World Journal of Clinical Pediatrics

Online Submissions: http://www.wjgnet.com/esps/
wjcp@wjgnet.com
doi:10.5409/wjcp.v2.i3.16

World J Clin Pediatr 2013 August 8; 2(3): 16-25
ISSN 2219-2808 (online)

REVIEW

Potential for treatment of severe autism in tuberous sclerosis complex

Tanjala T Gipson, Gwendolyn Gerner, Mary Ann Wilson, Mary E Blue, Michael V Johnston

Tanjala T Gipson, Gwendolyn Gerner, Michael V Johnston, Tuberous Sclerosis Clinic, Kennedy Krieger Institute, Baltimore, MD 21205, United States
Tanjala T Gipson, Gwendolyn Gerner, Michael V Johnston, Department of Neurology and Developmental Medicine, Kennedy Krieger Institute, Baltimore, MD 21205, United States
Tanjala T Gipson, Gwendolyn Gerner, Michael V Johnston, Clinical Trials Unit, Kennedy Krieger Institute, Baltimore, MD 21205, United States
Mary Ann Wilson, Mary E Blue, Michael V Johnston, Neuroscience Laboratory, Hugo W. Moser Research Institute, Inc., Kennedy Krieger Institute, Baltimore, MD 21205, United States
Mary Ann Wilson, Mary E Blue, Michael V Johnston, Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, United States
Mary Ann Wilson, Mary E Blue, Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205, United States
Michael V Johnston, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD 21205, United States
Michael V Johnston, Department of Physical Medicine and Rehabilitation, Johns Hopkins University School of Medicine, Baltimore, MD 21205, United States
Author contributions: Gipson TT, Gerner G, Wilson MA, Blue ME and Johnston MV solely contributed to this paper.
Supported by Grant 2K12NS001696-11A1 (to Gipson TT) from the National Institute of Neurological Disorders and Stroke; Grant 5T32HD007414-18 (to Gerner G) from the National Institute of Child Health and Human Development
Correspondence to: Tanjala T Gipson, MD, Tuberous Sclerosis Clinic, Kennedy Krieger Institute, 716 North Broadway, Baltimore, MD 21205, United States. gipson@kennedykrieger.org
Telephone: +1-443-9232746 Fax: +1-443-9239165
Received: February 22, 2013 Revised: May 26, 2013
Accepted: June 1, 2013
Published online: August 8, 2013

Abstract

The Food and Drug Administration (FDA) has approved two mechanism-based treatments for tuberous sclerosis complex (TSC)-everolimus and vigabatrin. However, these treatments have not been systematically studied in individuals with TSC and *severe* autism. The aim of this review is to identify the clinical features of severe autism in TSC, applicable preclinical models, and potential barriers that may warrant strategic planning in the design phase of clinical trial development. A comprehensive search strategy was formed and searched across PubMed, Embase and SCOPUS from their inception to 2/21/12, 3/16/12, and 3/12/12 respectively. After the final search date, relevant, updated articles were selected from PubMed abstracts generated electronically and emailed daily from PubMed. The references of selected articles were searched, and relevant articles were selected. A search of clinicaltrials.gov was completed using the search term "TSC" and "tuberous sclerosis complex". Autism has been reported in as many as 60% of individuals with TSC; however, review of the literature revealed few data to support clear classification of the severity of autism in TSC. Variability was identified in the diagnostic approach, assessment of cognition, and functional outcome among the reviewed studies and case reports. Objective outcome measures were not used in many early studies; however, diffusion tensor imaging of white matter, neurophysiologic variability in infantile spasms, and cortical tuber subcategories were examined in recent studies and may be useful for objective classification of TSC in future studies. Mechanism-based treatments for TSC are currently available. However, this literature review revealed two potential barriers to successful design and implementation of clinical trials in individuals with severe autism-an unclear definition of the population and lack of validated outcome measures. Recent studies of objective outcome measures in TSC and further study of applicable preclinical models present an opportunity to overcome these barriers.

Key words: Autism; Self-injury; Aggression; Tuberous

sclerosis complex; Intellectual disability

Core tip: Children with severe behaviors and cognitive impairment may benefit from newly available mechanism-based treatments; however, several factors warrant consideration in clinical trial design and implementation.

Gipson TT, Gerner G, Wilson MA, Blue ME, Johnston MV. Potential for treatment of severe autism in tuberous sclerosis complex. *World J Clin Pediatr* 2013; 2(3): 16-25 Available from: URL: http://www.wjgnet.com/2219-2808/full/v2/i3/16.htm DOI: http://dx.doi.org/10.5409/wjcp.v2.i3.16

INTRODUCTION

Tuberous sclerosis complex (TSC) is a leading cause of syndromic autism characterized by multi-system hamartomas. Clinically, the diagnosis is classified as definite, probable, or possible TSC based on the presence of a specific combination of major and minor features (Table 1)[1] or genetically by mutations in TSC1 or TSC2. Although autism has been reported in as many as 61% of individuals with TSC[2,3], severity is not frequently reported. Existing neurologic comorbidities include epilepsy in 60%-90%[4-6], intellectual disability in 45%[7], self-injury in 10%[8], and severe aggression in 13%[9]. The contribution of these neurologic comorbidities or the features of TSC to the severity of autism in this population is unknown.

The neurobiology underlying this condition has been established. TSC1 and TSC2 encode hamartin and tuberin respectively, which indirectly inhibit mammalian target of rapamycin (mTOR). This enzyme is an essential component of two complexes, mTORC1 and mTORC2, which have distinct, wide-ranging effects on gene transcription, protein translation and cell proliferation. Excessive mTOR activity results from a mutation in either TSC1 or TSC2. Disrupted synaptic plasticity, characterized by excessive glutamate activity, may occur through downstream effects of this excessive mTOR activity on ribosomal s6 and EIF4E. Targeted drug development based on this neurobiology has resulted in FDA approval of the mTOR inhibitor, everolimus, and the irreversible inhibitor of GABA transaminase, vigabatrin, for individuals with TSC. While everolimus targets the underlying mechanism of TSC and may have wide-ranging effects, vigabatrin, in addition to anti-seizure effects, may also have an impact on glutamatergic mechanisms important in brain development, synaptic plasticity and learning.

TSC is considered a cause of syndromic autism; however, causality and determinants of severity are unknown. Additionally, the risk/benefit profile of approved treatments in this population has not been tested in a clinical trial. The aim of this review is to identify the clinical features of severe autism in TSC, applicable preclinical models, and potential barriers that may warrant strategic planning in the design phase of clinical trial development.

In order to identify key research, a comprehensive search strategy was formed and then searched across PubMed, Embase and SCOPUS from their inception to 2/21/12, 3/16/12, and 3/12/12 respectively. Database-specific controlled vocabulary terms were combined with keyword terms and phrases for each concept. These terms and phrases were then combined and translated for use in each database. The results were limited to studies conducted on humans and to those written in English. References from key papers were also reviewed and key studies, including preclinical studies were included.

AUTISM

Autism is a syndrome clinically defined by the presence of stereotyped, repetitive behavior and impairments in language and social interaction with onset prior to the age of 3[10]. TSC, the first identified cause of autism, is now considered a leading cause of syndromic autism[2,3,11,12]. The prevalence of autism in TSC is 26%-61% with an average prevalence of 32%[2,3,9,13-23]. Variability in operational definitions of autism and study design, in particular the approach to individuals with intellectual disability, may contribute to variation in these estimates. We did not discover any studies of the severity of autism in TSC. However, self-injury was observed in 10% of a large clinical population. Self-injury, one of the main parental concerns in TSC, may signal a severe form of autism in this subgroup[24].

