Targeted treatment trials for tuberous sclerosis and autism: no longer a dream

Mustafa Sahin
The F. M. Kirby Neurobiology Center, Department of Neurology, Children's Hospital Boston, Harvard Medical School, Boston, MA 02115, USA

Abstract

Genetic disorders that present with a high incidence of autism spectrum disorders (ASD) offer tremendous potential both for elucidating the underlying neurobiology of ASD and identifying therapeutic drugs and/or drug targets. As a result, clinical trials for genetic disorders associated with ASD are no longer a hope for the future but rather an exciting reality whose time has come. Tuberous sclerosis complex (TSC) is one such genetic disorder that presents with ASD, epilepsy, and intellectual disability. Cell culture and mouse model experiments have identified the mTOR pathway as a therapeutic target in this disease. This review summarizes the advantages of using TSC as model of ASD and the recent advances in the translational and clinical treatment trials in TSC.

Introduction

Autism spectrum disorders (ASD) affect approximately 1% of children in the United States, and are characterized by defects in social interaction, language delay, and repetitive interests or behaviors. ASD is a major public health problem that disrupts families and leads to significant disability, resulting in a total annual societal cost of ~$35 billion to care for and treat autistic individuals [1]. When initially described by Kanner in the 1940s, researchers believed that this disorder of behavior and cognition resulted from emotional deprivation in infancy [2]. This notion has long since been discarded, and rather it has been recognized that neuronal dysfunction occurs in early brain development in individuals affected with ASD [3–5]. In the last decade or so, we have started to undertake the studies needed to understand the underlying etiology of ASD. Genes play a greater role in the risk of ASD than in any other common neurodevelopmental disorder, with estimates of heritability as high as 60–90% [6•,7•]. However, the genetic cause is unknown in most cases. Collectively, rare copy number variants (CNVs) account for the largest category (6–8%) of known genetic causes of ASD. Combining recurrent CNVs and single gene Mendelian disorders, known genetic causes currently account for less than 15% of ASD cases [8•,9]. Since each of the rare CNVs associated with autism is identified in a very small number of individuals with ASD, leads to alterations in dosage of a relatively large number of genes, and demonstrates phenotypic variability of expression, it is not yet clear how we will be able to utilize this knowledge to develop therapeutics. Identifying the key gene(s) in CNVs is likely to take some time. In contrast, single-gene disorders associated with ASD appear to provide us with a critical opportunity to understand and develop treatments for ASD since they provide both a much larger number and more homogenous group of patients to study. Thus, ASD studies that leverage findings from these single-gene disorders have the potential to elucidate both the underlying neurobiology of ASD and to identify therapeutic drugs and/or drug targets.

Corresponding author: Sahin, Mustafa (mustafa.sahin@childrens.harvard.edu).

Until recently, Mendelian etiologies with high penetrance of ASD such as fragile X syndrome, tuberous sclerosis complex (TSC), and Rett syndrome had been relatively ignored in autism research. For several decades, most of the research in the ASD field was focused on 'pure' or 'idiopathic' autism. In fact, much of the imaging and cognitive neuroscience studies were performed on a relatively small segment of the ASD population with 'high functioning autism' and without known genetic syndromes. This was an unusual approach in neuroscience since research on diseases such as Alzheimer's, Parkinson's and amyotrophic lateral sclerosis have benefited enormously from the study of familial cases with known genetic causes. However, ASD researchers have recently turned to the opportunities provided by the Mendelian disorders strongly associated with autism. A case in point is TSC. This review summarizes the recent progress in TSC translational and clinical research, as an example of how a Mendelian disorder can be used to investigate early detection and development of effective treatments for ASD.

The case for tuberous sclerosis complex in ASD research

TSC is a multisystem genetic disorder in which 90–95% of the affected individuals have CNS involvement. Epilepsy occurs in 80–90% of these patients and can be medically refractory [10,11]. Approximately 45% of TSC patients have mild-to-profound intellectual disability, and ASD occurs in up to 50% of TSC individuals [10,12]. The neuropathological findings in the TSC brain typically include subependymal nodules (SENs), subependymal giant cell astrocytomas (SEGAs) and cortical tubers [13]. Accumulating evidence suggests that TSC patients have non-tuber abnormalities that significantly contribute to the development of the neurological phenotype, including disorganization of axon tracts, aberrant myelination, and defects in synaptic plasticity (see below).

