Guest Editorial

Tuberous Sclerosis Complex: Future Research Directions

Bernard L. Maria, MD, MBA

Scientific advances of the past decade have improved our understanding of the pathogenesis of many disorders characterized by neurologic dysfunction. In several of these disorders, new diagnostic tools and therapies have been developed, tested, and adopted. However, the pace of discovery in the basic and clinical neurosciences has made it challenging for child neurologists to determine what molecular diagnostic strategies, sophisticated neuroimaging tools, or innovative therapies should be adopted to manage not only common ailments but also the estimated 1500 rare diseases that cause neurologic dysfunction.

In 2001, the National Institutes of Health funded a 5-year conference grant titled "Neurobiology of Disease in Children." The overall goal was to bring together clinicians, scientists, caregivers, and National Institutes of Health program officers to determine how research findings can be translated to enhance clinical understanding and affect clinical practice.

Tuberous sclerosis complex was selected as the topic for the third symposium (neurofibromatosis and leukodystrophy were the topics of the first and second conferences, respectively). The conference format included formal presentations, question-and-answer sessions, panel discussions, and open discussions to directly address conference objectives. The symposia directors were Drs David Gutmann and Steve Roach, who are highly respected leaders in the field of tuberous sclerosis complex. The panel included speakers and moderators, each an expert in the field, selected by the directors to ensure authoritative presentations on key topics and productive discussions on research directions. The question-and-answer sessions after each set of talks encouraged brainstorming between the panelists and audience of child neurologists about the best directions of future research and how clinicians can contribute.

The first presentation, "Current Approaches to Diagnosis," described current approaches to the diagnosis of tuberous sclerosis and progress toward understanding brain abnormalities, imaging findings, and behavioral consequences. The second session, "Treatment and Management of Complications," addressed the problems of epilepsy and its medical and surgical management and the non-neurologic complications of tuberous sclerosis. These presentations provided the audience with an understanding of the spectrum of clinical disease in tuberous sclerosis complex and the controversies in the management of neurologic and non-neurologic complications. The topic of the third session, "Genetic Strategies," provided the audience with an understanding of clinical and molecular genetic aspects and information about tuberous sclerosis complex genes and mouse models. The fourth session, "Future Directions and Innovative Therapies," discussed approaches for therapy and current or proposed clinical trials and questions germane to planning such trials (eg, natural history, measurement of outcomes). This last session included a panel discussion moderated by Drs Bob Finkelstein (National Institutes of Health) and Vicky Whittemore (Tuberous Sclerosis Alliance), which provided insights into the implementation of advances for the benefit of patients and families.

I wish to express my sincere appreciation to the National Institute of Neurologic Disorders and Stroke, the Child Neurology Society (St. Paul, MN), and the Tuberous Sclerosis Alliance for cosponsoring the conference. Also, I thank Drs Gutmann and Roach for directing a superb symposium on tuberous sclerosis complex.

One of the specific aims of the "Neurobiology of Disease in Children" conferences is to disseminate the proceedings of the symposia to ensure that clinicians and basic scientists are informed about scientific advances, current research initiatives, and future directions. This issue of the *Journal of Child Neurology* features a series of papers prepared by conference participants and the verbatim transcript of the conference's question-and-answer sessions, panel discussions, and open discussions.

Rett syndrome has been selected as the topic for the fourth "Neurobiology of Disease in Children" conference, which will be held on October 13, 2004, in Ottawa, Ontario. In keeping with the objectives of the original proposal, the program directors, Drs Alan Percy and Carolyn Schanen, have prepared a superb agenda that includes a panel of the most highly respected leaders in the field of Rett syndrome. We look forward to seeing you in Ottawa!

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Discussion

Tuberous Sclerosis Complex: Pathogenesis, Diagnosis, Strategies, Therapies, and Future Research Directions

Bernard L. Maria, MD, MBA; Kathleen McCann Deidrick, PhD; E. Steve Roach, MD; David H. Gutmann, MD, PhD

INTRODUCTION AND STATEMENT OF SYMPOSIUM GOALS

Bernard L. Maria, MD, MBA, Executive Director of the Children's Research Institute; Jeffrey Edwin Gilliam Chair and Professor, Medical University of South Carolina, Charleston, SC

The symposium on tuberous sclerosis complex was the third in a series of five symposia titled "Neurobiology of Disease in Children." The conference was supported by the National Institute of Neurological Disorders and Stroke (NINDS), the Tuberous Sclerosis Alliance, and the Child Neurology Society. Dr Maria outlined the following goals for the series:

- Identify research findings that have the potential to enhance clinical practice in child neurology
- Identify future research needed to improve diagnostic accuracy and to develop safe and effective therapies
- Enhance collaboration between basic and clinical scientists
- Introduce students to research on neurologic disease in children

Objectives specific to the tuberous sclerosis complex symposium included description of diagnostic strategies, review of pathogenesis, discussion of therapies and clinical trials, definition of future research directions, and publication of the proceedings.

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From the Children's Research Institute (Dr Maria), Medical University of South Carolina, Charleston, SC; the Department of Psychology (Dr Deidrick), University of Missouri, Columbia, MO; the Department of Neurology (Dr Roach), Wake Forest University School of Medicine, Winston-Salem, NC; and the Department of Neurology (Dr Gutmann), Washington University School of Medicine, St. Louis, MO.

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CURRENT APPROACHES TO DIAGNOSIS OF TUBEROUS SCLEROSIS COMPLEX

Moderator: E. Steve Roach, MD, Professor of Neurology, Pediatric Neurology, and Pediatrics, Wake Forest University Baptist Medical Center, Winston-Salem, NC

Overview and Approaches to Diagnosis

E. Steve Roach, MD, Wake Forest University Baptist Medical Center, Winston-Salem, NC

Dr Roach reviewed the criteria for clinical and genetic diagnosis of tuberous sclerosis complex. In his presentation, he outlined the associated clinical features of the disease. Tuberous sclerosis complex is an autosomal dominant neurocutaneous disorder with a prevalence of 1 in 6000 to 9000 people. Although a positive family history of tuberous sclerosis complex is frequently observed, spontaneous genetic mutations are common (65–75% of cases). Genetic studies have resulted in the identification of two genes implicated in the disease: *TSC1* and *TSC2*.

Definite tuberous sclerosis complex, as defined by the 1998 consensus conference sponsored by the Tuberous Sclerosis Alliance and the National Institutes of Health (NIH), is diagnosed when at least two major or one major plus two minor features are present. Probable tuberous sclerosis complex includes one major and one minor feature. Possible tuberous sclerosis complex includes one major or two or more minor features. Major features include skin manifestations (ie, facial angiofibromas, ungual fibroma, more than three hypomelanotic macules, and shagreen patch), brain and eye lesions (ie, cortical tuber, subependymal nodules, subependymal giant cell astrocytomas, multiple retinal nodular hamartomas), and tumors in other organs (ie, cardiac rhabdomyoma, lymphangioleiomyomatosis, renal angiomyolipoma). Minor features include multiple randomly distributed pits in dental enamel, rectal polyps, bone cysts, cerebral white-matter migration abnormalities on brain imaging, gingival fibromas, nonrenal hamartomas, retinal achromic patches, confetti skin lesions, and multiple renal cysts. The 1998 criteria do not include symptoms such as seizures or mental retardation to avoid "double counting" (ie, central nervous system lesions cause seizures, and including both in the

criteria leads to counting the same symptom twice). Associated neurologic features also include seizures, autism or pervasive developmental disorders, mental retardation, and various learning and behavioral disorders.

The clinical criteria outlined above are useful, despite the availability of a genetic test for tuberous sclerosis complex, because they are quick, accurate, and inexpensive. The genetic test for tuberous sclerosis complex has a false-negative rate of 20%, partly because of complications in the way in which the disease is transmitted. Specifically, in 2% of patients, the mutated *TSC* gene is not detected in either parent because of germline mosaicism. This might be more frequent when the mother is the carrier and when *TSC2* mutations are involved. Despite these difficulties, genetic testing is still indicated when the clinical diagnosis is unclear and/or when parents are making decisions about family planning. Phenotypic variability can be related to various factors: (1) the stronger phenotypic presentation in patients with *TSC2* gene mutations, (2) somatic mosaicism, and (3) the specific type of genetic mutation.

Brain Abnormalities in Tuberous Sclerosis Complex

Francis J. DiMario Jr, *MD*, *Professor of Pediatrics and Neurology*, *University of Connecticut School of Medicine*, *Farmington*, *CT*; *Associate Chair for Academic Affairs and Faculty Development*, *Department of Pediatrics*, *Chief*, *Division of Pediatric Neurology*, *Connecticut Children's Medical Center*, *Hartford*, *CT*

Dr DiMario described the central nervous system lesions in tuberous sclerosis complex, the relationship between tuberous sclerosis complex and neuronal migration disorders, and morphometric abnormalities observed in brain imaging studies. Ninety percent of people with tuberous sclerosis complex exhibit at least one supratentorial brain lesion, including cortical tubers, subependymal nodules, subependymal giant cell astrocytomas, white-matter linear migration lines, corpus callosum agenesis or dysplasia, and transmantle cortical dysplasia. Infratentorial brain lesions are less common (< 2% of patients) and can include linear and gyriform cerebellar folia calcifications, cerebellar nodular white-matter calcifications, agenesis and hypoplasia of the cerebellar hemispheres and vermis, enlargement of the cerebellar hemispheres, and subependymal nodules and tubers in the brain stem and fourth ventricle.

Brain imaging structural abnormalities can occur owing to disturbed neural migration, leading to changes in the number, size, and/or thickness of cortical gyri; heterotopic neuronal aggregates; and variable degrees of cortical cytoarchitectural disorganization (eg, aberrant columnar and laminar arrangement). Specifically, hamartomas are thought to represent focal areas of improperly proportioned cellular components that result from poor cellular organization or differentiation. Tubers, subependymal nodules, and subependymal giant cell astrocytomas result from focal tissue dysplasia, whereas a transmantle cortical dysplasia represents a more diffuse tissue dysplasia.

Dr DiMario and colleagues examined morphometric measures in patients with tuberous sclerosis complex to determine whether abnormalities in neural migration affect whole brain development and function. The authors found that 92% of participants with tuberous sclerosis complex had focal abnormalities (mean 5.7 subependymal nodules, 6.7 tubers). Abnormalities were most common in the periventricular and frontal region, including larger ventricles and a corresponding decrease in gray and white matter. The number of subependymal nodules was associated with increased posterior ventricle size, whereas the number of tubers was associated with increased anterior ventricle size. Subependymal nodules and tubers were also associated with increased frequency of seizures, cognitive impairment, and adaptive functioning. Regional morphometric brain disturbances were observed both in conjunction with and separate from periventricular migration lesions.

Structural Magnetic Resonance Imaging of Tuberous Sclerosis Complex

Edward Bullmore, *PhD*, *Professor of Psychiatry; Khanum Ridler*, *PhD*, *Research Associate*, *University of Cambridge*, *Cambridge*, *UK*

Dr Bullmore reviewed the work of Dr Khanum Ridler describing morphometric magnetic resonance imaging (MRI) studies in individuals affected with tuberous sclerosis complex. Two traditional approaches are used in examining morphometry. The first is the "region of interest" approach, in which the volume of the area most likely to be affected by tuberous sclerosis complex is estimated. The benefits of the approach include accessibility to expert information, definition of regions as native space, and support of regional-level factorial modeling. The disadvantages include the need for a prior hypothesis, a lack of comprehensiveness, the intensity of human labor required, and less-than-perfect interrater reliability. Recent advances have led to computational approaches in which an axial image of the brain is obtained, the brain image is lifted from the rest of the image, and estimates of brain tissues are made. This approach requires no prior knowledge of the brain abnormality; is comprehensive, robust, and reliable; and supports voxel and cluster level computational modeling. Drawbacks include a lack of accessibility to expert information and the use of standard space modeling. Automated anatomic modeling is a new approach that combines aspects of region of interest and computational methods. In this method, brain maps are put into the space, but a template divides the gray matter into 45 regions of interest in each hemisphere.