Operational definitions of autism in TSC

The Diagnostic Statistical Manual and the International Classification of Disease have provided checklist criteria for defining autism. The study with the highest reported prevalence of autism and 3 additional studies used these checklists[3,10,19-21,25]. Other instruments have been developed to assist professionals in identifying individuals with autism, and combined use of the Autism Diagnostic Observation Schedule (ADOS) and Autism Diagnostic Interview (ADI, ADI-R) has been widely accepted as the gold standard. However, the original sample used in developing these instruments did not include individuals with profound intellectual disability[26,27]. The Social Responsiveness Scale (SRS) and the Social Communication Questionnaire (SCQ) are screening instruments for autism; however, the presence of intellectual disability decreases the specificity of SCQ from 80% to 67%[28,29]. The Hunt and Dennis Questionnaire is a screening instrument designed for use in individuals with TSC, behavioral difficulties, and profound intellectual disability. Autism is evaluated by 13 out of 321 questions; however, psychometric properties have not been reported to date[9].

Prevalence study designs

The diagnostic approach used in each of the 19 reviewed studies is indicated in Table 2. Multiple sequenced evaluations were used in 3 studies. The highest reported preva-

Table 1 Criteria for clinical diagnosis of tuberous sclerosis complex

Major features	Minor features
Cortical tubers	Dental enamel pits
Subependymal nodules	Hamartomatous rectal polyps
Subependymal giant cell astrocytoma	Bone cysts
Hypomelanotic macules (3 or more)	Cerebral white matter radial migration lines
Shagreen patch	Gingival fibromas
Facial angiofibromas or forehead plaque	Nonrenal hamartoma
Multiple renal nodular hamartomas	Retinal achromatic patches
Nontraumatic ungual or periungual fibromas	"Confetti" skin lesions
Cardiac rhabdomyoma, 1 or >	Multiple renal cysts
Pulmonary Lymphangiomyomatosis and/ or renal angiomyolipomas	

Tuberous sclerosis complex (TSC) can be clinically diagnosed as definite, probable, or possible based on the presence or absence of a specific combination of major and minor features. Definite TSC: two major features or one major and two minor features; Probable TSC: one major and one minor feature; Possible TSC: one major feature or two or more minor features.

lence of autism in TSC was determined using 3 structured interviews, Childhood Autism Rating Scale (CARS), Autistic Behavior Checklist, and review of DSM Ⅲ-R criteria[3]. In addition to variation in diagnostic approach, inclusion of children with severe or profound intellectual disability varied among these studies and may contribute to variability in prevalence estimates.

Risk factors

Potential risk factors and clinical associations of autism in TSC include epilepsy, structural or neurophysiological abnormalities, and/or joint effects of these categories, but causality has not been established. Intellectual disability and autism were not uniformly characterized among the studies, making it difficult to compare studies focused on risk factors associated with autism or ASD to those focused on risk factors associated with cognitive impairment. Among studies using standard measures to assess cognition, the choice of measure was often based on the assessed functional level or age of the individual. However, this approach was not routinely applied to assessments for autism or ASD. Therefore, we included studies of risk factors for autism and/or intellectual disability in TSC.

Epilepsy

Epilepsy affects 60%-90% of individuals with TSC[2,6,30-38]. Age of epilepsy onset, seizure type and/or severity have been evaluated as potential risk factors for autism and/or intellectual disability in children with TSC. Early age of seizure onset[2,33,34,36-40] and infantile spasms were most commonly identified as risk factors[2,14,17,34,34-42,41-46].

Epilepsy intractability and/or severity was characterized using measures such as development of multiple seizure types after infantile spasms, time to epilepsy control, duration of seizure control, and/or EEG features and was reported as a potential risk factor in 6 of 8 studies that characterized severity[14,37,38,41,46,47].

Neuropathology

Early studies focused solely on lesion location[34,39,40,43,45,48] as a potential risk factor for autism in TSC. Temporal lobe location was linked to poor neurodevelopmental outcomes, including severe language impairment, autism and intellectual disability in these studies; however, this approach did not fully explain the neurodevelopmental phenotype of TSC. Newer studies, illuminating the neurobiology of classic brain lesions in TSC through detailed structural and functional analysis, have expanded knowledge regarding the potential impact of these lesions on the neurodevelopmental phenotype in TSC[21,49-51].

Different approaches to analysis of brain lesions in TSC have identified features associated with autism. Cortical tuber and/or white matter lesion load has been identified as a potential risk factor for poor neurodevelopmental outcomes[14,33,36,42,50]. Cortical lesions seem to worsen the neurological phenotype. The mechanism by which this occurs is unclear, but may be associated with epilepsy and/or circuit disruption or reorganization. T1, T2 and FLAIR imaging have been used to identify specific characteristics of white matter that are associated with autism[51]. White matter abnormalities have also been detected using diffusion tensor imaging fractional anisotropy (FA), a measure of white matter integrity. Lower FA values indicate loss of the typically restricted diffusion found in normal white matter, and are thought to represent deficits in white matter. Autism spectrum disorder in 12 individuals with TSC was associated with lower average FA when compared to 28 individuals with TSC without autism spectrum disorder and 29 age-matched controls[52]. A subsequent study compared the FA of the arcuate fasciculus, which interconnects language areas in the temporal and frontal lobes, among individuals with TSC, with or without autism, to typical controls. FA in this key pathway for language was lowest in those individuals with TSC and autism[53].

Functional analysis has been carried out using EEG, PET, and diffusion tensor imaging (DTI). Across all of these studies, temporal lobe abnormalities were most commonly identified as a risk factor for poor neurodevelopmental outcome. In a retrospective study of 19 individuals, temporal lobe epileptiform activity and seizure onset within the first 36 mo of life were independently associated with autism and PDD[39]. Interictal temporal lobe spikes increased the likelihood of autism spectrum disorder by a factor of 15 in another group[20]. Poor neurodevelopmental outcome was also associated with a subtype of hypsarrhythmia[47]. PET studies revealed reduced glucose metabolism (a measure of neuronal activity) in temporal lobe of individuals with autism and TSC, but increased glucose metabolism in deep cerebellar nuclei

Table 2 Prevalence studies for autism in tuberous sclerosis complex

Instrument	Administration method(time)	Children with severe or profound ID included (Y/N)	Measured prevalence of autism in TSC (N)	Ref.
ADOS	Observation schedule (20-30 min)	N	29% (28) +ADI 15-33% (4 age-based groups of 12-15)	[18]
ADI	Structured Interview (2-3 h)	Y-severe	54% (13) 20% (20)	[15,16]
SRS	65-item Screening Questionnaire (15-20 min)	N (37 w/IQ data)	52% (21)	[22]
SCQ	40-item Screening Questionnaire (15-20 min)	Y-severe	43% (21)	[22]
Hunt and Dennis Questionnaire	321 item interview/13-item subset for autism (1-4 h)	Y-estimates only	50% (90) 26% (23) 24% (21) 5% (131)	[2,9,14,23]
DSM Ⅲ-R	Checklist (10-20 min)	Y-severe	61% (28)	[3]

These are reviewed studies reporting epidemiology of autism in tuberous sclerosis complex (TSC). ADI: Autism diagnostic interview; DSM: Diagnostic and statistical manual of mental disorders; ADOS: Autism diagnostic observation schedule; SRS: Social responsiveness scale; SCQ: Social communication questionnaire; Y: Yes; N: No.

that was associated with intellectual disability, stereotypical behavior, communication impairments, and impaired social interaction[43]. Subsequent PET studies examined tubers in cerebellar cortex and found decreased glucose metabolism associated with symptoms of autism[54]. Output from cerebellar cortex normally inhibits the deep cerebellar nuclei, and decreased activity in cerebellar cortex would be expected to result in increased activity in the deep cerebellar nuclei, as reported in the earlier study. The effect of tuber location within the cerebellum has also been examined, and children with right cerebellar tubers showed increased social isolation and deficits in communication and development, compared to children with left cerebellar tubers[54]. The output of the right cerebellum influences activity in the left cerebral cortex, where language areas are located in most individuals, and taken together these findings support a role for cerebellar deficits in TSC-associated autism.