The proteins encoded by the *TSC1* and *TSC2* genes, respectively known as hamartin and tuberin, bind together to form a protein complex that plays a critical role in the regulation of a serine-threonine kinase mammalian target of rapamycin (mTOR), a key regulator of protein synthesis [14]. Thus, without a functional TSC complex, mTOR is hyperactive, resulting in disinhibited protein synthesis and subsequent cell growth [15,16]. Importantly, mutations in at least two other genes that regulate protein translation (*FMR1* and *PTEN*) are also associated with ASD. Taken together these findings strongly suggest that defects in translational regulation may represent one common mechanism leading to ASD phenotypes [17].

Multiple factors make TSC patients a unique population to study the early development of ASD. First, many TSC patients may be diagnosed before birth or at the time of birth due to the presence of rhabdomyomas in the heart. A fetus or a newborn with multiple cardiac tumors has a 95% chance of having TSC [18]. Increased use of prenatal ultrasounds has significantly increased the detection of TSC in the fetus such that cardiac manifestations have become the most common presenting sign of TSC [19], providing a 'newborn imaging screen' to detect individuals with TSC very early in life. Second, approximately 50% of TSC patients are affected with ASD symptoms. This is a much higher incidence than other high-risk groups that can be identified early in life, such as those who are siblings of affected individuals. Third, significant understanding of the aberrant cellular mechanisms in TSC already exists. Among single gene disorders frequently associated with ASD, TSC is one of the best characterized at the cellular level. Finally, the mTOR pathway has been studied from various medical perspectives, including those of oncology, immunology, and transplantation. As a result, there are now three FDA-approved mTOR specific inhibitors. Taken together, these four factors make TSC a unique genetic disorder for the study of the early development of ASD.

Basic cell biology of TSC in neurons

Studies performed both *in vitro* and *in vivo* using mouse models have demonstrated that the Tsc1 and Tsc2 proteins play crucial roles not only in cell growth, but also in axonal, dendritic and synaptic development and function. On the axonal side, TSC1/2 proteins regulate axon specification, guidance, myelination and regeneration. In cultured hippocampal neurons undergoing early neuronal polarity determination, TSC pathway components are expressed in a polarized manner, much higher in nascent axons than dendrites [20–22]. Over-expression of Tsc1 and Tsc2 suppresses axon formation while loss of either gene results in increased axon number [20]. In addition, Tsc2 haploinsufficiency in retinal ganglion cells leads to aberrant neuronal projections, due to the crucial role TSC1/2 proteins play in ephrin/Eph receptor signaling [23•]. Finally, neuronal expression of Tsc1 or Tsc2 is crucial for the proper myelination of axons. Loss of *Tsc1* in neurons causes a block in myelination, consistent with crucial developmental interaction between neurons and oligodendrocytes [24].

On the dendritic side, the TSC/mTOR pathway also regulates dendritic arborization and spine morphogenesis [25–27]. Loss of either Tsc1 or Tsc2 expression increases spine length and head width and reduces spine density in hippocampal slice cultures [27] and *in vivo* [28]. In addition, the mTOR pathway plays a role in postsynaptic AMPA receptor expression [29]. In Tsc1 deficient hippocampal neurons, the AMPA/NMDA receptor current ratio is increased, suggesting a relative enhancement of synaptic AMPA receptors [27]. Furthermore, loss of Tsc1 or Tsc2 in the mouse hippocampus abolishes mGluR-de-pendent long-term depression (LTD), but not NMDA receptor-dependent LTD [30••,31••]. Tsc2+/− rats also display increased paired-pulse facilitation and reduced long-term potentiation [32]. Together, the roles that the TSC1/2 proteins play in axonal, dendritic development and synaptic function strongly indicate that loss of TSC1/2 is likely to result in defects in synapse formation and plasticity, likely correlating with the neurocognitive and behavioral symptoms of TSC. Since the ASD are starting to emerge as 'developmental disconnection syndromes,' [33,34] TSC is especially suited to understand the contribution of axonal, dendritic and synaptic defects in ASD.

Mouse models of TSC can be treated with mTOR inhibitors

Several mouse models of TSC have been generated and all of them display neurocognitive deficits [24,28,35–37], many of which can be reversed with treatment with mTOR inhibitors [28,35]. For example, *Tsc1*^{+/−} mice show impaired learning in hippocampal-dependent learning tasks and impaired social behavior, supporting a model in which haploinsufficiency for the TSC genes leads to aberrations in neuronal functioning resulting in impaired learning and social behavior [36]. Similarly, *Tsc2*^{+/−} mice show deficits in synaptic plasticity, learning, and memory [35]. These deficits emerge in the absence of gross structural brain abnormalities or seizures, demonstrating that other disease mechanisms are involved. Notably, a brief 5-day treatment with rapamycin in adult mice rescued not only the synaptic plasticity, but also learning and memory deficits in this animal model [35]. More severe mouse models of TSC have been generated by homozygous inactivation of the *Tsc1* gene in neurons or astrocytes [24,28]. In both cases, mice develop epilepsy and premature death, which can be prevented or reversed by rapamycin. These experiments demonstrate that postnatal mTOR inhibition prevents the neurological and behavioral defect in TSC-deficient mice.