Dr Bullmore described the results of two case-controlled studies that used computational morphometry to study patients with tuberous sclerosis complex. These studies collectively suggested that bilateral symmetric deficits in subcortical gray matter and intrahemispheric white matter could be considered markers of tuberous sclerosis complex. Tubers were distributed symmetrically and were most often located in the frontal lobe. The density of tubers was greatest in the parietal lobes, and tubers were more frequent in the cingulate gyrus than expected. Tuber volume was positively correlated with the number of subependymal nodules

and negatively correlated with gray-matter volume. However, the location of tubers and subependymal nodules was unrelated to the location of gray- or white-matter deficits. Furthermore, when brain volume was corrected for the volume of tubers and subependymal nodules, gray-matter deficits were still apparent. In a group of patients of average intelligence, cognitive deficits in working memory and immediate and delayed recall were noted. In contrast, recognition memory, inhibition, and processing speed were intact. Memory scores were correlated with subcortical gray-matter volume. However, tuber and subependymal nodule volumes were unrelated to cognitive processing skills. The authors concluded: "Computational morphometry can complement and add value to anatomical characterization of neurodevelopmental syndromes."

Behavioral and Cognitive Aspects of Tuberous Sclerosis Complex

Penny Prather, PhD, Department of Neurology and Psychiatry, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Dr Prather reviewed the literature that suggests that tuberous sclerosis complex can be considered a risk factor for cognitive and developmental problems. Early descriptions of tuberous sclerosis complex emphasized the presence or absence of mental retardation or "delay." Although few studies examine cognition closely, as more patients are identified with tuberous sclerosis complex, it becomes apparent that cognitive outcomes vary more than was previously reported. Studies largely using parent surveys and reports of functional abilities estimate that 37 to 65% of children with tuberous sclerosis complex exhibit mental retardation. In one of only two studies to use structured tests, intellect was normally distributed, with the exception of a large cluster of children (30%) functioning in the severely deficient range.

Neuropsychologic studies ($N = 5$) suggest additional deficits in executive functioning and attention in children with tuberous sclerosis complex. In addition, children with tuberous sclerosis complex are at risk of symptoms of autism (≥ 40% of patients) and exhibit an increased risk of disruptive behavior disorders (eg, impulsivity and aggression) and social problems (eg, poor judgment and social awkwardness). Early onset of seizures (< 3 years of age; infantile spasm) and/or intractable seizures appear to be associated with an increased risk of neurodevelopmental and cognitive problems. Moreover, the number of tubers present on brain MRI is positively correlated with age at seizure onset and negatively correlated with global cognitive functioning. In this respect, it is unclear whether tubers, seizures, or both are specifically related to the learning deficits seen in children with tuberous sclerosis complex. Similar factors can be associated with autism in tuberous sclerosis complex. Specifically, Bolton and colleagues (2002) found that the concurrent finding of tubers in the temporal lobe and the early onset of temporal lobe seizures (< 12 months of age) were associated with the diagnosis of autism, implicating disruption of subcortical-orbitofrontal circuits.

Dr Prather summarized her research on neuropsychologic profiles in a sample of individuals affected with tuberous sclerosis complex ranging in age from 3 months to 50 years. Fifty-eight percent of the individuals in her sample were described as having cognitive function within normal limits, whereas 42% were described as delayed. Among children aged 6 to 18 years who completed a full testing battery (21%), deficits in regulation or executive functioning (62%), visual-motor organization and planning (54%), memory or information retrieval (38%), language (24%), and spatial (19%) abilities were observed. She concluded with a summary of consensus recommendations for neuropsychologic evaluation of children with tuberous sclerosis complex. Guidelines emphasize the importance of repeated evaluation at times associated with developmental shifts (ie, infancy, toddlerhood, preschool, grade-school entry, entry to upper primary grades, and adolescence).

Autism and Tuberous Sclerosis Complex

Max Wiznitzer, MD, Associate Professor of Pediatrics, Division of Pediatric Neurology, Case Western Reserve University, Rainbow Babies and Children's Hospital, Cleveland, OH

Dr Wiznitzer reviewed the core symptoms of autism, including impaired social interaction and communication and repetitive and restricted interests present before the age of 3 years. Much evidence for comorbidity between autism spectrum disorders and tuberous sclerosis complex comes from an extensive series of case studies. However, the methodology in many of these case studies is limited (eg, incomplete evaluation of tuberous sclerosis complex or autism, waxing and waning of the symptoms of autism). In group studies of autism, 1 to 4% of children exhibit tuberous sclerosis complex. Conversely, in group studies of tuberous sclerosis complex, 25% or more of children exhibit autistic features. However, several factors make it difficult to interpret this research. These include unrepresentative samples, failure to use "gold standard" assessment tools, and the presence of other disorders associated with symptoms of autism (eg, mental retardation and seizures). In tuberous sclerosis complex, an equal number of boys and girls are diagnosed with autism. This is in contrast to studies of classic autism, in which boys outnumber girls.

Several possible reasons for the increased risk of autism in children with tuberous sclerosis complex were discussed. First, the genes associated with tuberous sclerosis complex can directly affect the development of brain regions associated with autism. Research indicates that the *TSC* gene products are expressed in regions of the brain implicated in autism (ie, frontal and temporal lobes). Second, autism spectrum disorders can occur in association with tuberous sclerosis complex–related brain disorganization, including cognitive impairment, seizures, and tuber location. Symptoms of autism are prevalent in children with mental retardation, regardless of etiology. Given that mental retardation is prevalent in this population, autism spectrum disorders could be related to intellect. Alternatively, seizures could disrupt central nervous system organization, resulting in symp-

toms of autism spectrum disorders that could wax and wane along with seizure activity. The presence of tubers in specific brain regions also could be explanatory, although these findings were not conclusive. Finally, there could be linkage between the *TSC* genes and genes related to autism spectrum disorders. A genome scan implicated chromosome 16 in autism spectrum disorders. In that the *TSC2* gene is located on chromosome 16, these genetic studies suggest that linkage between autism spectrum disorder and tuberous sclerosis complex might be possible. However, this seems unlikely because of differences in gender ratio, the spectrum of cognitive impairment, and the frequency of seizure disorders between the tuberous sclerosis complex and autism spectrum disorder populations. Dr Wiznitzer suggested that physicians should consider the possibility of tuberous sclerosis complex in individuals with autism, particularly if seizures and/or mental retardation are present. Conversely, monitoring individuals with tuberous sclerosis complex for autism spectrum disorders is also indicated, with particular attention to the possibility of emergence or worsening of symptoms associated with seizure onset.

Question and Answer Session 1

Dr Roach: Can you elaborate on how the morphometric analysis was performed on the scans?

Dr DiMario: The measures were conducted primarily on axial sections at the level of the caudate.

Audience Member: Why does there seem to be frontal predominance of lesions in tuberous sclerosis complex?

Dr DiMario: There is no good answer for that question, but there must be a biologic explanation relating to the ventricular zone in that region.

Audience Member: Do you know if changes occur over time in tuberous sclerosis? In attention-deficit hyperactivity disorder (ADHD) and schizophrenia, changes can be shown in longitudinal studies.

Dr DiMario: We haven't looked at it ourselves, but patients with frequent seizures taking antiepileptic drugs could well have progressive cortical changes.

Dr Roach: The classic dogma has been that lesions develop before 22 weeks' gestation, and everything' is then "set in stone." On the other hand, a normal brain is continuing to evolve after birth, and some patients seem to acquire lesions. There is often a question about whether the imaging technology has become more sensitive or whether a lesion is truly progressive.

Audience Member: There appears to be no anatomic correlation between gray-matter deficits and the distribution of the tubers, but is it possible that reciprocal connections between the two areas could account for the gray-matter deficits?

Dr Bullmore: I think that's a very interesting point. Generally, these structures are part of circuits, and presumably that is why damage to a region is associated with impairment of so many different aspects of working memory. One of the things we'd like to do is take the data analysis forward a little bit and see if we can understand whether there are differences between the cases, not just on a region-by-region basis but in terms of the circuits. I think the idea that there might be some kind of remote connections or correlations between anatomically remote areas of regional deficit is absolutely correct.

Dr Maria: Why do you think serial recognition memory seems to be much more robust? You showed us very impressive thalamic involvement. One would have expected serial recognition memory to be as impaired. Any thoughts on why that might be?

Dr Bullmore: No, it's very difficult to explain that, and I think I should make a number of points. One is that this general approach to taking structural data and psychological test scores and trying to understand one in terms of the other is a rich approach and could be used to look at various psychopathologies and to understand abnormal variation. There isn't a great hypothesis about what is expected. It would be very nice in a few years' time to be able to come back to the question.

Dr Roach: That's a pretty remarkable group of tuberous sclerosis patients with an average IQ of 114. Have you studied more typical individuals with IQs of 50 to 60?

Dr Bullmore: I think the choice of higher IQ participants was pragmatic because we wanted good quality imaging data to examine the relationship between structure and function. But it would be certainly very interesting and important to know more about the anatomic profile and also structure and function, with perhaps a more representative sample of patients.

Audience Member: Do the data show that the presence of infantile spasms is a risk factor for cognitive dysfunction in tuberous sclerosis? The numbers seem small to draw that conclusion.

Dr Prather: I agree that the data do not yet show a clear association between the presence of infantile spasms and cognitive dysfunction in tuberous sclerosis.

Dr Roach: That's a good point. There was an earlier paper by Charles Shepherd and Manny Gomez that looked at number and variety of spasms. Quite clearly, if there are many lesions, spasms are more likely, but cognitive dysfunction could be related to the number of lesions in the first place.

Dr Maria: Are there any longitudinal data in the way of neuropsychological assessment that might give us some sense of natural history? That's my first question. And my second question is what would you recommend as an approach to neuropsychological investigation? Are we saying now that every child who would be entering kindergarten would be required to have a full neuropsychological evaluation? What would you generally recommend?

Dr Prather: There are some consensus guidelines coming out, and I think this is in the "forewarned is forearmed" requirement, but I think it's very helpful for a number of reasons to have re-evaluations at regular intervals in tuberous sclerosis. I believe that children with tuberous sclerosis should be evaluated when entering school, in the fourth grade, and again in the ninth grade.

Audience Member: Dr Wiznitzer, I wanted to address your comments about linkage studies on chromosome 16. You were a little bit less enthusiastic with reference to the *TSC2* gene because of the difference in the gender ratio.

Dr Wiznitzer: No, what I meant is that if we're looking for an independent linkage between an autism gene and autism just goes along for the ride, we probably would expect to see the male:female ratio that we see in autism.

Audience Member: Dr Wiznitzer, you provided a very elegant and extensive survey of this tuberous sclerosis association. Recognizing that your population of pediatric neurologic patients com-

prises several thousand children with autistic spectrum disorders, how common is tuberous sclerosis in your population of patients?

Dr Wiznitzer: In my patient population, I have yet to identify a patient with autism who did not have other clinical features of tuberous sclerosis, including epilepsy.

Audience Member: Can tuberous sclerosis present with strictly behavioral problems?

Dr Wiznitzer: It would be unusual for behavioral problems to be a presenting complaint in the absence of developmental delay. According to the American Academy of Neurology guidelines, children with unexplained global developmental delay should have MRI. But even before considering an MRI, it is important to conduct a dermatologic examination. In my opinion, the physical examination is paramount to making the diagnosis of tuberous sclerosis. I usually do not order neuroimaging for individuals with autism.

Audience Member: Do brain lesions in tuberous sclerosis shrink or disappear, as has been reported for cardiac lesions?

Dr Roach: The heart lesions shrink within 2 to 3 years, but lesions in a 6 year old are not likely to vanish. However, postmortem exams can show residual cardiac lesions that were too small for imaging resolution. There is no information about central nervous system lesions disappearing.