Joint effects: neuropathology and epilepsy

Almost half of the reviewed studies simultaneously investigated features of both epilepsy and neuropathology as potential risk factors. In the majority of those studies, autism was associated with joint effects of these two risk factors[33,34,36,39,40,42,43,45,47,48]. Thus, multiple factors should be considered in a risk assessment for autism in individuals with TSC. Development of a standard, generalizable approach to assessing these complex risk factors would be informative for clinical trial design in this population.

NEUROBIOLOGY OF TSC RELATED TO DEVELOPMENTAL DISABILITIES

An excellent and comprehensive review recently outlined effects of TSC proteins and the mTOR pathway in the nervous system[55]. A working knowledge of basic neuroscience research as it pertains to TSC is critical in considering therapeutic targets for humans. Highlighted here are discoveries most relevant to patients with severe manifestations of TSC: central nervous system effects of TSC, animal models with phenotypes that mimic particular manifestations of TSC, and effects of rapamycin in these models. Autism, epilepsy and cognitive impairment have all been modeled preclinically, and treatment with inhibitors of mammalian target of rapamycin, such as rapamycin and everolimus, has demonstrated potentially beneficial effects in some models (Table 3).

TSC is an autosomal dominant condition that occurs when there is a mutation in either TSC1, which is located on chromosome 9q34 and encodes the protein hamartin, or TSC2, which is located on chromosome 16 and encodes the protein tuberin. Normally, TSC1 (hamartin) and TSC2 (tuberin) form a complex in which TSC1 stabilizes TSC2 by blocking its ubiquitination and degradation. The TSC1/TSC2 complex inactivates Rheb, which otherwise stimulates mammalian target of rapamycin (mTOR) activity. Mutations in TSC1/TSC2 lead to increased mTOR activity and dysregulation of gene transcription, metabolism, and cell proliferation. Although most cells in individuals with TSC have a single germline mutation in TSC1 or TSC2, recent studies discovered a second somatic mutation limited to giant cells within cortical tubers or to subependymal giant cell astrocytomas (SEGAs). These cells with mutations in both alleles of TSC1 or TSC2 were characterized by hyperactivation of mTOR[56,57]. These studies suggest that a two-hit mechanism may underlie the formation of cortical tubers or SEGAs, but the contribution of this mechanism to development of autism/ASD remains unclear.

Animal models have been developed to investigate the effects of heterozygous mutation of TSC1 or TSC2, or of conditional deletion of one or both alleles in different populations of neurons or in astrocytes. The characteristics and major findings obtained with selected models are summarized in Table 3.

Heterozygous Tsc1 and Tsc2 models

Mouse lines that are *Tsc1*$^{+/-}$ or *Tsc2*$^{+/-}$ and a rat strain with a spontaneous mutation in Tsc2 (Eker rat) have been

Table 3 Selected preclinical models of tuberous sclerosis complex

Model	Genetic manipulation	Behavioral effects	Seizures	Autism	Synaptic plasticity	Neuropathology	Rapamycin	Ref.
Tsc1$^{+/-}$ mice	Heterozygous *Tsc1* deletion, exons 6-8	↓Hippocampal dependent learning	None	↓Social interaction	-	None	-	[58]
Tsc2$^{+/-}$ Eker rat	Spontaneous autosomal dominant (Heterozygous)	-	-	-	↓LTP, ↓LTD, ↑PPF	-	-	[61]
Tsc2$^{+/-}$ Eker rat	Spontaneous autosomal dominant (Heterozygous)	No learning and memory deficits	No spontaneous	↓Social interaction, ↓↓ after seizure induction	-	-	-	[59]
Tsc2$^{+/-}$	Heterozygous disruption in second exon	↓Hippocampal dependent learning	-	-	↓Threshold for L-LTP	-	Reversed all	[93]
Tsc2$^{+/-}$ or WT pups from *Tsc2*$^{+/-}$ or WT dams	Heterozygous *Tsc2* deletion	↑Maternal care by *Tsc2*$^{+/-}$ dams	-	↑Vocalization in WT and *Tsc2*$^{+/-}$ pups of *Tsc2*$^{+/-}$ dams	-	-	-	[60]
Tsc2$^{+/-}$	Heterozygous *Tsc2* deletion	-	-	-	↓mGluR-LTD, ↓Arc synthesis	-	Reverses deficits in protein-synthesis-dependent mGluR-LTD	[62]
Tsc1$^{fl/fl}$ *Nestin-rtTA(+)TetOp-cre(+)*	Mosaic homozygous *Tsc1* deletion in cortical neural progenitors	-	Yes^{+}	-	-	Heterotopias with enlarged, pS6+ neurons White matter nodules	↑Survival, ↓seizures, ↓neuropath	[94]
Tsc1^{fl}/-Syn1-Cre	Homozygous *Tsc1* deletion in neurons from mid-gestation; (heterozygous in all other cells)	-	Yes^{+}	-	-	Dysplastic neurons ↓Cortical organization ↓Myelin	↑Survival, ↑myelination, ↑body weight, ↓neurologic impairment	[95,96]
Tsc1$^{fl/fl}$ Emx1-Cre	Homozygous *Tsc1* deletion in early embryonic neural progenitors	-	Yes^{+}	-	-	↓Cortical organization ↑Brain size ↓Myelin tHick astrocyte processes	↓Seizures, ↑survival, ↓glial abnl, ↑weight, ↓brain size	[97]
Tsc1$^{fl/fl}$ Nestin-Cre	Homozygous *Tsc1* deletion in differentiating neurons	-	-	-	↑EPSCs ↑AMPA	↑Cell size ↑Spine width ↑Spine length ↓Spine density	↓Spine width ↑spine length	[98]
Tsc1$^{fl/fl}$ Syn1-Cre	Homozygous *Tsc1* deletion in neurons	-	No spontaneous Epileptiform discharges	-	↑EPSCs ↑AMPA	No gross abnormalities	-	[99]
Tsc1$^{fl/fl}$ GFAP-Cre	Homozygous *Tsc1* inactivation in glial-fibrillary acidic protein (GFAP)-+ cells		Yes^{+}, ↓Glt1			↑Astrocytes ↑Brain size ↓Hippocampal organization	↑Glt1, ↑survival ↓neuropath Early: prevented epilepsy, Late: decreased seizure frequency	[100,101]
Tsc2$^{fl/fl}$ GFAP-Cre	Homozygous *Tsc2* inactivation in GFAP + cells	-	Yes^{++}, ↓Glt1	-	-	↑Astrocytes ↑Brain size ↓Hippocampal organization	↑Survival, ↓seizures, ↓neuropath	[102]
Tsc1$^{fl/+}$ *L7-Cre, or* *Tsc1*$^{fl/fl}$ *L7-Cre*	Heterozygous or homozygous *Tsc1* deletion limited to cerebellar Purkinje cells	Normal acquisition, ↓ reversal of spatial learning in homozygous mutants	-	↓social interaction in both genotypes ↑ grooming, vocalization	PC ↓excitability in heterozygous, ↓↓ in homozygous	PC loss in homozygous, ↑PC dendritic spine density in both heterozygous and homozygous	Reversed pathological and behavioral abnormalities	[64]
Tsc2$^{fl/-}$ *, or Tsc2*$^{fl/-}$ *Pcp2-Cre*	Heterozygous *Tsc2* deletion (global), or homozygous *Tsc2* deletion in cerebellar Purkinje cells, heterozygous in other cells	-	-	↑repetitive behavior in homozygous, ↓social interaction in both genotypes	-	PC loss in homozygous,	Reversed social deficits	[63]

These are existing preclinical models designed to re-capitulate neurologic features of tuberous sclerosis complex (TSC). - Not examined; +: Mild seizure severity; ++: Moderate seizure severity.

characterized in a number of studies. Although there are no neuropathologic findings and no spontaneous seizures reported in these heterozygous mutants, behavioral abnormalities include social interaction deficits[58,59], abnormal vocalization[60] and learning deficits that may be related to altered synaptic plasticity[58,61]. Both synaptic and behavioral learning deficits were reversed by acute rapamycin treatment. One study found that social interaction deficits in the Eker rat were exacerbated by seizure induction in the postnatal period[59]. Thus, in animal models with heterozygous mutations in Tsc1 or Tsc2, social interaction deficits and altered maternal-pup interactions that model some aspects of autism occur in the absence of cortical lesions or epilepsy, but can be exacerbated by seizures induced early in postnatal brain development. The latter findings support intervention to control seizures in infants with TSC.