Another brain pathology in TSC disease is the presence of SENs and SEGAs. SENs are lesions found along the wall of the lateral ventricles in the brain. These benign lesions do not cause any medical problems for patients; however, in 5–10% of cases, a SEN can grow into

SEGA. SEN-like structures in mice were only recently achieved by loss of Tsc1 specifically in postnatal neural stem/progenitor cells [38•]. Recently, huge progress has been achieved in testing the efficacy of mTOR inhibitors on SEGA volume in patients (see below).

Human clinical trials with mTOR inhibitors in TSC

In parallel with the basic neurobiology research in the role of TSC1/2 in brain development, there has been major progress in the use of mTOR inhibitors in several aspects of medical care (Figure 1). Following the discovery of rapamycin in the 1970s, rapamycin and related mTOR inhibitors have been developed for several clinical indications; first to prevent solid organ transplant rejection, and later to prevent stenosis of artificial coronary stents and treatment for several types of cancer. While *TSC1* and *TSC2* were identified in the 1990s as causative genes in TSC, it was not until 2002 that evidence demonstrating a role for TSC1/2 in mTOR regulation emerged [39–43]. At that time, David Franz and colleagues began to consider the possibility that rapamycin and related mTOR inhibitors might have clinical benefit for various clinical features in TSC [44]. They initially treated SEGAs in patients who had failed previous surgical resections or were otherwise unsuitable for conventional surgery. For these studies, the size of the SEGA on brain imaging provided an objective primary outcome measure, as commonly used in oncology treatment trials. The initial case series, in which all 5 patients responded to treatment with rapamycin [44], was followed up with a prospective, phase I/II clinical trial with the mTOR inhibitor everolimus. In that study 21 of 28 (75%) TSC patients showed a reduction in SEGA volume of at least 30%, and none progressed [45••]. The latter trial led to the approval of everolimus for SEGAs in TSC by the FDA in 2010 (US) and EMEA in 2011 (Europe). Other studies have reported on the efficacy of mTOR inhibitors for treatment of additional manifestations of TSC, including renal angiomyolipomas [46,47•,48•], pulmonary lymphangioleiomyomatosis [46,47•,48•,49•], cardiac rhabdomyoma [50•] and facial angiofibromas [47•,51–55]. In addition to providing clinical evidence of benefit for these TSC-related tumors, these trials provided crucial safety data on the use of mTOR inhibitors in TSC patients. Consequently, evidence of major benefit of rapamycin and related drugs for neurobehavioral phenotypes in mouse models and evidence of both benefit and overall tolerance in human clinical trials on tumor manifestations of TSC have led to the design of clinical treatment trials aimed at neurocognitive deficits in TSC.

One of the lessons of the early clinical trials of mTOR inhibitors is that the selection of inclusion and exclusion criteria has to be carefully tailored for each outcome measure. In other words, one cannot necessarily look at all outcome measures in one group of subjects. For example, a group of individuals with TSC that has SEGAs may not have the ability to participate in neurocognitive testing batteries because of age or intellectual ability. Therefore, a separate trial needed to be designed to investigate whether mTOR inhibition could improve neurocognition in children with TSC (*clinicaltrials.gov*: NCT01289912). This placebo-controlled double blind trial of everolimus is currently enrolling children with TSC between the ages of 6 and 21 years, with IQ greater than or equal to 60, who are stable on an anti-seizure medication regimen and have no evidence of SEGAs. The primary endpoint is improvement on neurocognitive tests while autism, seizure frequency, and sleep habits are evaluated as secondary endpoints. This trial highlights one of the biggest challenges in treatment trials in neurodevelopmental disorders: How do we demonstrate that a treatment has had an effect? What are the most sensitive outcome measures that are dynamic within a reasonable time span of treatment? Does improvement in an outcome measure correlate with better function in real life? Significant attention has to be paid to choosing the most sensitive, reliable and quantitative outcome measures appropriate for each group of subjects.