Audience Member: And you don't know why the heart lesions can be smaller?

Dr Roach: The cardiac lesions are rich in glycogen, and there's speculation that the glycogen is used up.

Dr Maria: I would normally think about the functional disability as related to the cortical lesions, but Dr Bullmore's data suggest a relation with deep gray-matter structures such as the thalamus. Please clarify.

Dr Bullmore: The functional consequences resulting from given lesions may be attributed to disruption of neuronal circuits rather than direct involvement of tissue by lesions. Although we can document functional problems from focal deficits, the function is probably normally served not just by regions but by circuits of which they're a constituent part. And I think the other thing to say, as Dr Roach was pointing out, is that particular sample was not a very impaired sample, thus perhaps not representative of usual tuberous sclerosis.

Dr Prather: Just to add to that, the statistics tell what's most frequent. It may just be that the frontotemporal is most common; that's what most people have, but they have other things too.

Dr DiMario: I think also the circuitry is at issue. Even areas that seem normal on MRI may be microscopically affected.

Dr Roach: I think that's part of the issue; you have to distinguish between what you find normal or above normal and then consider the whole question: Is there a correlation in someone that's poorly functioning and someone that's better functioning?

Dr Roach: Dr Wiznitzer, if you're having trouble sorting out the relative contribution to autism of lesion location, lesion type, and so on and cognitive function, do you see enough people with tuberous sclerosis who have autism who are not functioning to do a big study and just not include anyone who's retarded?

Dr Wiznitzer: They have to be out there. I have not seen any, no, but then we have to assume that if you're doing a background or if you're dealing with a tuberous sclerosis association…I would guess that few people have a high number of individuals in their patient population who have autistic features and tuberous sclerosis complex but have normal intelligence and have epilepsy.

TREATMENT AND MANAGEMENT OF COMPLICATIONS

Moderator: E. Steve Roach, MD

Managing Epilepsy in Tuberous Sclerosis Complex

Elizabeth A. Thiele, MD, PhD, Director, Tuberous Sclerosis Complex Comprehensive Clinical Program; Director, Pediatric Epilepsy Program, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Dr Thiele described epilepsy as "the most common disorder in tuberous sclerosis complex." Seizures occur in up to 92% of people with tuberous sclerosis complex and are most common during the first year of life. All seizure types, except typical absence seizures, can be observed in children with tuberous sclerosis complex. Although increased numbers of tubers are associated with seizures, 10% of people with tuberous sclerosis complex who have intractable tuberous sclerosis complex exhibit normal MRI findings. Abnormal electroencephalograms (EEGs), such as those with epileptiform features (ie, focal spike or sharp-wave discharges, multifocal epileptiform abnormalities, hypsarrhythmia, and generalized spike-and-wave discharges) and diffuse slowing, are commonly observed. The presence of seizures is associated with a poorer cognitive outcome. Treatments are similar to those offered to patients without tuberous sclerosis complex who have epilepsy, including medication, vagus nerve stimulation, the ketogenic diet, and surgery. However, some children with tuberous sclerosis complex and epilepsy respond poorly to conventional antiseizure therapies.

Tuberous sclerosis complex is the leading cause of infantile spasms, accounting for 25% of all cases. At least one third of all patients with tuberous sclerosis complex have infantile spasms, typically between 4 and 6 months of age. For one third of children with tuberous sclerosis complex, spasms are preceded by partial seizures. These spasms can be difficult to detect and can manifest as subtle minor changes in infant behavior (eg, indifference to the social environment, irritability). EEG abnormalities do not necessarily include hypsarrhythmia and often demonstrate one or more foci. Research has suggested that vigabatrin is the preferred treatment for infantile spasm, and in many studies, vigabatrin eliminated spasms in most children with tuberous sclerosis complex. Vigabatrin is an irreversible γ-aminobutyric acid (GABA) transaminase inhibitor typically prescribed in doses of 100 to 200 mg/day titrated up over several days. Treatment usually lasts for 1 year, but it is unclear whether shorter treatment periods (eg, 4–6 months) are also effective. Unfortunately, vigabatrin can result in ophthalmologic toxicity in 5 to 20% of patients and include the reduction of 30 Hz flicker cone b-wave amplitude in full-field electroretinography, concentric visual field deficits, and reversible effects on the outer retina. Little is known about the effects of dose and length of treatment on toxicity and whether all of the ophthalmologic effects are reversible. Accordingly, electroretinographic examinations are obtained periodically and used to plan treatment. If other seizure

types are present, additional medications might be necessary. Early treatment of infantile spasm might preserve cognitive functioning in some children. However, even without treatment, one third of children with tuberous sclerosis complex and infantile spasms show generally good overall cognitive outcomes. Dr Thiele noted that vigabatrin is not approved by the US Food and Drug Administration (FDA), limiting its availability for treatment in the United States. The session ended with a review of the remaining questions on etiology and the nature of seizures in tuberous sclerosis complex.

Epilepsy Surgery for Children with Tuberous Sclerosis Complex

Howard L. Weiner, MD, Daniel Miles, MD, Orrin Devinsky, MD, New York University Comprehensive Epilepsy Center, New York University School of Medicine, New York, NY

Surgical treatment of epilepsy is challenging in people with tuberous sclerosis complex because multiple bilateral lesions located in eloquent cortex are common. Thus, many children with tuberous sclerosis complex are rejected for surgical treatment. However, independent studies (~ 50) suggest that surgery is generally effective. These studies are limited by their inclusion of predominantly older children or adolescents, resections of single lesions, and the failure to use invasive monitoring strategies to locate the epileptogenic area.

Dr Weiner advocates the use of innovative approaches in children with tuberous sclerosis complex when the surgery is safe, effective for both seizure control and promotion of good child development, and conducted within a comprehensive epilepsy center. He described multistage surgery for epilepsy. This approach has been used on a selected group of children with tuberous sclerosis complex and can be used with children who exhibit one lesion, multiple lesions, or no lesions. Monitoring strategies include grid, strip, and depth electrode placement. For bilateral lesions, electrodes are placed to locate the epileptogenic area, and the lesion is resected. Electrodes are then replaced to determine whether the epileptogenic region was completely resected, and the lesion is resected again if necessary. This technique helps identify adjacent or distal epileptogenesis and is especially useful when presurgical data suggest eloquent cortex or bihemispheric involvement.

This procedure has been evaluated in a small series of 16 children (5 boys), in which 13 received the three-stage surgery. Ten of these children are seizure free at follow-up, three have rare seizures (two have seizures associated with high fever), and two exhibited a 50% reduction in seizure frequency. Three of these children had unilateral seizure onset that could not be detected without invasive monitoring. All but one child required the removal of two or more tubers, and two children underwent bilateral resections. In this sample, one child suffered an infection related to the operation and three experienced transient hemiparesis. Dr Weiner ended by emphasizing that MRI often misses lesions, that lesionectomy is rarely sufficient alone, and that invasive monitoring is critical to the success of surgical treatment for seizure disorders in this population.

Non-Neurologic Manifestations of Tuberous Sclerosis Complex

David N. Franz, MD, Associate Professor of Pediatrics and Neurology, Director, Tuberous Sclerosis Clinic, University of Cincinnati College of Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Dr Franz described the non-neurologic aspects of tuberous sclerosis complex, including dental, cardiac, renal, and pulmonary manifestations. Dr Franz noted the presence of dental pits and craters in permanent teeth in 100% of people with tuberous sclerosis complex and the frequent presence of gingival fibromas. He stated that dental examinations are useful in screening for tuberous sclerosis complex because multiple dental pits or craters and gingival fibromas are diagnostic of tuberous sclerosis complex. People with tuberous sclerosis complex also can exhibit fibromas in the toes.

Potential cardiac symptoms include ventricular tachycardia and fibrillation, arrhythmia, bradycardia, and heart block. In the case of cardiac rhabdomyoma, Dr Franz emphasized the importance of managing patients medically and allowing time for improvement in those whose tumors are not obstructive, rather than pursuing surgical approaches, because many of these fibromas tend to regress over time.

Kidney complications are the second most common cause of morbidity in people with tuberous sclerosis complex (after central nervous system complications). Angiomyolipomas are also observed, and polycystic kidney disease occurs in 5 to 8% of patients. Although not typically malignant, angiomyolipomas can grow and destroy kidney tissue, resulting in complications such as hemorrhage, hematuria, hypertension, and renal failure. Angiomyolipomas vary in composition, consisting of some combination of fat, smooth muscle, and blood vessels. Because abnormal vessels are present in angiomyolipomas, the risk of hemorrhage increases as lesion size increases (3.5–6 cm). Treatment options include observation, embolization, and total or partial nephrectomy. Dr Franz recommended embolization because it preserves kidney tissue, prevents hemorrhage, and reduces kidney size and tumor volume. The risks include embolization of unwanted targets, infection, and postembolization syndrome, which can be reduced by presurgical corticosteroid treatment.

Lung manifestations include lymphangioleiomyomatosis, multifocal cysts, and multifocal micronodular pneumocyte hyperplasia (a benign condition sometimes mistaken for malignancy). Lymphangioleiomyomatosis in tuberous sclerosis complex can be distinguished from sporadic lymphangioleiomyomatosis, an uncommon, uninherited condition (incidence 0.0001%). Sporadic lymphangioleiomyomatosis is more likely to be progressive than lymphangioleiomyomatosis seen in individuals with tuberous sclerosis complex. Both types of lymphangioleiomyomatosis occur almost exclusively in women (up to 40% of women with tuberous sclerosis complex) and are associated with angiolipomas. Symptoms include cystic lung disease, dyspnea, cough, hemoptysis, recurrent pneumothoraces, lymphatic obstruction, and retroperitoneal and hilar adenopathy. Dr Franz concluded by speculating whether treatment with

rapamycin inhibitors will be effective in treating some of the clinical manifestations of tuberous sclerosis complex.

Question and Answer Session 2

Audience Member: I have a practical question. What do you do about the availability of vigabatrin? We've been grappling with this in the practice community.

Dr Thiele: The question is a good one. I think this is a major question for the child neurology community. Vigabatrin is one of the few effective drugs I use that has not been approved by the Food and Drug Administration. However, the use of the drug is associated with a financial burden on families and a liability burden on practitioners. We do have some colleagues who don't feel comfortable prescribing vigabatrin, which I think is fine and readily defendable. And then, oftentimes, patients are referred to physicians outside this country. That's also fine. My concern with that is there may be a month or 2-month wait before the child is started on vigabatrin. I know a lot of our patients get vigabatrin from various patients, and I know in Mexico you can buy it at Sam's over the counter. There are different ways of getting it. I think there needs to be strong advocacy for having the drug made available in the United States.

Dr Roach: Written documentation of the benefits of vigabatrin could help address liability issues.

Audience Member: Are there any data regarding age of exposure to vigabatrin and risk of retinal toxicity? And perhaps a related question, have any of your patients developed electroretinogram abnormalities on the drug? If so, how has that been managed?

Dr Thiele: It is not known what the risk of retinal toxicity is in relation to age of exposure to vigabatrin. I think when the reports first came out, they were in older individuals, and those people could be readily tested. I think now that we're relying on electroretinograms, it is not clear what the significance of those changes are, especially in younger children. At this point, we deal with patients on a case-by-case basis.

Audience Member: Is there any information about the natural history of disease in children with tuberous sclerosis, epilepsy, and behavioral problems?

Dr Thiele: There is no published natural history study in tuberous sclerosis. I can tell you from my experience with my patients, plenty of these kids do outgrow seizures and continue to go on infinitum without recurrence of the seizures. That clearly plays a role when you're looking at your way of treating it; should surgery be a strong option or not? As with other causes of epilepsy, I think seizure control is really of paramount importance in tuberous sclerosis.

Audience Member: Please discuss the methodology used in obtaining electroretinograms.

