressed, and pinpointing the specific cell types, including excitatory and inhibitory neurons, as well as glial cells.

Strategies tailored to target the specific mechanisms underlying TSC are likely to be adapted to individual patients soon. Deconstructing TSC into its multiple biological components can suggest precision medicine strategies that could achieve higher success rates, not only in controlling seizures but also in addressing associated developmental encephalopathy.

The use of mTOR inhibition in TSC is expected to become more widespread in clinical practice. However, determining the optimal timing for initiating treatment for this multisystem disorder remains a significant challenge. Further research is needed to explore whether early suppression of abnormal mTOR signalling with mTOR inhibitors before seizure onset could be a more effective antiepileptogenic and disease-modifying strategy in infants with TSC. Future studies should also clarify if altered lamination and neuronal morphology disrupt local and long-range connectivity and whether treatment with mTOR inhibitors can reverse these abnormalities. There is an urgent need for collaborative efforts. Future international, prospective, long-term trials that consider not only seizure outcomes, but also cognitive and behavioural symptoms could clarify whether mechanism-based preventive strategies for DEE can improve long-term disease trajectories in children with TSC.

Search strategy.

References for this Review were identified by searches of PubMed between January 2017 and November 2024, and references from relevant articles. The search terms used were "tuberous sclerosis" AND (as individual combinatory terms) "mTOR pathway", "epileptogenesis", "epilepsy", "intellectual disability", "autism", "biomarkers", "diagnosis", "targeted therapy" were used. There were no language restrictions. The final reference list was generated on the basis of relevance to the topics covered in this Review. Relevant historical references outside the search timeframe were also included.

Outstanding questions

- What is the optimal timing and duration of preventive treatment (e.g., vigabatrin, mTOR inhibitors) in infants with TSC to effectively alter long-term neurodevelopmental outcomes?
- Can individualized risk stratification models, integrating genetic, EEG, imaging, and molecular biomarkers, reliably predict which infants with TSC will develop drug-resistant epilepsy, ASD, or intellectual disability?
- Are there early biomarkers—beyond EEG and genotype—that can guide targeted interventions for non-seizure comorbidities such as ASD and cognitive impairment?
- What is the long-term safety and efficacy of early mTOR inhibition, especially when initiated during the prenatal or neonatal period?
- How can gene therapy and gene editing technologies be translated from preclinical models into safe and effective treatments for infants and children with TSC?
- Can non-pharmacological interventions (e.g., environmental enrichment, parent-mediated therapy) be standardized and integrated early enough to meaningfully influence cognitive and social outcomes?
- What role do epigenetic factors and modifier genes play in phenotypic variability in TSC, and can they be leveraged to personalise therapeutic approaches?
- Will large-scale longitudinal trials be able to demonstrate that biomarker-guided, mechanism-based interventions truly shift the developmental trajectory of children with TSC?

Contributors

NS contributed to writing the manuscript, with literature search and preparation of figures and Tables.

VDM contributed to writing the manuscript, with literature search and preparation of Tables.

EA contributed to writing the manuscript, with literature search and preparation of figures and Tables.

MS contributed to writing the manuscript, and preparation of figures.

PC designed the article, contributed to writing the manuscript, reviewed the literature search and coordinated the writing of the article as a whole.

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

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