TSC and Fragile X syndrome may share neurobiology related to intellectual disability and autism, since these conditions have been found to result in opposite effects on metabotropic glutamate receptor (mGluR)-mediated protein synthesis in a recent preclinical study. In *Tsc2*$^{+/-}$ mice, mGluR-mediated long term depression was abnormally decreased, but in *Fmr*$^{/y}$ mice it was increased. These alterations were rescued by up or down modulation of mGluR5 activity, respectively, and by rapamycin treatment in *Tsc2*$^{+/-}$ mice. Investigators also crossed the two mouse lines and observed reversal of memory deficits in offspring expressing both mutations[62].

Conditional homozygous deletion of Tsc1 or Tsc2

Mice with conditional deletion of both alleles of Tsc1 or Tsc2 in cortical neurons exhibit a variety of structural abnormalities such as increased brain and cell size, dysplastic neurons and deficits in cortical organization; mosaic homozygous inactivation of Tsc1 produced heterotopias and white matter nodules that resemble some cortical lesions in individuals with TSC. Spontaneous epileptiform discharges, seizures and increased excitatory synaptic activity have also been reported in these mice. Conditional homozygous deletion of Tsc1 in GFAP-expressing cells, which include astrocytes and possibly adult neural progenitors in neurogenic niches such as the hippocampal subgranular zone, decreased the expression of the astrocyte transporter for glutamate, Glt1. These mice exhibited spontaneous seizures, altered hippocampal organization, as well as increased astrocyte number and brain size. Thus, homozygous deletion of Tsc1 or Tsc2 produced more severe effects on brain structure and function and also reduced survival; rapamycin treatment reversed or ameliorated glial and neuronal abnormalities and increased survival. In the mice with homozygous deletion of Tsc1 in astrocytes and GFAP-positive neural progenitors, early rapamycin treatment prevented seizures and later treatment reduced seizure frequency. None of these studies of mice with homozygous deletion in cortical neurons or astrocytes examined social behavior.

Two recent studies examined the behavioral effects of conditional loss of one or both alleles of Tsc1 or Tsc2 that was limited to cerebellar Purkinje cells (PCs)[63,64]. Homozygous deletion caused a progressive loss of PCs, and both heterozygous and homozygous deletion mutants exhibited a striking increase in PC dendritic spine density and reduced PC excitability. Autistic-like behaviors were observed in both heterozygous and homozygous mutants, including deficits in social approach, response to social novelty, altered vocalization during a limited postnatal period, and increased grooming. Chronic rapamycin treatment initiated at about 1 wk of age reversed the neuropathologic and behavioral effects in these mice. These animal models provide important insights into the role of the cerebellum in autism caused by TSC, which was initially proposed based on human neuropathologic and PET imaging studies.

These animal models of TSC exhibit the neuropathological, neurophysiological and behavioral abnormalities characteristic of TSC. They have been used to evaluate therapies based on mTOR inhibition, antiepileptic medications, and positive modulation of mGluR5 activity, and provide essential platforms for developing and preclinical testing of new therapies.

IMPLICATIONS FOR TREATMENT OF AUTISM

Currently available behavioral and pharmacological treatment options for children with TSC and autism do not differ from treatments available to children without TSC[65,66]. Mutations in TSC1 or TSC2 lead to increased activity of mTOR. Two mechanism-based treatments have been FDA-approved for use in individuals with TSC, everolimus for tumors (SEGAs and renal angiomyolipomas) and vigabatrin for infantile spasms. It is unknown whether either treatment would be effective for severe autism.

The protein kinase mTOR is incorporated into two protein complexes, mTORC1 and mTORC2, which have distinct downstream effects on gene transcription, metabolism, cell proliferation, and synaptic plasticity. Everolimus targets excess mTOR activity in the protein complex mTORC1 (mTORC2 is unaffected). After 12-18 mo of treatment, everolimus has been shown to increase abnormally low FA in an open-label study of 20 individuals with TSC and SEGAs[67], indicating an improvement in white matter structure. A clinical trial of everolimus for neurocognition in high-functioning individuals with TSC is ongoing and may include individuals with autism.

Vigabatrin, an irreversible inhibitor of γ-aminobutyric acid (GABA) transaminase, targets excessive levels of glutamate (the brain's most common excitatory neurotransmitter) by increasing levels of GABA (the brain' s inhibitory neurotransmitter). To date, there are no active clinical trials of vigabatrin for neurocognition or autism. Directly targeting the neurobiology of TSC may be therapeutic for children with severe autism; however, definitive clinical trials are warranted. Successful design

and implementation of these trials will require careful planning. In this review, we identified 4 areas of need: (1) validation of severe autism as an endophenotype in TSC; (2) natural history of severe autism in TSC; (3) selection of reliable, valid outcome measures in this population; and (4) assessment of recruitment and protocol compliance (feasibility factors relevant to trial implementation).

DISCUSSION

To date, discovery of mechanism-based treatments for TSC has not impacted the clinical and educational intervention offered to children with autism and TSC. A severe form of autism characterized by self-injurious behavior and/or aggression may exist in some individuals with TSC; however, few data are available about this potential endophenotype. Level of parental concern, danger to self and others, decreased quality of life, and lack of full participation in the community provide a rationale for prioritizing inclusion of this population in clinical trials. Clearly, improved treatment options for individuals with extreme behavioral manifestations of TSC are needed. Targeting the neurobiology directly may result in greater improvement than non-specific treatments.

This structured review of TSC literature revealed important clinical and preclinical features of TSC, including autism, intellectual disability, self-injury, and/or aggression in individuals with TSC. Several clinical associations were without clear causation, such as location of cortical tubers and autism. However, clinical trials demonstrating the efficacy of vigabatrin in individuals with TSC and infantile spasms[68] and of everolimus for non-surgically resectable SEGAs[69] have led to FDA approval for these indications. A comprehensive phenotypic analysis of patients with TSC can be achieved using measures that provide an assessment of neural activity, such as EEG and PET scans, newer MRI methods that detect subtle structural abnormalities, and appropriate behavioral assessments. This approach may also prove useful for evaluating the efficacy of mechanism-based treatments.

Review of the basic science literature highlighted the biological pathways and downstream effects of increased mTOR activity in TSC, alterations in neurochemistry in TSC, effects of pharmacological agents that directly impact the mTOR pathway, and described several animal models that recapitulate important features of TSC in humans. Although an animal model that exhibits all of the severe manifestations of this disorder does not currently exist, further study of the current models will continue to lead to important discoveries.

FUTURE DIRECTIONS

Shared deficits in neurobiology related to neurodevelopmental disabilities may represent an opportunity to generalize findings from research in TSC to individuals affected by related conditions. Abnormalities in mTOR signaling and associated pathways, as seen in TSC, have been identified in focal cortical dysplasia type Ⅱ, hemimegalencephaly[70], phosphatase and tensin homologue hamartoma syndromes, such as autism associated with mutations in PTEN[71-73], neurofibromatosis-1[74-76], and autism associated with mutations in EIF4E (eukaryotic translation initiation factor 4E)[77-79]. Targeting pathophysiological mechanisms has resulted in phenotypic rescue in animal models of several other single gene disorders, such as Lhermitte-Duclos[80], Fragile X syndrome[81], Angelman syndrome[82], Rett syndrome[83], Neurofibromatosis I [84,85], Down syndrome[86,87], and Rubenstein-Taybi[88-90].