Dr Thiele: We refer to the ophthalmologist, who will first try to do an unsedated electroretinogram. Regrettably, many children require sedation to obtain good data.

Audience Member: How long should one treat with vigabatrin?

Dr Thiele: I typically keep kids on it for a year. I know there is the hope that a shorter duration of treatment will at least be effective in some kids.

Audience Member: Dr Weiner, what was the age of your youngest surgical candidate with tuberous sclerosis? Did you use any positron emission tomography (PET) studies to correlate studies?

Dr Weiner: The youngest patient is the one I have in the hospital now, a 10-month-old boy. Most patients referred come with PET imaging data, but I have not found the information critically important in planning surgery.

Audience Member: I was unclear in terms of your technique; is this dependent upon finding interictal sites? How are you doing that with the very young child, who, in my experience, rarely tolerates being placed in the monitoring unit for several days to record the seizures?

Dr Weiner: All this work is really done with ictal data. I don't think any of the children in this series were done primarily on interictal spikes. And it's a challenge for the child, it's a challenge for the parents. I can tell you that, with this recent fresh experience with the 10 month old, he tolerated it quite well. His mother tolerated it less well. Basically, they come through it okay. But most of the patients come in with families with fears that with behavioral abnormalities, they're not going to be able to tolerate lying in bed for 2 weeks at a time.

Audience Member: Do you use sedation?

Dr Weiner: We don't use sedation at all. We use some pain management, light pain management with the pediatric intensive care unit staff.

Dr Roach: In younger patients, seizure frequency is high and is driving the surgical option. Thus, monitoring often does not have to be for a prolonged period.

Audience Member: What are the preinvasive criteria to go on to the invasive monitoring?

Dr Miles: Most of these children probably had two or three extensive monitoring sessions.

Audience Member: Can you comment on the patients for whom surgery was not an option?

Dr Weiner: The patients I am seeing have been carefully screened for surgical eligibility. I can tell you that three of these patients went in for bilateral strip studies. We generally placed four holes and a small craniotomy at the top to just place survey strips. In those cases, I was very guarded about the possibility of going forward and conveyed that to the parents, and I was very surprised that we were able to lateralize the seizures. So, in general, I'm not seeing the patients, obviously, who are being rejected for surgery. I think they don't really even come to bilateral study.

Audience Member: I have a question about routine surveillance of sonograms; how are they influenced by the patient's age? And the last one is, as they cut down the frequency, is there any evidence of whether computed tomography (CT) or MRI is better? Is there a certain frequency?

Dr Roach: The closest thing in the literature that specifically addresses that is from the consensus conference, a series of recommendations on routine follow-ups once the diagnosis has been made. And that was in the *Journal of Child Neurology* of 1999, I think a year after the diagnostic criteria. And that came from basically the same group in Annapolis that did the diagnostic criteria division, and the approach is trying to do routine surveillance targeted toward features where early identification offers some advantage. And also where you have relatively noninvasive means of monitoring and also where you have some treatment. So, using that kind of scheme, you more or less took away extra eye exams, we took away chest CTs, and so on. This was not so much evidence, but at least the consensus was, at least among children, some routine imaging is merited. I don't think there's too much evidence to favor CT versus MRI; both are going to pick up enlargement, so I don't think there's too much indication to favor one versus the other. I think if it's

available, MRI certainly shows it more eloquently. And the recommendation was to scan every 1 or 3 years, realizing that we're not in a position evidence-wise to come down real strong on the recommendation it had to be done every year, which, for a lot of these kids, clearly is too much. Even then, if you do it yearly and a child gets a giant cell tuber, it can enlarge and become symptomatic during that time. That same paper basically has the same recommendation: ultrasonography every 1 to 3 years. And most of us tend to shape that a little bit, depending on what the last finding is.

Dr Franz: If you do an initial sonogram, and it looks absolutely clean, you may be at lower risk of having an angiomyolipoma early on, but we still screen those kids. When you get to a lesion above 4 cm, we like to do a magnetic resonance angiogram to assess the presence of aneurysms. There is also a recommendation for the screening for lymphangioleiomyomatosis in women with tuberous sclerosis when they reach adulthood. Some people think, "What's the point; there's no treatment for lymphangioleiomyomatosis, and there's nothing you can do about it." I think that's a very worthwhile thing to do for a couple of reasons. Number one, the care for adults with tuberous sclerosis complex is rather abysmal. Lymphangioleiomyomatosis is misdiagnosed; they're told they have emphysema or they're told they have lung disease. The other thing is there's a number of lines of evidence that say if you have lymphangioleiomyomatosis, estrogen exposure may accelerate it or make it worse. I think it's worth suggesting to a person they may not want to take estrogen contained in contraceptives and things like that.

Dr Roach: One additional point: the 1998 paper that we published in the *Journal of Child Neurology* was a longitudinal study of ultrasonography of kidneys in 60 some patients with tuberous sclerosis complex. One thing that stood out is the idea that this old notion that if your initial sonogram of the kidney doesn't show a tumor, then you're home free is clearly wrong. The people who had normal renal sonograms at the time of diagnosis, or at least at the time of a perfect ultrasound study, fully half of those later developed a recognizable tumor. My guess is there would be a time when you could say, "Well, if you haven't developed one of these by the time you're 20 or something, your chances of getting one go down."

Audience Member: My question is about vigabatrin and infantile spasms. How often do you use vigabatrin by itself without other anticonvulsants in tuberous sclerosis complex patients?

Dr Thiele: If it's infantile spasms, I typically start kids on it as initial therapy. If they respond, then it's vigabatrin and only vigabatrin. If they don't respond and have spasms, then other treatments for spasm, such as adrenocorticotropic hormone (ACTH)? If they develop partial seizures, like I said, I haven't found vigabatrin particularly effective. I guess with all the cases I have with spasms and tuberous sclerosis, I would say two thirds of them are on vigabatrin alone.

Audience Member: When you withdraw the vigabatrin, do they stay off all anticonvulsants?

Dr Thiele: That would be the goal, that they continue to be seizure free. If they do develop other seizure types, sometimes they go back on the anticonvulsants. If they were already on anticonvulsants, once they've been seizure free for a year, I'd get them off vigabatrin. Sometimes they are off the other medication before or after that time mentioned.

Audience Member: What about after staying off all medicine, after you withdraw?

Dr Thiele: That's a small number of kids. I would think I probably have started maybe 50 or 60 kids on vigabatrin for tuberous sclerosis, and I would guess that maybe 40% of those kids might be off medications and seizure free.

Dr Maria: As you know, one of the things we're trying to do today is to define some priorities in the way of future directions. My question is for Dr Franz. From a neurosurgical perspective, what are the important questions remaining, and how would you prioritize those?

Dr Franz: I didn't really have time to touch on that. What we're involved in is coordinating a national study looking at all the surgical cases that have been done in the United States up until now as a basis for proposing a prospective study. And one thing we're interested in looking at obviously is outcome, not only seizure outcome but also functional outcome. And then trying to correlate some of these issues such as imaging findings and how that relates to surgery and how that relates to outcome. So, in other words, I think the trend in this small series is that we're pushing the envelope somewhat, and the question is "Are we having any real impact on the disease?" That's the real question. So those are some of the things we're focusing on with this retrospective study. We're looking at radiology, we're looking at pathology, we're trying to correlate with a number of operations as to whether that has any overall impact, trying to correlate the sort of risk factors with outcome. But I think that noninvasive modalities for trying to understand the epileptic tubers are going to be critical and obviously molecular studies as well.

Audience Member: We had a patient in yesterday, and I wonder if anybody else had had the experience of managing a patient with abnormal signal in the pancreas or thyroid gland?

Dr Roach: Yes, I think each of those has been described. As I recall in Dr Gomez's book, one of the editions had mentioned at least both pancreatic and thyroid. Liver was very common. We didn't talk about the liver today; we looked at that in Dallas. About 25% of the children overall have benign liver lesions. As David mentioned, the lung is probably the most common one that we didn't talk about.

Audience Member: What are the statistics for getting rid of anticonvulsants in patients with tuberous sclerosis?

Dr Roach: That paper basically came out a couple of weeks ago. I think 15 patients, or 40%, managed to get off medicine.

GENETIC STRATEGIES IN TUBEROUS SCLEROSIS COMPLEX

Moderator: David H. Gutmann, MD, PhD, Donald O. Schnuck Family Professor of Neurology, Washington University School of Medicine, St. Louis, MO

Clinical Genetics of Tuberous Sclerosis Complex

Hope Northrup, MD, Director, Division of Medical Genetics, Professor, Department of Pediatrics, The University of Texas Medical School at Houston, Houston, TX

Dr. Northrup noted that tuberous sclerosis complex was first identified as a genetic disorder over 100 years ago. In 1935, Gunther and Penrose identified tuberous sclerosis complex as an autosomal dominant disorder but thought that 50% of cases were sporadic. However, today we think that 70% of tuberous sclerosis complex cases are sporadic,

whereas 30% are familial. In the 1980s, it became apparent that the disease is heterogeneous, showing linkage to more than one gene on two different chromosomes. Linkage of the *TSC1* gene on chromosome 9 was reported in 1987. This discovery was followed by the discovery of linkage for the *TSC2* gene (chromosome 16) in 1992. Currently, we know that the *TSC2* gene is located on the short arm of chromosome 16 and is about 41 exons in size. Two of the exons are alternatively spliced, but mutations have never been discovered in these exons. The *TSC1* gene is located on the long arm of chromosome 9 and is 23 exons in length. The first two exons are alternatively spliced. The gene products of the *TSC1* (hamartin) and *TSC2* (tuberin) regulate key intracellular growth control pathways, suppress the actions of ribosomal S6 kinase, and inhibit the mammalian target of rapamycin (mTOR). Hamartin and tuberin form a single signaling complex, which is critical for regulating a number of important cellular processes related to cell growth and size.

Dr Northrup described her work using data on mutations in more than 400 people with tuberous sclerosis complex to provide prognostic information. More than 1000 mutations are known to exist. Most mutations in the *TSC1* gene constitute inactivating or nonsense mutations. In contrast, missense mutations constitute close to 25% of mutations. In families, close to 50% have mutations involving the *TSC1* gene; however, *TSC2* gene mutations are overrepresented in sporadic patients. Mosaicism occurs on an infrequent basis (1–2%) in tuberous sclerosis complex. Dr Northrup described her research indicating that phenotypes with neurologic deficits were more closely associated with mutations in the *TSC2* gene than in the *TSC1* gene. Similarly, more severe additional features of tuberous sclerosis complex were related to *TSC2* gene mutations. These findings were consistent with prior literature in suggesting a more severe phenotype for people with *TSC2* gene mutations.

Molecular Genetics of Tuberous Sclerosis Complex: What Is New in 2003?

Elizabeth Petri Henske, MD, Member, Medical Science Division, Fox Chase Cancer Center, Philadelphia, PA

Dr Henske described findings suggesting that cells in the brain that lack *TSC2* can have an unusual ability to migrate, as seen in lymphangioleiomyomatosis, and an unusual ability to differentiate, as seen in renal tuberous sclerosis complex. The authors investigated the possibility of a mutation in the *TSC2* gene in patients with sporadic lymphangioleiomyomatosis, suggesting that sporadic lymphangioleiomyomatosis was either a mild form of tuberous sclerosis complex or reflected genetic mosaicism. Preliminary evidence suggested mutations in the *TSC2* gene in angiomyolipomas of women with sporadic lymphangioleiomyomatosis. In a study of five patients with no mutations in their angiomyolipomas, researchers found no mutations in the peripheral blood, ruling out the mild tuberous sclerosis complex hypothesis. Furthermore, the only abnormal genes were found in abnormal lung and kidney tissue, arguing against genetic mosaicism. A third model suggested that cells were actually metastasizing from the kidney to the lungs, causing lymphangioleiomyomatosis. This was hard to understand because angiomyolipomas are benign tumors. The researchers examined a single case in which lymphangioleiomyomatosis recurred after transplant. A mutation was found in the lymphangioleiomyomatosis before transplant, and the mutation was not present in normal lung tissue. After transplant, the same mutation was found in a recurring lymphangioleiomyomatosis, suggesting the spread of lymphangioleiomyomatosis cells. More research is necessary to determine how these findings relate to the central nervous system, but researchers now wonder if tubers might result from abnormal migration in the central nervous system.