A second lesson from these early trials has been the need for multi-center collaborations. For longitudinal studies in a rare disease such as TSC, traveling long distances for research visits at one center is extremely taxing for families, especially when their children are affected with ASD, epilepsy, sleep, and behavioral disorders, as is often the case in TSC. Therefore, multi-center collaborations are necessary to recruit sufficient subjects and to gather the large amount of data required to test the hypotheses in a reasonable time frame. This is best accomplished in concert with national advocacy organizations — in this case the Tuberous Sclerosis Alliance. Such advocacy organizations have been crucial in supporting basic, translational and clinical studies in TSC and other neurodevelopmental disorders, not only by directly funding research and informing families about study opportunities which is essential for recruitment, but also by bringing together leaders of the field and encouraging collaborations.

Need for biomarkers

An abundance of clinical and basic science evidence suggests that mTOR inhibitors represent a rational candidate for the treatment of neurodevelopmental disabilities in TSC. However, mTOR inhibitors can have side effects, such as immuno-suppression and endocrine dysfunction. Given the prominent role of the mTOR pathway in many normal physiological functions, there is also the theoretical risk of adverse effects on growth and development, especially early in life. Despite these risks, steps can be taken to minimize unnecessary exposure and risk of mTOR inhibitors in the context of drug treatment of ASD in TSC patients. Since not all patients with TSC develop ASD or intellectual disability, an early biomarker that can reliably identify at a young age those children who will develop severe neurodevelopmental deficits would be very helpful. A recent study suggests that treating TSC patients that have an abnormal EEG before onset of infantile spasms with vigabatrin may improve neurological outcome [56••], but the use of EEG as a reliable biomarker of future ASD or even epilepsy has not been rigorously validated. Diffusion tensor imaging (DTI) appears promising as a biomarker of neurological complications and ASD [57•,58••], but needs to be studied in a larger group of children with TSC and preferably in a prospective trial. With a predictive biomarker, the highest risk TSC patients who are most appropriate for a drug treatment (e.g. a clinical trial) could be selected, thus sparing the low-risk TSC patients exposure to a drug with potential risk and likely increasing the efficacy of the treatment for a targeted population. Furthermore, sensitive biomarkers can potentially accelerate treatment trials as quantitative surrogate endpoints. Lastly, mechanistically based biomarkers could help identify subgroups of 'idiopathic' ASD patients that can benefit from treatments similar to those effective in homogenous populations like TSC.

Conclusions: other barriers and future directions

While the excitement over our ability to finally offer mechanism-based treatment options for rare genetic diseases is warranted, we should recognize that there are still several obstacles to performing treatment trials in neurodevelopmental disorders. One obvious difficulty is the risk-benefit analysis. For many parents whose children are affected with ASD and other neurodevelopmental disorders, treatment trials offer the hope of their child having more effective communication or enhanced social skills. However, for institutional review boards (IRBs), weighing the risk-benefit ratio of a treatment trial is much more difficult in the area of neurodevelopmental disorders than in oncology. This reluctance to intervene may subside once treatment trials in neurodevelopmental disorders become more common, and if there are therapeutic successes. Second, the number of physician scientists trained in clinical trials for neurodevelopmental disorders is extremely low. As the targets for treatment trials in neuroscience increase (and there is no question they will increase with the general

application of next-generation sequencing), the number of physician scientists with this expertise also has to be increased. This will require not only additional mentoring and guidance, but also providing the incentives for young clinicians to choose this path.

Similar to the TSC trials described here, targeted treatment trials have recently been initiated for other known genetic disorders associated with ASD such as fragile X, Rett and Down syndromes (Table 1). There is no doubt that the number of potential treatment targets in neurodevelopmental disorders will increase based on investigations of single-gene disorders; for instance, it was recently discovered that a common anti-cancer drug (topotecan) can turn on the normally dormant paternal *Ube3a* allele in the brain of the Angelman syndrome mouse model [59••]. Recent evidence from mouse models indicate that even disorders that appear quite similar in terms of cell biology (i.e. regulation of protein synthesis) such as TSC and FXS may have diametrically opposite physiological phenotypes under certain circumstances [31••]. Therefore, a detailed understanding of the circuitry and cellular abnormalities associated with each single-gene disorder is crucial to select the most effective treatment. Otherwise, one type of therapy that relieves neurological symptoms in one disease could potentially be harmful in the other. Until we have better biomarkers to predict the treatment response, using these therapies in idiopathic (genetically undefined) autism is similarly risky. Finally, just like in other areas of clinical medicine, we have to be mindful of the fact that not all trials will succeed. Despite the challenges and barriers, carefully designed treatment trials in genetically defined neurodevelopmental disorders are one of the most exciting avenues of research in science and medicine today, and we hope that they will have a positive impact, improving the lives of children and families affected with these disorders in the near future.