Although usually considered to be chronic, life-long conditions, there is hope for children and adults affected by TSC and other diseases that are associated with severe neurological manifestations, such as self-injurious behavior, aggression, intellectual disability, autism and seizures. Rescue of the synaptic plasticity deficits that underlie these manifestations may eventually be achieved with comprehensive treatment by pairing available mechanism-based treatments with evidence-based educational and behavioral interventions[91,92].

ACKNOWLEDGEMENTS

The authors thank clinical librarian Victoria Goode, MLIS, for assistance with development of search strategies, research students Raechel Mattison and Nassira Bougrab for assistance, and colleagues Louis Hagopian, PhD, Lee Wachtel, MD, and Dejan Budimirovic for helpful discussions.

REFERENCES

1 **Roach ES**, DiMario FJ, Kandt RS, Northrup H. Tuberous Sclerosis Consensus Conference: recommendations for diagnostic evaluation. National Tuberous Sclerosis Association. *J Child Neurol* 1999; **14**: 401-407 [PMID: 10385849 DOI: 10.1177/088307389901400610]

2 **Webb DW**, Fryer AE, Osborne JP. Morbidity associated with tuberous sclerosis: a population study. *Dev Med Child Neurol* 1996; **38**: 146-155 [PMID: 8603782 DOI: 10.1111/j.1469-8749.1996.tb12086.x]

3 **Gillberg IC**, Gillberg C, Ahlsén G. Autistic behaviour and attention deficits in tuberous sclerosis: a population-based study. *Dev Med Child Neurol* 1994; **36**: 50-56 [PMID: 8132114 DOI: 10.1111/j.1469-8749.1994.tb11765.x]

4 **Jansen AC**, Sancak O, D'Agostino MD, Badhwar A, Roberts P, Gobbi G, Wilkinson R, Melanson D, Tampieri D, Koenekoop R, Gans M, Maat-Kievit A, Goedbloed M, van den Ouweland AM, Nellist M, Pandolfo M, McQueen M, Sims K, Thiele EA, Dubeau F, Andermann F, Kwiatkowski DJ, Halley DJ, Andermann E. Unusually mild tuberous sclerosis phenotype is associated with TSC2 R905Q mutation. *Ann Neurol* 2006; **60**: 528-539 [PMID: 17120248 DOI: 10.1002/ana.21037]

5 **Rosner M**, Hanneder M, Siegel N, Valli A, Hengstschläger M. The tuberous sclerosis gene products hamartin and tuberin are multifunctional proteins with a wide spectrum of interacting partners. *Mutat Res* 2008; **658**: 234-246 [PMID: 18291711 DOI: 10.1016/j.mrrev.2008.01.001]

6 **Gomez M,** Sampson JR, Whittemore VH, editors. Tuberous sclerosis complex. 3rd ed. Oxford: Oxford University Press,

1999
7 **Joinson C**, O'Callaghan FJ, Osborne JP, Martyn C, Harris T, Bolton PF. Learning disability and epilepsy in an epidemiological sample of individuals with tuberous sclerosis complex. *Psychol Med* 2003; **33**: 335-344 [PMID: 12622312 DOI: 10.1017/S0033291702007092]
8 **Staley BA**, Montenegro MA, Major P, Muzykewicz DA, Halpern EF, Kopp CM, Newberry P, Thiele EA. Self-injurious behavior and tuberous sclerosis complex: frequency and possible associations in a population of 257 patients. *Epilepsy Behav* 2008; **13**: 650-653 [PMID: 18703161 DOI: 10.1016/j.yebeh.2008.07.010]
9 **Glowinski J**, Niecoullon A, Chéramy A. Regulations of the activity of the nigrostriatal dopaminergic pathways by cortical, cerebellar, and sensory neuronal afferences. *Adv Biochem Psychopharmacol* 1978; **19**: 75-87 [PMID: 358788]
10 **American Psychiatric Association.** Diagnostic and statistical manual of mental disorders: DSM-IV-TR. 4th ed. Washington, DC: American Psychiatric Association, 2000
11 **Critchley M EC.** Tuberous sclerosis and allied conditions. *Brain* 1932; **55**: 311-346
12 **Kanner L**. Autistic disturbances of affective contact. *Acta Paedopsychiatr* 1968; **35**: 100-136 [PMID: 4880460]
13 **Hunt A**, Shepherd C. A prevalence study of autism in tuberous sclerosis. *J Autism Dev Disord* 1993; **23**: 323-339 [PMID: 8331050]
14 **Jambaqué I**, Cusmai R, Curatolo P, Cortesi F, Perrot C, Dulac O. Neuropsychological aspects of tuberous sclerosis in relation to epilepsy and MRI findings. *Dev Med Child Neurol* 1991; **33**: 698-705 [PMID: 1916024]
15 **Smalley SL**, Tanguay PE, Smith M, Gutierrez G. Autism and tuberous sclerosis. *J Autism Dev Disord* 1992; **22**: 339-355 [PMID: 1400103]
16 **Baker P**, Piven J, Sato Y. Autism and tuberous sclerosis complex: prevalence and clinical features. *J Autism Dev Disord* 1998; **28**: 279-285 [PMID: 9711484]
17 **Gutierrez GC**, Smalley SL, Tanguay PE. Autism in tuberous sclerosis complex. *J Autism Dev Disord* 1998; **28**: 97-103 [PMID: 9586771]
18 **Jeste SS**, Sahin M, Bolton P, Ploubidis GB, Humphrey A. Characterization of autism in young children with tuberous sclerosis complex. *J Child Neurol* 2008; **23**: 520-525 [PMID: 18160549 DOI: 10.1177/0883073807309788]
19 **Calderón González R**, Treviño Welsh J, Calderón Sepúlveda A. [Autism in tuberous sclerosis]. *Gac Med Mex* 1994; **130**: 374-379 [PMID: 7607368]