In addition, researchers wished to know whether cells deficient in *TSC2* gene expression also had an unusual ability to differentiate. They sought to determine whether the three types of cells found in an angiomyolipoma (ie, fat, muscle, and blood vessel) resulted from the same precursor cell. First, researchers examined whether blood vessels in angiomyolipomas were neoplastic or reactive. Five types of blood vessels were identified, and vessels were both reactive (those without loss of heterozygosity) and neoplastic (those with loss of heterozygosity). Thus, it seems that kidney cells lacking *TSC2* gene expression are unusually plastic and are therefore able to differentiate into these three cell types. Whether central nervous system lesions result from abnormal differentiation during cortical development is not yet known. Dr Henske closed by reviewing research suggesting that rapamycin might be effective in treating tuberous sclerosis complex.

***TSC* Genes and the Brain**

Peter B. Crino, MD, PhD, PENN Epilepsy Center, Department of Neurology, University of Pennsylvania Health System, Philadelphia, PA

Dr Crino began by pointing out that the link between loss of function of hamartin or tuberin and the change from a normal progenitor cell to a giant cell is unknown. This is important because tubers are characterized by disorganized cortical lamination, aberrant dendritic arbors and axonal projections, astrocytic proliferation, and abnormal cell morphology (ie, dysplastic and heterotopic neurons and giant cells).

Dr Crino described the mechanism for tuber formation during brain development. Gene mutations in either of the two *TSC* genes influence neural precursors between weeks 7 and 20 of gestation to result in disrupted cell division, abnormal cell differentiation, dysregulated cell size control, and abnormal cellular migration. However, it is not known whether this effect develops via haploinsufficiency (single germline mutation) or through a second "hit" caused by a stochastic or random DNA event. Dr Crino's group hypothesized that giant cells occur as a result of a second hit, whereas adjacent dysplastic cells are haploinsufficient and exhibit only the germline *TSC* gene mutation. To test this hypothesis, tubers were obtained intraoperatively from patients undergoing surgery for seizures. Examination of the tubers indicated that two tuber sections exhibited evidence of S6 kinase hyperactivation, with the giant cells being the only cells with this abnormal S6 kinase activation. In addi-

tion, researchers examined perituberal tissue and did not find this abnormal S6 kinase activation. They then dissected out individual giant cells and sequenced the messenger ribonucleic acid. In these experiments, Dr Crino and colleagues detected only the one mutation in dysplastic neurons. In the cerebellum, dysplastic cells express tuberin, but giant cells do not.

Tubers are thought to arise during embryogenesis within the ventricular zone, where a second somatic mutation occurs, affecting the neuroprogenitor cell. The mTOR pathway is disrupted, resulting in dysregulated cell size and proliferation. It is hypothesized that these cells then migrate in an abnormal fashion to the cortex to generate in abnormal collections of inappropriately positioned neurons. In conclusion, Dr Crino described recently published data suggesting that cortical tubers express markers of recent cell proliferation in both the Eker rat brain nodules and human subependymal giant cell astrocytomas. Dr Crino suggested that giant cells, tubers, and subependymal nodules derive from the same population of progenitor cells.

Mouse Models

David H. Gutmann, MD, PhD, Department of Neurology, Washington University School of Medicine, St. Louis, MO

Dr Gutmann began by describing the advantages and disadvantages of using animal models for the study of tuberous sclerosis complex. Animal models are quite instructive for understanding the molecular and cellular pathogenesis of disease, identifying targets for therapeutic drug design, preclinical evaluation of potential therapies, and developing more refined methods for following human disease. He described four methods for creating mouse models. First, human tissue can be implanted in mice. Because this method allows for the use of human tissue in a natural genetic or cellular situation, it can more accurately predict human responses to therapies. However, this approach is not useful for studying nontumor tissue. The second option is to use naturally occurring animal models (eg, the Eker rat; *Tsc2* gene mutation). The benefit of the naturally occurring rat model is the presence of a natural genetic or cellular situation that is potentially manipulable. However, the condition in a rat might differ from the human condition and might not recapitulate all aspects of human disease, including exhibiting a different response to therapeutic agents. A third option is to ablate the disease gene to generate mice that are genetically similar to humans. However, this approach has similar drawbacks to those of the natural rat model, including the problem that loss of *TSC* gene expression results in embryonic lethality. Finally, tissue-specific inactivation of the disease gene in question can be employed. The benefits are similar to those of gene ablation, but this method potentially eliminates the problem of embryonic lethality. In this method, only one tissue type is targeted.

Dr Gutmann described research from his laboratory in which an enzyme that mediates DNA recombination and excision was specifically expressed in astrocytes to develop a mouse that lacks Tsc1 expression in the brain. The researchers studied astrocytes from these Tsc1-deficient mice to learn more about the molecular pathogenesis of tuberous sclerosis complex. In their studies, Dr Gutmann and colleagues found evidence of abnormal migration and differentiation of neurons. In addition, loss of Tsc1 expression resulted in increased astrocyte proliferation.

The *Tsc1* conditional knockout mice exhibited abnormal behavior, beginning around 1 month of age. By 2 months of age, rhythmic jerking of the front paws was noted, suggesting the development of seizures. Mouse EEG confirmed that this rhythmic jerking represented seizures. Although it was not immediately obvious how Tsc1 loss in astrocytes might result in neuronal hyperexcitability, recent studies by Dr Gutmann and Dr Michael Wong have suggested that Tsc1-deficient astrocytes have reduced glutamate transport. These findings might be useful in the design of future therapies aimed at treating epilepsy in children with tuberous sclerosis complex.

Question and Answer Session 3

Dr Gutmann: Let me start out with an incredibly unfair question. If the model is that the *TSC1* and *TSC2* genes are interacting to form a functional complex, how would you suppose that patients with *TSC1* or *TSC2* would present with a severe phenotype, or does it matter?

Dr Northrup: Some of the things that I've been reading suggest they probably have some functions that are more related to one of the proteins versus the other, that are more related to hamartin and more related to tuberin. I would postulate that a tuberin mutation affecting the *TSC2* gene may be involved in other functions that are more critical and more likely to cause more severe phenotype.

Dr Bullmore: Does anybody have any idea why there should be a high mutation rate in these two genes? What are the data like on the variation of these genes in the normal population?

Dr Northrup: I'm going to answer your second question first. These genes are extremely variable, and that's been a really tricky thing for us in trying to do mutational analysis. There are polymorphic variants all over the place, even within exons, so it's really difficult. When we see what we may end up classifying as a missense mutation in a child, we then have to trace back to parents, look at our whole data set, has it ever been described. Unless it's a protein mutation, it's up in the air as to whether we feel comfortable calling it a missense mutation or just calling it a variation. In terms of your second question, I don't think we really understand why there is a high mutation rate.

Dr Gutmann: We do not know why that particular region is a hot spot.

Dr Bullmore: Have you found any mutations in both these genes, and do any mutations in the binding regions have any different phenotype that relate to the combination of these two proteins? Do specific mutations relate to the phenotype?

Dr Northrup: No mutations have been concurrently found in both genes. Because of the large number of mutations, phenotype-genotype correlations have not been studied.

Audience Member: Are your cells missing both copies of the *PS2* gene?

Dr Henske: The question is whether in the sporadic lymphangioleiomyomatosis, are there patients with both copies of the *PS2* gene, and yes, there are, which actually alludes to the one question in the explanation of one hit or two hits.

Audience Member: You mentioned *TSC2* but not *TSC1* with the angiomyolipoma. Is there anything about that?
Dr Henske: I just used *TSC2* as an example. *TSC1* also causes angiomyolipoma, although we have found very few *TSC1* mutations in the sporadic lymphangioleiomyomatosis in the population.
Audience Member: Why don't men with angiomyolipoma get lung disease?
Dr Henske: The question of my career is why don't men get lymphangioleiomyomatosis. We would love to have the answer. Men get angiomyolipoma, but they virtually never get pulmonary manifestations. That must be telling us something about hormone regulation and the *TSC* genes.
Audience Member: My question is, you have the giant cells in the tubers that clearly have two hits, and you have dysplastic cells in the tuber that have one hit, and you have the normal cortical neurons that have one hit. What differentiates dysplastic cells and cortical neurons? Why aren't cortical neurons dysplastic?
Dr Crino: That's a fantastic question. What it suggests is at the molecular genetic level, the molecular genetic phenotype of the cell is supposedly the normal cortex, is exactly identical to a cell within an aberrant morphology within a tuber. What that suggests is that potentially the reason dysplastic neurons form does not have to do with the cell itself. It may not be a downstream cause. But it may be a contextual event because you have these large giant cells forming early on in the development. Maybe the lineage of giant cells has to do with neuronal migration. You're quite right that, in fact, the gene type of dysplastic neurons seems to be identical to a completely normal and unaffected cortex.
Audience Member: My question is, do you think the second-hit event relates somehow to the calcifications?
Dr Crino: We have some evidence in the lab to suggest there may be, in keeping with migration of cells into tubers, enhanced cell death within tubers that could theoretically account for calcifications from an exhausted blood supply. However, there is no final answer to the question of why tubers calcify.
Audience Member: Dr Crino, I thought the questions that you ended up with were great about the second hit; is it the same second hit in one tuber and two different tubers possibly having the same hit? Do you think you're going to be able to nail that down at the DNA level given the limitations of microdissection?
Dr Crino: It's obviously very dependent on getting tuber specimens from patients. Howard Weiner has been incredibly generous in helping me out. In a couple of cases, we've been able to do two resections in the same patient. What we're going to try to do is identify the germline in the patients and go into the tubers and see if the second hit is indeed the same or different.
Dr Maria: Is there a reason why you picked *TSC1* rather than *TSC2*?
Dr Gutmann: You know the old story of why the drunk looks for his keys under the lamppost. We picked *TSC1* to start with. We're now in collaboration with David Dukovski's group; we know the *TSC2* knockouts have been crossed. They're only 6 weeks old. At least at this point, they look fairly indistinguishable from *Tsc1* mice at that age, but only time will tell.
Audience Member: Do you have any data on preneural precursors? Have you ever tried to knock it out there rather than the astrocytes?
Dr Gutmann: Well, that would completely upset my rule of few. We have not focused at all on that. David Dukovski's group has some exciting data in various stages of evolution, but we have focused on the astrocyte mostly because of our original hypothesis.

FUTURE DIRECTIONS AND INNOVATIVE THERAPIES

Moderators: Robert Finkelstein, PhD, Program Director, Neurogenetics Cluster, National Institute of Neurological Disorders and Stroke, Bethesda, MD; Vicky Holets Whittemore, PhD, Medical Director, Tuberous Sclerosis Alliance, Silver Spring, MD

Dr Vicky Whittemore joined the scientists to conduct a panel discussion. The panelists identified these crucial issues for future study:

- Investigate the cellular basis of tuberous sclerosis complex.
  - Identify the molecular pathways that tuberin and hamartin regulate and use this knowledge to develop targeted therapies. Possible pathways include mTOR (rapamycin) and Rheb (farnesyltransferase inhibitors).
  - Conduct a natural history study to investigate cell lineage, identify the point at which cortical development of tuberous sclerosis complex is initiated, and determine whether these lesions continue to evolve after birth.
  - Determine whether a "second hit" occurs in brain lesions associated with tuberous sclerosis complex.
  - Investigate the etiology of the female predominance of lymphangioleiomyomatosis.
  - Look for modifier genes that might explain heterogeneity in outcomes.
- Study the clinical characteristics of the disease and predictors of clinical outcomes.
  - Understand the mechanisms of epileptogenesis.
  - Determine whether a classic behavioral phenotype exists and, if so, why.
  - Clarify the relationship between brain structure and outcome.
  - Identify predictors of good quality of life.
- Set priorities for treatment research.
  - Prioritize interventions and organize therapeutic trials.
  - Determine which of the therapies available are most useful.
  - Determine whether early aggressive surgical treatment for epilepsy improves outcome and which factors are related to good surgical outcome.
  - Increase the availability of appropriate medications.
  - Investigate the effectiveness of rapamycin therapy.
- Enhance research, collaboration among scientists, and communication of findings.
  - Develop a large multicenter database to allow for better study of the natural history of the disorder.
  - Develop a network of investigators to facilitate patient access to clinical trials and studies. Networks in oncology and autism were provided as examples.
  - Use mechanisms such as the Tuberous Sclerosis Alliance newsletter to disseminate findings to the public.