Acknowledgments

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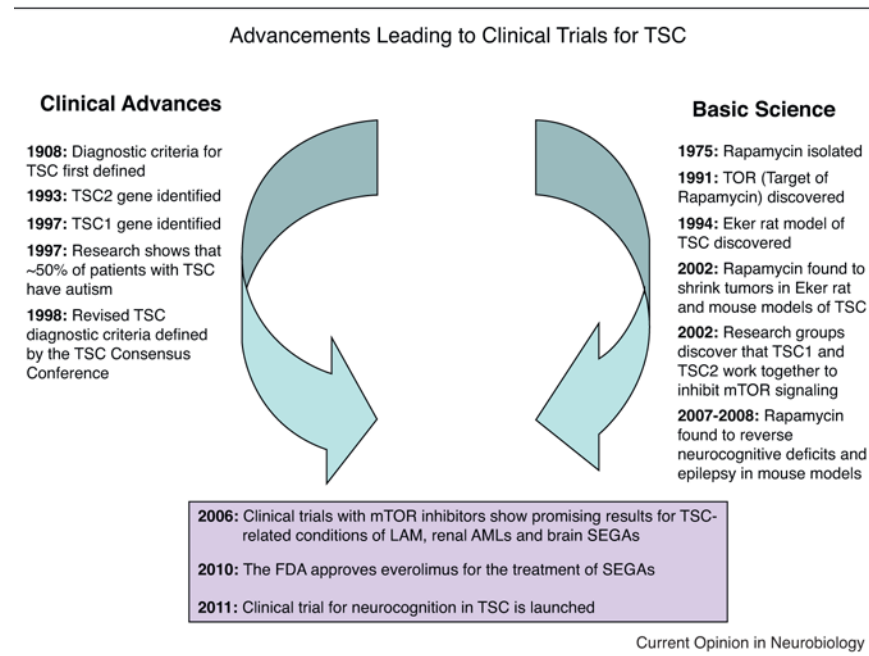
Advancements Leading to Clinical Trials for TSC

Clinical Advances

1908: Diagnostic criteria for TSC first defined

1993: TSC2 gene identified

1997: TSC1 gene identified

1997: Research shows that ~50% of patients with TSC have autism

1998: Revised TSC diagnostic criteria defined by the TSC Consensus Conference

Basic Science

1975: Rapamycin isolated

1991: TOR (Target of Rapamycin) discovered

1994: Eker rat model of TSC discovered

2002: Rapamycin found to shrink tumors in Eker rat and mouse models of TSC

2002: Research groups discover that TSC1 and TSC2 work together to inhibit mTOR signaling

2007-2008: Rapamycin found to reverse neurocognitive deficits and epilepsy in mouse models

2006: Clinical trials with mTOR inhibitors show promising results for TSC-related conditions of LAM, renal AMLs and brain SEGAs

2010: The FDA approves everolimus for the treatment of SEGAs

2011: Clinical trial for neurocognition in TSC is launched

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Figure 1.
Some milestones in defining the pathophysiology of tuberous sclerosis complex (TSC). The current therapeutic efforts in the neurocognitive phenotype of TSC patients stem from the contributions from two independent lines of research: clinical advances in genetics and phenotyping of TSC (left timeline) and basic research on the TSC-mTOR pathway and mouse models (right timeline). See text for details.

Table 1

Targeted clinical trials for genetic disorders associated with ASD (from clinicaltrials.gov 1/13/12)

Genetic disorder associated with ASD	Study drug	ASD related outcome measures	Clinicaltrials.gov study ID
Tuberous sclerosis complex (TSC)	Everolimus (RAD001)	ASD, memory, language skills, cognition, behavior, sleep	NCT01289912
Rett syndrome	rhIGF-1	Behavior, cognition, motor function, language skills	NCT01253317
Fragile X syndrome	(1) Acamprosate	(1) Hyperactivity, language impairment, irritability, social deficits, and cognitive delay	(1) NCT01300923
	(2) Arbaclofen (STX209)	(2) Social withdrawal	(2) NCT01325220
	(3) Minocycline Hydrochloride	(3) Language, behavior, cognition	(3) NCT01053156
	(4) AFQ056 and RO4917523	(4) Learning, cognition, behavior	(4) NCT01253629 and NCT01015430
Down syndrome	(1) Memantine	(1) Cognition, memory, language	(1) NCT01112683
	(2) Rivastigmine	(2) Language, memory, and executive function	(2) NCT01084135
	(3) RG1662	(3) Cognition, memory	(3) NCT01436955