20 **Numis AL**, Major P, Montenegro MA, Muzykewicz DA, Pulsifer MB, Thiele EA. Identification of risk factors for autism spectrum disorders in tuberous sclerosis complex. *Neurology* 2011; **76**: 981-987 [PMID: 21403110 DOI: 10.1212/WNL.0b013e3182104347]
21 **Bolton PF**, Griffiths PD. Association of tuberous sclerosis of temporal lobes with autism and atypical autism. *Lancet* 1997; **349**: 392-395 [PMID: 9033466]
22 **Granader YE**, Bender HA, Zemon V, Rathi S, Nass R, Macallister WS. The clinical utility of the Social Responsiveness Scale and Social Communication Questionnaire in tuberous sclerosis complex. *Epilepsy Behav* 2010; **18**: 262-266 [PMID: 20554253 DOI: 10.1016/j.yebeh.2010.04.010]
23 **Hunt A**. Development, behaviour and seizures in 300 cases of tuberous sclerosis. *J Intellect Disabil Res* 1993; **37** (Pt 1): 41-51 [PMID: 7681710]
24 **Hunt A**. Tuberous sclerosis: a survey of 97 cases. III: Family aspects. *Dev Med Child Neurol* 1983; **25**: 353-357 [PMID: 6192027]
25 Report of the ICD-10 Task Force. American Medical Record Association. *J Am Med Rec Assoc* 1987; **58**: 51-56 [PMID: 10284181]
26 **Lord C**, Rutter M, Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord* 1994; **24**: 659-685 [PMID: 7814313]
27 **Lord C**, Risi S, Lambrecht L, Cook EH, Leventhal BL, DiLavore PC, Pickles A, Rutter M. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord* 2000; **30**: 205-223 [PMID: 11055457]
28 **Rutter M,** Bailey A, Lord C. Social communication questionnaire. Melton South: Western Psychological Services, 2003
29 **Constantino JN**, Abbacchi AM, Lavesser PD, Reed H, Givens L, Chiang L, Gray T, Gross M, Zhang Y, Todd RD. Developmental course of autistic social impairment in males. *Dev Psychopathol* 2009; **21**: 127-138 [PMID: 19144226 DOI: 10.1017/S095457940900008X]
30 **Osborne JP**, Fryer A, Webb D. Epidemiology of tuberous sclerosis. *Ann N Y Acad Sci* 1991; **615**: 125-127 [PMID: 2039137]
31 **Cross JH**. Neurocutaneous syndromes and epilepsy-issues in diagnosis and management. *Epilepsia* 2005; **46** Suppl 10: 17-23 [PMID: 16359466 DOI: 10.1111/j.1528-1167.2005.00353.x]
32 **Curatolo P**, Bombardieri R, Verdecchia M, Seri S. Intractable seizures in tuberous sclerosis complex: from molecular pathogenesis to the rationale for treatment. *J Child Neurol* 2005; **20**: 318-325 [PMID: 15921233]
33 **Jansen FE**, Vincken KL, Algra A, Anbeek P, Braams O, Nellist M, Zonnenberg BA, Jennekens-Schinkel A, van den Ouweland A, Halley D, van Huffelen AC, van Nieuwenhuizen O. Cognitive impairment in tuberous sclerosis complex is a multifactorial condition. *Neurology* 2008; **70**: 916-923 [PMID: 18032744 DOI: 10.1212/01.wnl.0000280579.04974.c0]
34 **Kaczorowska M**, Jurkiewicz E, Domańska-Pakieła D, Syczewska M, Lojszczyk B, Chmielewski D, Kotulska K, Kuczyński D, Kmieć T, Dunin-Wąsowicz D, Kasprzyk-Obara J, Jóźwiak S. Cerebral tuber count and its impact on mental outcome of patients with tuberous sclerosis complex. *Epilepsia* 2011; **52**: 22-27 [PMID: 21204819 DOI: 10.1111/j.1528-1167.2010.02892.x]
35 **Bolton PF**. Neuroepileptic correlates of autistic symptomatology in tuberous sclerosis. *Ment Retard Dev Disabil Res Rev* 2004; **10**: 126-131 [PMID: 15362169 DOI: 10.1002/mrdd.20021]
36 **Shepherd CW**, Houser OW, Gomez MR. MR findings in tuberous sclerosis complex and correlation with seizure development and mental impairment. *AJNR Am J Neuroradiol* 1995; **16**: 149-155 [PMID: 7900584]
37 **Shepherd CW**, Stephenson JB. Seizures and intellectual disability associated with tuberous sclerosis complex in the west of Scotland. *Dev Med Child Neurol* 1992; **34**: 766-774 [PMID: 1526347]
38 **Chu-Shore CJ**, Major P, Camposano S, Muzykewicz D, Thiele EA. The natural history of epilepsy in tuberous sclerosis complex. *Epilepsia* 2010; **51**: 1236-1241 [PMID: 20041940 DOI: 10.1111/j.1528-1167.2009.02474.x]
39 **Bolton PF**, Park RJ, Higgins JN, Griffiths PD, Pickles A. Neuro-epileptic determinants of autism spectrum disorders in tuberous sclerosis complex. *Brain* 2002; **125**: 1247-1255 [PMID: 12023313 DOI: 10.1093/brain/awf124]
40 **Zaroff CM**, Barr WB, Carlson C, LaJoie J, Madhavan D, Miles DK, Nass R, Devinsky O. Mental retardation and relation to seizure and tuber burden in tuberous sclerosis complex. *Seizure* 2006; **15**: 558-562 [PMID: 16935530 DOI: 10.1016/j.seizure.2006.06.010]
41 **Goh S**, Kwiatkowski DJ, Dorer DJ, Thiele EA. Infantile spasms and intellectual outcomes in children with tuberous sclerosis complex. *Neurology* 2005; **65**: 235-238 [PMID: 16043792 DOI: 10.1212/01.wnl.0000168908.78118.99]
42 **O'Callaghan FJ**, Harris T, Joinson C, Bolton P, Noakes M, Presdee D, Renowden S, Shiell A, Martyn CN, Osborne JP. The relation of infantile spasms, tubers, and intelligence in tuberous sclerosis complex. *Arch Dis Child* 2004; **89**: 530-533