Special Article

Diagnosis of Tuberous Sclerosis Complex

E. Steve Roach, MD; Steven P. Sparagana, MD

ABSTRACT

Tuberous sclerosis complex is a dominantly inherited disorder affecting multiple organs; because of its phenotypic variability, the diagnosis of tuberous sclerosis complex can be difficult in the young or in individuals with subtle findings. Recently revised consensus diagnostic criteria for tuberous sclerosis complex reflect an improved understanding of its clinical manifestations and its genetic and molecular mechanisms. The diagnostic criteria are based on the premise that there are probably no truly pathognomonic clinical signs for tuberous sclerosis complex; signs that were once regarded as specific occur as isolated findings in individuals with no other clinical or genetic evidence of tuberous sclerosis complex. Consequently, the revised criteria require tuberous sclerosis complex–associated lesions of two or more organ systems or at least two dissimilar lesions of the same organ to confirm the diagnosis. The addition of DNA testing complements clinical diagnosis and allows more precise genetic counseling and, in some individuals, prenatal diagnosis. Nevertheless, the 15% false-negative rate for DNA testing and the occurrence of germline mosaicism in about 2% of individuals with tuberous sclerosis complex make it difficult to exclude the diagnosis of tuberous sclerosis complex in family members. (*J Child Neurol* 2004;19:643–649).

Tuberous sclerosis complex is a dominantly inherited disorder of cellular differentiation and proliferation that variably affects the brain, skin, kidneys, heart, and other organs. Because of its striking variability of clinical expression and severity, the diagnosis of tuberous sclerosis complex can be difficult, especially in young individuals or in those with subtle findings.[1] The genetics and biologic mechanisms of tuberous sclerosis complex are not nearly as straightforward as once believed. For these reasons, the diagnosis of tuberous sclerosis complex can be challenging.[1–3]

In 1998, a panel of international experts revised the diagnostic criteria for tuberous sclerosis complex at the Tuberous Sclerosis Complex Consensus Conference in Annapolis, Maryland.[4,5] The revised criteria (Table 1) reflect an improved understanding of the clinical manifestations of tuberous sclerosis complex and its genetic and molecular mechanisms. Fundamental to the revised criteria was the agreement among the experts that there are no truly pathognomonic clinical signs for tuberous sclerosis complex; the signs that were once regarded as specific sometimes occur as isolated findings in individuals with no other clinical or genetic evidence of tuberous sclerosis complex. Consequently, the revised criteria require tuberous sclerosis complex–associated lesions of two or more organ systems or at least two dissimilar lesions of the same organ to confirm the diagnosis.

Another departure from earlier tuberous sclerosis complex diagnostic criteria was the panel's decision not to include epilepsy and mental retardation as indicators of tuberous sclerosis complex. The group concluded that epilepsy and mental retardation were both so common in the general population and their causes so numerous that neither had sufficient specificity to be useful in the criteria. Additionally, most patients with epilepsy and mental retardation have cortical brain lesions on neuroimaging studies, and the number of such lesions tends to increase in rough proportion to the severity of the neurologic problems. There was concern that including both the symptoms and the lesions that caused the symptoms amounted to counting the same item twice.[4]

The consensus criteria were designed to establish a consistent and reliable standard for the diagnosis of tuberous sclerosis complex. It is much more difficult, however, to devise criteria that will exclude the diagnosis in affected

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Table 1. Diagnostic Criteria for Tuberous Sclerosis Complex

Major features
Facial angiofibromas or forehead plaque
Nontraumatic ungual or periungual fibroma
Hypomelanotic macules (more than three)
Shagreen patch (connective tissue nevus)
Cortical tuber*
Subependymal nodule
Subependymal giant cell astrocytoma
Multiple retinal nodular hamartomas
Cardiac rhabdomyoma, single or multiple
Lymphangiomyomatosis†
Renal angiomyolipoma†
Minor features
Multiple randomly distributed pits in dental enamel
Hamartomatous rectal polyps‡
Bone cysts§
Cerebral white-matter "migration tracts"*§
Gingival fibromas
Nonrenal hamartoma‡
Retinal achromic patch
"Confetti" skin lesions
Multiple renal cysts‡

Adapted from Roach ES et al.[4]
*When cerebral cortical dysplasia and cerebral white-matter migration tracts occur together, they should be counted as one rather than two features of tuberous sclerosis.
†When both lymphangiomyomatosis and renal angiomyolipomas are present, other features of tuberous sclerosis should be present before a definite diagnosis is assigned.
‡Histologic confirmation is suggested.
§Radiographic confirmation is sufficient.
Definite tuberous sclerosis complex: Either two major features or one major feature plus two minor features.
Probable tuberous sclerosis complex: One major plus one minor feature.
Suspect tuberous sclerosis complex: Either one major feature or two or more minor features.

individuals with few tuberous sclerosis complex signs or in younger patients who have yet to develop the full array of manifestations. Nevertheless, the revised criteria are still useful, even in very young patients or when the diagnostic evaluation is incomplete, because once sufficient evidence of tuberous sclerosis complex accumulates, a definite diagnosis can still be made. It is important, however, to periodically reassess these individuals over time or when more evidence becomes available.[6,7]

The revised diagnostic criteria (see Table 1) have been widely disseminated and have shown great utility in establishing the clinical diagnosis of tuberous sclerosis complex. Standardized criteria provide a quick, reliable, and economical method of establishing a diagnosis, and they help ensure uniformity in clinical tuberous sclerosis complex research. The addition of DNA-based testing complements clinical diagnosis and allows more precise genetic counseling and, in some cases, prenatal diagnosis. Our aim is to review the clinical features of tuberous sclerosis complex as they relate to diagnosis and the recent developments in confirmatory diagnosis.

CLINICAL FEATURES OF TUBEROUS SCLEROSIS COMPLEX

Skin Lesions

The cutaneous lesions of tuberous sclerosis complex include hypomelanotic macules, the shagreen patch, ungual fibromas, and facial angiofibromas (Figure 1). One or more of these skin lesions occur in over 90% of individuals with tuberous sclerosis complex, although none is pathognomonic.[4]

Hypomelanotic macules (ash leaf spots) are found in over 90% of patients with tuberous sclerosis complex (see Figure 1A). They are usually present at birth but are often difficult to see in the newborn without an ultraviolet light. Other pigmentary abnormalities include the "confetti" lesions (an area with stippled hypopigmentation, typically on the extremities) and poliosis of the scalp hair or eyelids. Hypomelanotic macules are not specific for tuberous sclerosis complex because one or two of these lesions are relatively common in normal individuals.[8]

Facial angiofibromas (see Figure 1B) occur in about three fourths of patients but often appear several years after the diagnosis has been established by other means. These lesions typically become apparent during the preschool years as a few small red papules on the malar region; they gradually become larger and more numerous, sometimes extending down the nasolabial folds or onto the chin. Angiofibromas contain both vascular and connective tissue elements. Although facial angiofibromas are a strong indication of tuberous sclerosis complex when found with other manifestations, these lesions also occur in individuals with multiple endocrine neoplasia type I and thus are not pathognomonic for either condition.

The shagreen patch (see Figure 1C) is most commonly found on the back or flank area; it is an irregularly shaped, slightly raised, or textured skin lesion. The lesion is found in 20 to 30% of patients with tuberous sclerosis complex and occasionally other individuals. It might not be apparent in young children.

Ungual fibromas (see Figure 1D) are nodular or fleshy lesions that arise adjacent to or from underneath the nails. The lesion is usually considered specific for tuberous sclerosis complex, although a single lesion occasionally occurs after trauma. Ungual fibromas are seen in about 20% of unselected patients with tuberous sclerosis complex and are more likely to be found in adolescents or adults than in younger children. Sometimes the fibroma itself is not visible but creates a prominent longitudinal groove in the fingernail, a finding that also has diagnostic significance.

Retinal Lesions

The frequency of retinal hamartomas in tuberous sclerosis complex varies with the expertise and technique of the examiner. Under ideal circumstances, up to 87% of patients with tuberous sclerosis complex have retinal lesions, but especially in uncooperative children, these lesions can be difficult to identify without dilating the pupils and the use of indirect ophthalmoscopy.[9] Retinal lesions vary from the classic mulberry lesions (Figure 2) adjacent to the optic disk to the plaquelike hamartoma or depigmented areas. Most retinal lesions are clinically insignificant, but occasional patients have visual impairment owing to a large macular lesion, and rare patients have visual loss owing to retinal detachment, vitreous hemorrhage, or hamartoma enlargement. Some patients have a pigmentary defect of the iris.