[PMID: 15155396 DOI: 10.1136/adc.2003.026815]
43 **Asano E**, Chugani DC, Muzik O, Behen M, Janisse J, Rothermel R, Mangner TJ, Chakraborty PK, Chugani HT. Autism in tuberous sclerosis complex is related to both cortical and subcortical dysfunction. *Neurology* 2001; **57**: 1269-1277 [PMID: 11591847 DOI: 10.1212/WNL.57.7.1269]
44 **Jozwiak S**, Goodman M, Lamm SH. Poor mental development in patients with tuberous sclerosis complex: clinical risk factors. *Arch Neurol* 1998; **55**: 379-384 [PMID: 9520012 DOI: 10.1001/archneur.55.3.379]
45 **Doherty C**, Goh S, Young Poussaint T, Erdag N, Thiele EA. Prognostic significance of tuber count and location in tuberous sclerosis complex. *J Child Neurol* 2005; **20**: 837-841 [PMID: 16417883]
46 **Chu-Shore CJ**, Major P, Montenegro M, Thiele E. Cyst-like tubers are associated with TSC2 and epilepsy in tuberous sclerosis complex. *Neurology* 2009; **72**: 1165-1169 [PMID: 19332694 DOI: 10.1212/01.wnl.0000345365.92821.86]
47 **Muzykewicz DA**, Costello DJ, Halpern EF, Thiele EA. Infantile spasms in tuberous sclerosis complex: prognostic utility of EEG. *Epilepsia* 2009; **50**: 290-296 [PMID: 18801034 DOI: 10.1111/j.1528-1167.2008.01788.x]
48 **Raznahan A**, Higgins NP, Griffiths PD, Humphrey A, Yates JR, Bolton PF. Biological markers of intellectual disability in tuberous sclerosis. *Psychol Med* 2007; **37**: 1293-1304 [PMID: 17335641 DOI: 10.1017/S0033291707000177]
49 **Curatolo P**, Cusmai R. Autism and infantile spasms in children with tuberous sclerosis. *Dev Med Child Neurol* 1987; **29**: 551 [PMID: 3678635]
50 **Chou IJ**, Lin KL, Wong AM, Wang HS, Chou ML, Hung PC, Hsieh MY, Chang MY. Neuroimaging correlation with neurological severity in tuberous sclerosis complex. *Eur J Paediatr Neurol* 2008; **12**: 108-112 [PMID: 17869556 DOI: 10.1016/j.ejpn.2007.07.002]
51 **Gallagher A**, Grant EP, Madan N, Jarrett DY, Lyczkowski DA, Thiele EA. MRI findings reveal three different types of tubers in patients with tuberous sclerosis complex. *J Neurol* 2010; **257**: 1373-1381 [PMID: 20352250 DOI: 10.1007/s00415-010-5535-2]
52 **Peters JM**, Sahin M, Vogel-Farley VK, Jeste SS, Nelson CA, Gregas MC, Prabhu SP, Scherrer B, Warfield SK. Loss of white matter microstructural integrity is associated with adverse neurological outcome in tuberous sclerosis complex. *Acad Radiol* 2012; **19**: 17-25 [PMID: 22142677 DOI: 10.1016/j.acra.2011.08.016]
53 **Lewis WW**, Sahin M, Scherrer B, Peters JM, Suarez RO, Vogel-Farley VK, Jeste SS, Gregas MC, Prabhu SP, Nelson CA, Warfield SK. Impaired language pathways in tuberous sclerosis complex patients with autism spectrum disorders. *Cereb Cortex* 2013; **23**: 1526-1532 [PMID: 22661408 DOI: 10.1093/cercor/bhs135]
54 **Eluvathingal TJ**, Behen ME, Chugani HT, Janisse J, Bernardi B, Chakraborty P, Juhasz C, Muzik O, Chugani DC. Cerebellar lesions in tuberous sclerosis complex: neurobehavioral and neuroimaging correlates. *J Child Neurol* 2006; **21**: 846-851 [PMID: 17005099 DOI: 10.1177/08830738060210100301]
55 **Han JM**, Sahin M. TSC1/TSC2 signaling in the CNS. *FEBS Lett* 2011; **585**: 973-980 [PMID: 21329690 DOI: 10.1016/j.febslet.2011.02.001]
56 **Chan JA**, Zhang H, Roberts PS, Jozwiak S, Wieslawa G, Lewin-Kowalik J, Kotulska K, Kwiatkowski DJ. Pathogenesis of tuberous sclerosis subependymal giant cell astrocytomas: biallelic inactivation of TSC1 or TSC2 leads to mTOR activation. *J Neuropathol Exp Neurol* 2004; **63**: 1236-1242 [PMID: 15624760]
57 **Crino PB**, Aronica E, Baltuch G, Nathanson KL. Biallelic TSC gene inactivation in tuberous sclerosis complex. *Neurology* 2010; **74**: 1716-1723 [PMID: 20498439 DOI: 10.1212/WNL.0b013e3181e04325]
58 **Goorden SM**, van Woerden GM, van der Weerd L, Cheadle JP, Elgersma Y. Cognitive deficits in Tsc1+/- mice in the absence of cerebral lesions and seizures. *Ann Neurol* 2007; **62**: 648-655 [PMID: 18067135 DOI: 10.1002/ana.21317]
59 **Waltereit R**, Japs B, Schneider M, de Vries PJ, Bartsch D. Epilepsy and Tsc2 haploinsufficiency lead to autistic-like social deficit behaviors in rats. *Behav Genet* 2011; **41**: 364-372 [PMID: 20927644 DOI: 10.1007/s10519-010-9399-0]
60 **Young DM**, Schenk AK, Yang SB, Jan YN, Jan LY. Altered ultrasonic vocalizations in a tuberous sclerosis mouse model of autism. *Proc Natl Acad Sci USA* 2010; **107**: 11074-11079 [PMID: 20534473 DOI: 10.1073/pnas.1005620107]
61 **von der Brelie C**, Waltereit R, Zhang L, Beck H, Kirschstein T. Impaired synaptic plasticity in a rat model of tuberous sclerosis. *Eur J Neurosci* 2006; **23**: 686-692 [PMID: 16487150 DOI: 10.1111/j.1460-9568.2006.04594.x]
62 **Auerbach BD**, Osterweil EK, Bear MF. Mutations causing syndromic autism define an axis of synaptic pathophysiology. *Nature* 2011; **480**: 63-68 [PMID: 22113615 DOI: 10.1038/nature10658]
63 **Reith RM**, McKenna J, Wu H, Hashmi SS, Cho SH, Dash PK, Gambello MJ. Loss of Tsc2 in Purkinje cells is associated with autistic-like behavior in a mouse model of tuberous sclerosis complex. *Neurobiol Dis* 2013; **51**: 93-103 [PMID: 23123587 DOI: 10.1016/j.nbd.2012.10.014]
64 **Tsai PT**, Hull C, Chu Y, Greene-Colozzi E, Sadowski AR, Leech JM, Steinberg J, Crawley JN, Regehr WG, Sahin M. Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. *Nature* 2012; **488**: 647-651 [PMID: 22763451 DOI: 10.1038/nature11310]
65 **National Autism Center.** National standards project: Findings and conclusions. Randolf, MA: NAC Office of Communications, 2009
66 Micromedex 2.0 [Internet], 2012. Available from: URL: http://www.micromedexsolutions.com
67 **Tillema JM**, Leach JL, Krueger DA, Franz DN. Everolimus alters white matter diffusion in tuberous sclerosis complex. *Neurology* 2012; **78**: 526-531 [PMID: 22262746 DOI: 10.1212/WNL.0b013e318247ca8d]
68 **Appleton RE,** Fryer AE. Neurological manifestations of tuberous sclerosis complex pathophysiology and drug treatment options. *CNS DRUGS* 1995; **3**: 174-185
69 **Krueger DA**, Care MM, Holland K, Agricola K, Tudor C, Mangeshkar P, Wilson KA, Byars A, Sahmoud T, Franz DN. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med* 2010; **363**: 1801-1811 [PMID: 21047224 DOI: 10.1056/NEJMoa1001671]
70 **Crino PB**. Focal brain malformations: seizures, signaling, sequencing. *Epilepsia* 2009; **50** Suppl 9: 3-8 [PMID: 19761448 DOI: 10.1111/j.1528-1167.2009.02289.x]
71 **Sunnen CN**, Brewster AL, Lugo JN, Vanegas F, Turcios E, Mukhi S, Parghi D, D'Arcangelo G, Anderson AE. Inhibition of the mammalian target of rapamycin blocks epilepsy progression in NS-Pten conditional knockout mice. *Epilepsia* 2011; **52**: 2065-2075 [PMID: 21973019 DOI: 10.1111/j.1528-1167.2011.03280.x]
72 **Kwon CH**, Luikart BW, Powell CM, Zhou J, Matheny SA, Zhang W, Li Y, Baker SJ, Parada LF. Pten regulates neuronal arborization and social interaction in mice. *Neuron* 2006; **50**: 377-388 [PMID: 16675393 DOI: 10.1016/j.neuron.2006.03.023]
73 **Rodríguez-Escudero I**, Oliver MD, Andrés-Pons A, Molina M, Cid VJ, Pulido R. A comprehensive functional analysis of PTEN mutations: implications in tumor- and autism-related syndromes. *Hum Mol Genet* 2011; **20**: 4132-4142 [PMID: 21828076 DOI: 10.1093/hmg/ddr337]
74 **Dasgupta B**, Yi Y, Chen DY, Weber JD, Gutmann DH. Proteomic analysis reveals hyperactivation of the mammalian target of rapamycin pathway in neurofibromatosis 1-associated human and mouse brain tumors. *Cancer Res* 2005; **65**: 2755-2760 [PMID: 15805275 DOI: 10.1158/0008-5472.CAN-04-4058]
75 **Banerjee S**, Crouse NR, Emnett RJ, Gianino SM, Gutmann DH. Neurofibromatosis-1 regulates mTOR-mediated astrocyte growth and glioma formation in a TSC/Rheb-indepen-