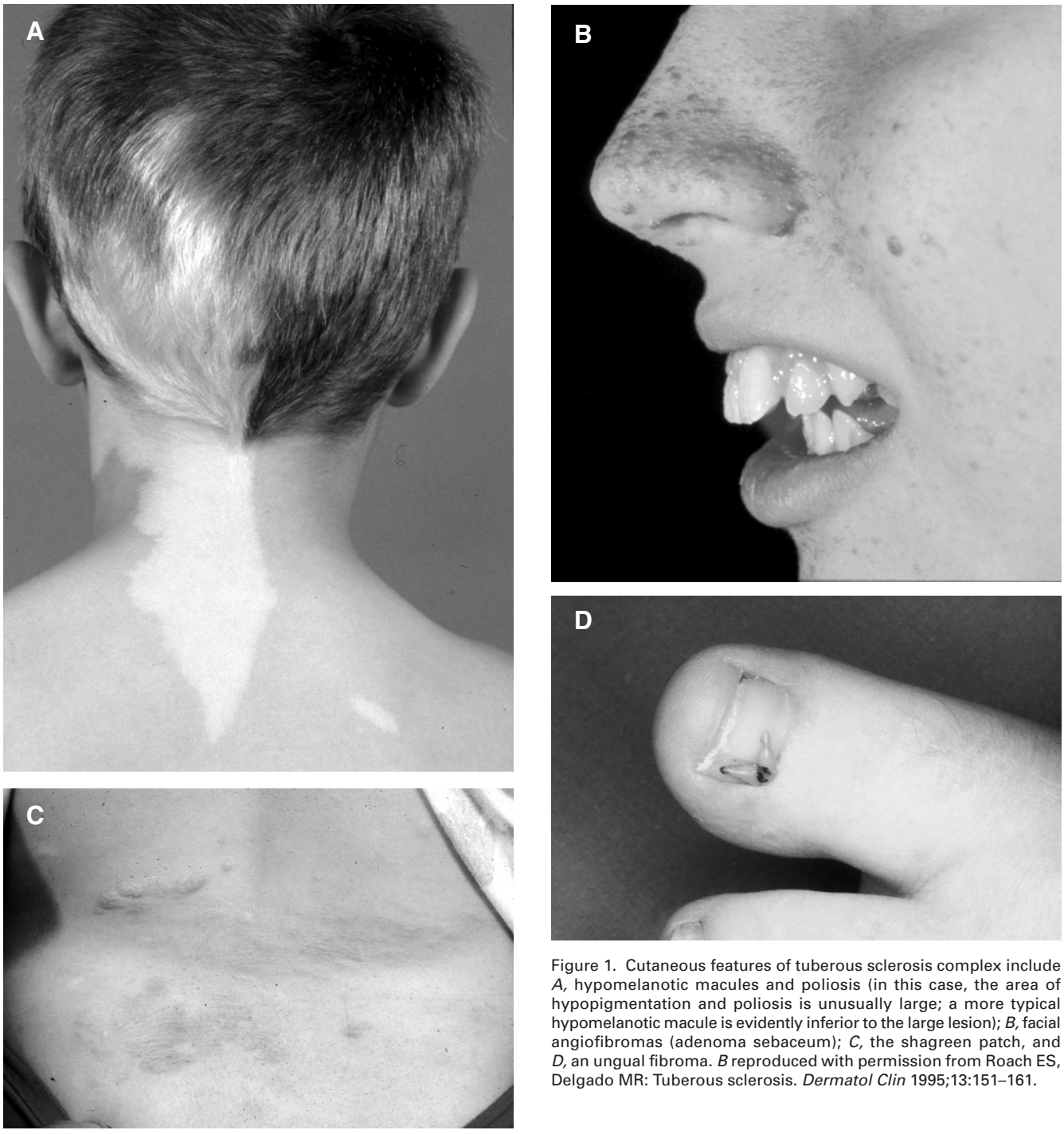

Figure 1. Cutaneous features of tuberous sclerosis complex include *A,* hypomelanotic macules and poliosis (in this case, the area of hypopigmentation and poliosis is unusually large; a more typical hypomelanotic macule is evidently inferior to the large lesion); *B,* facial angiofibromas (adenoma sebaceum); *C,* the shagreen patch, and *D,* an ungual fibroma. *B* reproduced with permission from Roach ES, Delgado MR: Tuberous sclerosis. *Dermatol Clin* 1995;13:151–161.

Cardiac Lesions

Roughly two thirds of newborns with tuberous sclerosis complex have one or more cardiac rhabdomyomas, but few of these lesions are clinically important. Cardiac rhabdomyomas are often multiple (Figure 3) but shrink over time and are identified much less often in older children and adults.[10] These lesions are sometimes evident on prenatal ultrasonographic testing, and most of the patients with cardiac dysfunction present soon after birth with heart failure, usually owing to either obstruction of blood flow by an intraluminal tumor or to inadequate normal myocardium to maintain perfusion. Some patients stabilize and improve after medical treatment; others require surgery. A few children later develop cardiac arrhythmias.

Renal Lesions

Renal angiomyolipomas occur in about 75 to 80% of patients with tuberous sclerosis complex over the age of 10 years; most of these lesions are histologically benign tumors with varying amounts of vascular tissue, fat, and smooth muscle (Figure 4).[11] Bilateral tumors or multiple tumors per kidney are typical. The prevalence of renal tumors increases with

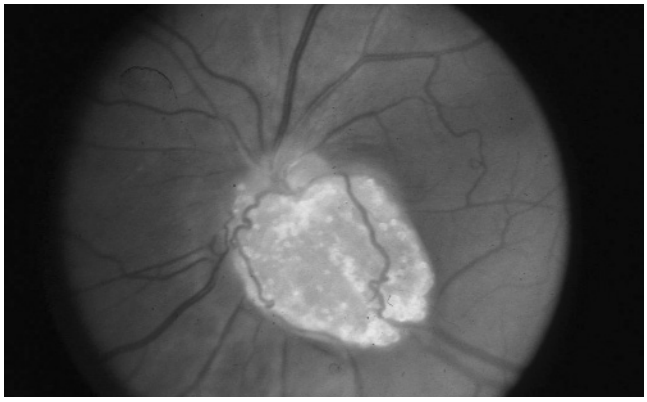

Figure 2. A retinal astrocytoma ("mulberry lesion") adjacent to the optic nerve is typical of those found with tuberous sclerosis complex. Reproduced with permission from Roach ES: Neurocutaneous syndromes. *Pediatr Clin North Am* 1992;39:591–620.

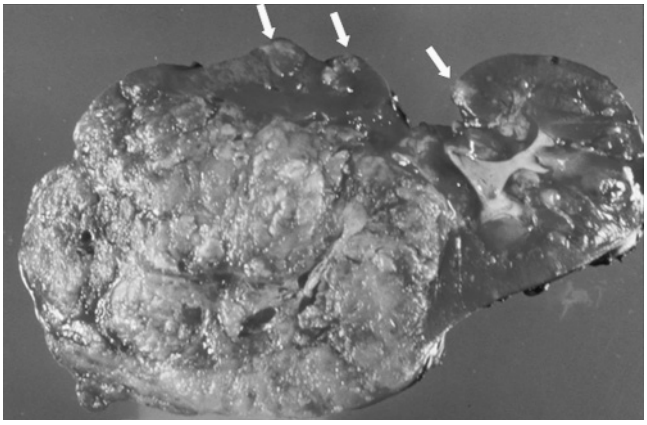

Figure 4. A large angiomyolipoma of the lower pole of a kidney removed at surgery; several smaller angiomyolipomas (*arrows*) are evident in the same specimen. Reproduced with permission from Weiner DM et al.[7]

age, and tumors larger than 4 cm are much more likely to become symptomatic than smaller tumors. Renal cell carcinoma or other malignancies are less common.

Single or multiple renal cysts are also a feature of tuberous sclerosis complex; these tend to appear earlier than the renal tumors. Larger cysts are easily identified with ultrasonography or computed tomography (CT), and the combination of renal cysts and angiomyolipomas is characteristic of tuberous sclerosis complex. Individual renal cysts can disappear.

Pulmonary Dysfunction

At least 1% of patients with tuberous sclerosis complex develop symptomatic pulmonary dysfunction, and many others probably have asymptomatic lung lesions on diagnostic studies later in life. The classic pulmonary lesion of tuberous sclerosis complex is lymphangioleiomyomatosis; other patients have multifocal micronodular pneumocyte hyperplasia. Pulmonary disease is five times more common in female than in male patients. Spontaneous pneumothorax, dyspnea, cough, and hemoptysis are typical symptoms of pulmonary tuberous sclerosis complex, although these do not often develop before the third or fourth decade. CT of the chest commonly demonstrates lung lesions even in adults without symptoms.

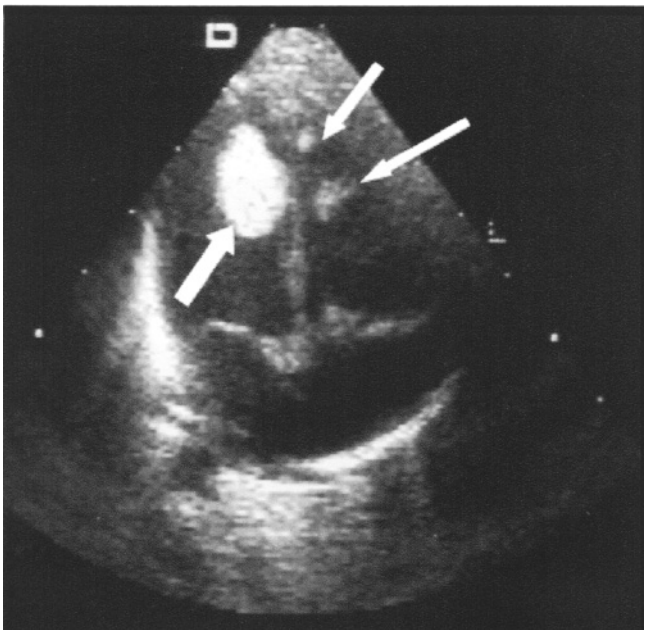

Figure 3. Ultrasonic demonstration of multiple cardiac rhabdomyomas (*arrows*). Reproduced with permission from Roach ES, Delgado MR: Tuberous sclerosis. *Dermatol Clin* 1995;13:151–161.

Both renal angiomyolipomas and pulmonary lymphangiomyomatosis are strongly associated with tuberous sclerosis complex, but neither is specific; moreover, they tend to occur together in the absence of other signs of tuberous sclerosis complex. Thus, when both conditions occur in the same patient, they should be counted as one major feature of tuberous sclerosis complex, not two. Either condition can be considered a major feature of tuberous sclerosis complex without the other, but the diagnosis should not rest solely on the presence of these two lesions.[4]

Neurologic Dysfunction from Tuberous Sclerosis Complex

The predominant neurologic manifestations of tuberous sclerosis complex are mental retardation, epileptic seizures, and behavioral abnormalities, but affected individuals with little or no neurologic impairment are common.[2,3] Neurologic lesions probably result from impaired neuronal migration along radial glial fibers and from abnormal proliferation of glial elements.

Neuropathologic lesions of tuberous sclerosis complex include subependymal nodules, cortical hamartomas, areas of focal cortical hypoplasia, and heterotopic gray matter.[7,12] Although all of the superficial cerebral lesions of tuberous sclerosis complex are sometimes lumped together as cortical tubers, the actual pathologic picture is more complex. A classic tuber is a dysplastic lesion of a gyrus that has a firm, nodular feel; it often occurs at the apex of a gyrus

but does not always enlarge the gyrus. However, areas of focal cortical dysplasia that are not nodular and are less sharply demarcated are common, ranging from grossly visible defects of the cortical mantle to microscopic disruption of the cytoarchitecture.

Various types of seizures occur in 80 to 90% of patients. Most patients with mental retardation have epilepsy, but there are exceptions. On the other hand, many people with tuberous sclerosis complex have epilepsy but have normal intelligence. The number of subependymal lesions does not correlate with the clinical severity of tuberous sclerosis complex, but patients with numerous lesions of the cerebral cortex shown by magnetic resonance imaging (MRI) tend to have more cognitive impairment and more difficulty with seizure control.[13,14] Children with infantile spasms are more likely to exhibit long-term cognitive impairment, but these patients, in turn, have more cortical lesions demonstrated by MRI.

The likelihood of mental retardation in patients with tuberous sclerosis complex is probably overestimated. Webb and colleagues, for example, found only 10 patients with mental retardation among 26 patients with tuberous sclerosis complex in a population survey.[15] The severity of intellectual dysfunction ranges from borderline to profound mental retardation. Autism and various behavioral disturbances, including hyperactivity, aggressiveness, and frank psychosis, are common, either as isolated problems or in combination with epilepsy or intellectual deficit.[16]

The calcified subependymal nodules (Figure 5) that characterize tuberous sclerosis complex are best demonstrated with cranial CT. Subependymal nodules arise adjacent to the ventricular wall and characteristically protrude into the ventricular lumen. They are most often found in the lateral ventricles and seem to occur more often anteriorly. Superficial cerebral lesions can sometimes be seen with cranial CT but are far more obvious with T_2-weighted MRI (Figure 6). Cerebellar anomalies occur in about a fourth of patients with tuberous sclerosis complex. Nodular subependymal lesions that have not yet calcified produce a high-signal lesion with T_2-weighted scans. Evidence of abnormal neuronal migration can be seen in some patients as a high-signal linear lesion running perpendicular to the cortex on T_2-weighted scans.