dent manner. *Proc Natl Acad Sci USA* 2011; **108**: 15996-16001 [PMID: 21896734 DOI: 10.1073/pnas.1019012108]
76 **Banerjee S**, Gianino SM, Gao F, Christians U, Gutmann DH. Interpreting mammalian target of rapamycin and cell growth inhibition in a genetically engineered mouse model of Nf1-deficient astrocytes. *Mol Cancer Ther* 2011; **10**: 279-291 [PMID: 21216928 DOI: 10.1158/1535-7163.MCT-10-0654]
77 **Schellenberg GD**, Dawson G, Sung YJ, Estes A, Munson J, Rosenthal E, Rothstein J, Flodman P, Smith M, Coon H, Leong L, Yu CE, Stodgell C, Rodier PM, Spence MA, Minshew N, McMahon WM, Wijsman EM. Evidence for multiple loci from a genome scan of autism kindreds. *Mol Psychiatry* 2006; **11**: 1049-1060, 979 [PMID: 16880825 DOI: 10.1038/sj.mp.4001874]
78 **Yonan AL**, Alarcón M, Cheng R, Magnusson PK, Spence SJ, Palmer AA, Grunn A, Juo SH, Terwilliger JD, Liu J, Cantor RM, Geschwind DH, Gilliam TC. A genomewide screen of 345 families for autism-susceptibility loci. *Am J Hum Genet* 2003; **73**: 886-897 [PMID: 13680528]
79 **Neves-Pereira M**, Müller B, Massie D, Williams JH, O' Brien PC, Hughes A, Shen SB, Clair DS, Miedzybrodzka Z. Deregulation of EIF4E: a novel mechanism for autism. *J Med Genet* 2009; **46**: 759-765 [PMID: 19556253 DOI: 10.1136/jmg.2009.066852]
80 **Kwon CH,** Zhu X, Zhang J, and Baker SJ. Proceedings of the National Academy of Sciences. USA: Dartmouth Journal Services, 2003: 12923-12928
81 **McBride SM**, Choi CH, Wang Y, Liebelt D, Braunstein E, Ferreiro D, Sehgal A, Siwicki KK, Dockendorff TC, Nguyen HT, McDonald TV, Jongens TA. Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a Drosophila model of fragile X syndrome. *Neuron* 2005; **45**: 753-764 [PMID: 15748850 DOI: 10.1016/j.neuron.2005.01.038]
82 **van Woerden GM**, Harris KD, Hojjati MR, Gustin RM, Qiu S, de Avila Freire R, Jiang YH, Elgersma Y, Weeber EJ. Rescue of neurological deficits in a mouse model for Angelman syndrome by reduction of alphaCaMKII inhibitory phosphorylation. *Nat Neurosci* 2007; **10**: 280-282 [PMID: 17259980 DOI: 10.1038/nn1845]
83 **Guy J**, Gan J, Selfridge J, Cobb S, Bird A. Reversal of neurological defects in a mouse model of Rett syndrome. *Science* 2007; **315**: 1143-1147 [PMID: 17289941]
84 **Costa RM**, Federov NB, Kogan JH, Murphy GG, Stern J, Ohno M, Kucherlapati R, Jacks T, Silva AJ. Mechanism for the learning deficits in a mouse model of neurofibromatosis type 1. *Nature* 2002; **415**: 526-530 [PMID: 11793011 DOI: 10.1038/nature711]
85 **Li W,** Cui Y, Kushner SA, Brown RA, Jentsch JD, Frankland PW, Cannon TD, Silva A. The HMG-CoA reductase inhibitor lovastatin reverses the learning and attention deficits in a mouse model of neurofibromatosis 1. *Curr biol* 2005: 1961-1967
86 **Fernandez F,** Morishita W, Zuniga E, Nguyen J, Blank M, Malenka RC, Garner CC. Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome. *Nat neurosci* 2007; **10**: 411-413
87 **Rueda N**, Flórez J, Martínez-Cué C. Chronic pentylenetetrazole but not donepezil treatment rescues spatial cognition in Ts65Dn mice, a model for Down syndrome. *Neurosci Lett* 2008; **433**: 22-27 [PMID: 18226451 DOI: 10.1016/j.neulet.2007.12.039]
88 **Bourtchouladze R**, Lidge R, Catapano R, Stanley J, Gossweiler S, Romashko D, Scott R, Tully T. A mouse model of Rubinstein-Taybi syndrome: defective long-term memory is ameliorated by inhibitors of phosphodiesterase 4. *Proc Natl Acad Sci USA* 2003; **100**: 10518-10522 [PMID: 12930888 DOI: 10.1073/pnas.1834280100]
89 **Alarcón JM**, Malleret G, Touzani K, Vronskaya S, Ishii S, Kandel ER, Barco A. Chromatin acetylation, memory, and LTP are impaired in CBP+/- mice: a model for the cognitive deficit in Rubinstein-Taybi syndrome and its amelioration. *Neuron* 2004; **42**: 947-959 [PMID: 15207239 DOI: 10.1016/j.neuron.2004.05.021]
90 **Ehninger D**, Li W, Fox K, Stryker MP, Silva AJ. Reversing neurodevelopmental disorders in adults. *Neuron* 2008; **60**: 950-960 [PMID: 19109903 DOI: 10.1016/j.neuron.2008.12.007]
91 **Landa RJ**, Kalb LG. Long-term outcomes of toddlers with autism spectrum disorders exposed to short-term intervention. *Pediatrics* 2012; **130** Suppl 2: S186-S190 [PMID: 23118250 DOI: 10.1542/peds.2012-0900Q]
92 **Jennett H**, Jann K, Hagopian LP. Evaluation of response blocking and re-presentation in a competing stimulus assessment. *J Appl Behav Anal* 2011; **44**: 925-929 [PMID: 22219542 DOI: 10.1901/jaba.2011.44-925]
93 **Ehninger D**, Han S, Shilyansky C, Zhou Y, Li W, Kwiatkowski DJ, Ramesh V, Silva AJ. Reversal of learning deficits in a Tsc2+/- mouse model of tuberous sclerosis. *Nat Med* 2008; **14**: 843-848 [PMID: 18568033 DOI: 10.1038/nm1788]
94 **Goto J**, Talos DM, Klein P, Qin W, Chekaluk YI, Anderl S, Malinowska IA, Di Nardo A, Bronson RT, Chan JA, Vinters HV, Kernie SG, Jensen FE, Sahin M, Kwiatkowski DJ. Regulable neural progenitor-specific Tsc1 loss yields giant cells with organellar dysfunction in a model of tuberous sclerosis complex. *Proc Natl Acad Sci USA* 2011; **108**: E1070-E1079 [PMID: 22025691 DOI: 10.1073/pnas.1106454108]
95 **Meikle L**, Pollizzi K, Egnor A, Kramvis I, Lane H, Sahin M, Kwiatkowski DJ. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. *J Neurosci* 2008; **28**: 5422-5432 [PMID: 18495876 DOI: 10.1523/JNEUROSCI.0955-08.2008]
96 **Meikle L**, Talos DM, Onda H, Pollizzi K, Rotenberg A, Sahin M, Jensen FE, Kwiatkowski DJ. A mouse model of tuberous sclerosis: neuronal loss of Tsc1 causes dysplastic and ectopic neurons, reduced myelination, seizure activity, and limited survival. *J Neurosci* 2007; **27**: 5546-5558 [PMID: 17522300 DOI: 10.1523/JNEUROSCI.5540-06.2007]
97 **Carson RP**, Van Nielen DL, Winzenburger PA, Ess KC. Neuronal and glia abnormalities in Tsc1-deficient forebrain and partial rescue by rapamycin. *Neurobiol Dis* 2012; **45**: 369-380 [PMID: 21907282 DOI: 10.1016/j.nbd.2011.08.024]
98 **Tavazoie SF**, Alvarez VA, Ridenour DA, Kwiatkowski DJ, Sabatini BL. Regulation of neuronal morphology and function by the tumor suppressors Tsc1 and Tsc2. *Nat Neurosci* 2005; **8**: 1727-1734 [PMID: 16286931 DOI: 10.1038/nn1566]
99 **Anderl S**, Freeland M, Kwiatkowski DJ, Goto J. Therapeutic value of prenatal rapamycin treatment in a mouse brain model of tuberous sclerosis complex. *Hum Mol Genet* 2011; **20**: 4597-4604 [PMID: 21890496 DOI: 10.1093/hmg/ddr393]
100 **Erbayat-Altay E**, Zeng LH, Xu L, Gutmann DH, Wong M. The natural history and treatment of epilepsy in a murine model of tuberous sclerosis. *Epilepsia* 2007; **48**: 1470-1476 [PMID: 17484760 DOI: 10.1111/j.1528-1167.2007.01110.x]
101 **Zeng LH**, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol* 2008; **63**: 444-453 [PMID: 18389497 DOI: 10.1002/ana.21331]
102 **Zeng LH,** Rensing NR, Zhang B, Gutmann DH, Gambello MJ, Wong M. Tsc2 gene inactivation causes a more severe epilepsy phenotype than Tsc1 inactivation in a mouse model of tuberous sclerosis complex. *Hum Mol Genet* 2011; **20**: 445-454 [PMID: 21062901 DOI: 10.1093/hmg/ddq491]

P- Reviewer Bordey A **S- Editor** Wen LL **L- Editor** A
E- Editor Lu YJ