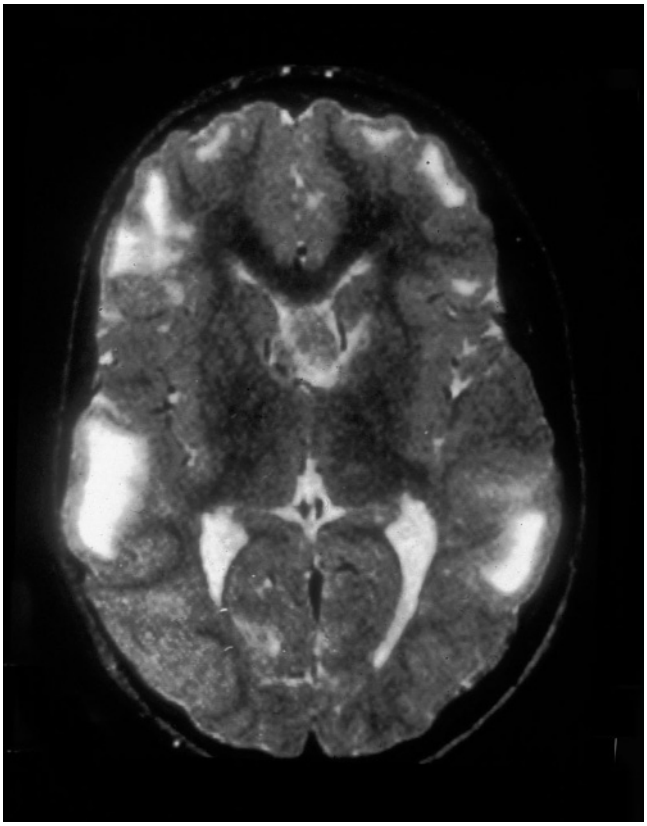

Figure 6. Noncontrast T_2-weighted magnetic resonance image from a child with tuberous sclerosis demonstrates extensive high-signal cortical lesions typical of tuberous sclerosis. Reproduced with permission from Miller VS, Roach ES: Neurocutaneous syndromes, in Bradley WG, Daroff RB, Fenichel GM, Marsden CD (eds): *Neurology in Clinical Practice,* 3rd ed. Newton, MA, Butterworth-Heinemann, 1999, 1665–1700.

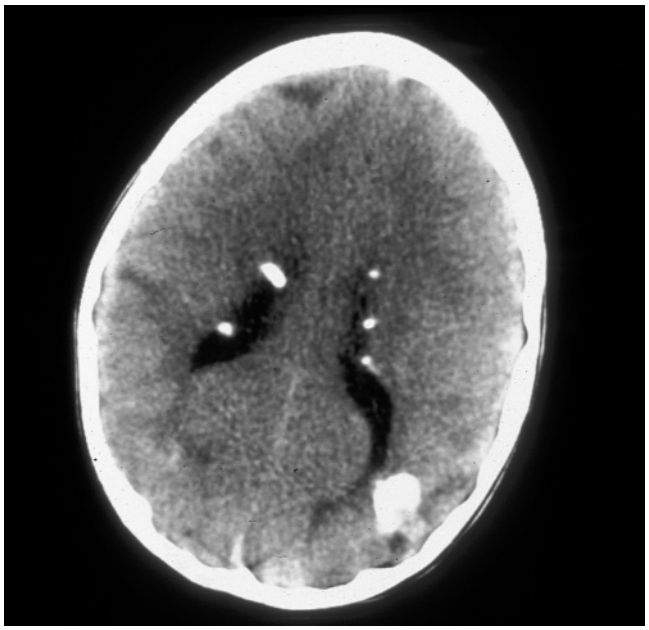

Figure 5. Computed tomographic scan from a child with tuberous sclerosis complex demonstrates typical calcified subependymal nodules, a large calcified parenchymal lesion, and smaller low-density cortical lesions. Reproduced with permission from Roach ES, Kerr J, Mendelsohn D, et al: Diagnosis of symptomatic and asymptomatic gene carriers of tuberous sclerosis by CCT and MRI. *Ann N Y Acad Sci* 1991;615:112–122.

Subependymal giant cell astrocytoma occurs in 6 to 14% of individuals with tuberous sclerosis complex and is more likely to develop during childhood. Unlike the more common cortical tubers, giant cell astrocytomas (Figure 7) can enlarge and cause symptoms such as increased intracranial pressure, new focal neurologic deficits, or deterioration of seizure control.[17] Acute or subacute onset of neurologic dysfunction rarely results from sudden obstruction of the ventricular system by an intraventricular subependymal giant cell astrocytoma or from hemorrhage into the tumor

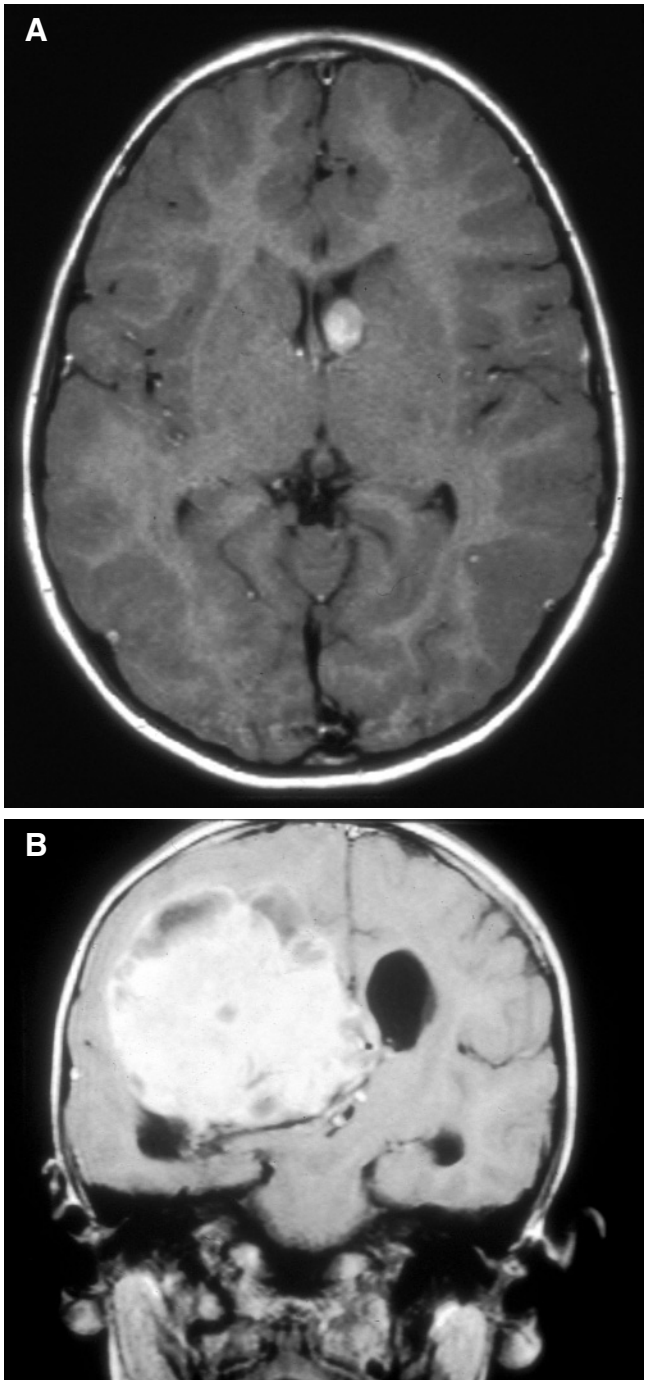

Figure 7. *A,* Gadolinium-contrast T_1-weighted magnetic resonance image (MRI) from a child with tuberous sclerosis complex shows an enhancing subependymal giant cell astrocytoma protruding into the left frontal horn. *B,* Another patient's MRI with gadolinium demonstrates a huge subependymal giant cell astrocytoma filling much of the lateral ventricle.

itself. Giant cell tumors are usually benign but locally invasive, and surgery, performed early, can be curative.

GENETICS OF TUBEROUS SCLEROSIS

Tuberous sclerosis complex was once thought to be a rare disease but is now known to occur in about 1 in 6000 live births,[18] making it not quite as common as neurofibromatosis type 1 (1 in 4,000 births) but more common than von Hippel-Lindau disease (1 in 36,000 births). Improved diagnostic techniques (especially in imaging and genetic testing), as well as an ever-growing awareness of tuberous sclerosis complex among physicians and the public, might, in time, reveal an even greater prevalence.

Tuberous sclerosis complex is inherited as an autosomal dominant trait with variable penetrance, and two thirds to three fourths of the individuals with tuberous sclerosis complex arise via a spontaneous mutation. Two different genes cause tuberous sclerosis complex: *TSC2*, which encodes tuberin, and *TSC1*, which encodes hamartin.[19,20] Multiple mutations of each gene have been identified. The phenotype for the two tuberous sclerosis complex genes overlap, although individuals with *TSC2* tend to have more severe neurologic impairment, and *TSC1* tends to be found more often in familial cases.[21] Tuberin and hamartin physically interact at the Golgi apparatus and function together as a single molecular complex, probably explaining why a mutation on either gene causes such a similar phenotype.[22–24]

DIAGNOSIS OF TUBEROUS SCLEROSIS COMPLEX BY DNA ANALYSIS

Testing for *TSC1* and *TSC2* mutations has been available from Athena Diagnostics, Inc. (Worcester, MA) since 2002. Confirmatory testing for tuberous sclerosis complex is helpful in individuals who fail to meet the criteria for definite tuberous sclerosis complex and to improve genetic counseling. Prenatal genetic testing for tuberous sclerosis complex is also possible when there is a defined tuberous sclerosis complex mutation in a specific family. Preimplantation genetic diagnosis is a method of determining the genetic characteristics of an embryo created by in vitro fertilization. Although preimplantation genetic diagnosis is feasible for tuberous sclerosis complex, its use is not widespread.[25–27]

Several issues limit the usefulness of confirmatory testing for tuberous sclerosis complex, beginning with the fact that most patients develop the disorder via a spontaneous mutation. And although there are few false-positive tests, as much as 15 to 20% of the time the test fails to demonstrate a disease-causing mutation. Nevertheless, confirmatory testing in an individual who already fulfills the diagnostic criteria for definite disease can help identify a mutation that can then be sought in other family members or for subsequent prenatal diagnosis.

Somatic and germline mosaicism also complicate confirmatory testing for tuberous sclerosis complex. Somatic mosaicism occurs when an individual has a mutation in some, but not all, cells and tissues. Such an individual often has milder manifestations of tuberous sclerosis complex or manifestations limited to a single organ system but can have more severely affected progeny.[28] Germline mosaicism occurs when an individual carries a mutation only in the germ cells and has no other signs or symptoms of the given disease. Germline mosaicism probably accounts for about 2% of the patients,[29] making genetic counseling

more difficult even when a specific mutation has been identified. Hence, a conservative recurrence risk for seemingly unaffected couples with a single affected child, even when they have no demonstrable tuberous sclerosis complex mutation, is 2%.

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Special Article

Brain Abnormalities in Tuberous Sclerosis Complex

Francis J. DiMario Jr, MD

ABSTRACT

Tuberous sclerosis complex is an autosomal dominant multisystem disorder. Spontaneous mutations occur in up to 60% of patients with gene loci located on chromosomes 9q34 (*TSC1*) and 16p13 (*TSC2*). Diagnosis is established with the identification of various neurocutaneous markers and multiple organ system hamartomas. The variable expression of severity, the potential for cognitive dysfunction, and epilepsy compound the clinical picture. The intracranial abnormalities include the identification of migration and hamartomatous brain lesions, such as tubers, subependymal nodules, and subependymal giant cell astrocytomas. A number of other neuroimaging and morphometric abnormalities coexist, which can be identified with current neuroimaging techniques. This review examines the spectrum of brain abnormalities encountered in tuberous sclerosis complex and presents them as not merely a collection of lesions but more cohesively in the context of a global neuronal migration disorder. (*J Child Neurol* 2004;19:650–657).

Tuberous sclerosis complex is one of the most commonly identified neurocutaneous disorders. Population studies have estimated a prevalence of approximately 1 per 6000 to 9000 individuals.[1,2] Although it is an autosomal dominant disorder, up to 60% of affected patients have spontaneous mutations. This produces approximately 40,000 Americans and about 2,000,000 people affected worldwide with tuberous sclerosis complex.[1,2] The diagnosis of tuberous sclerosis complex is based on the identification of multiple organ system hamartomas, in compliance with recent consensus conference clinical criteria of major and minor findings.[3]

The genetic underpinnings of tuberous sclerosis complex reside in the identification of two gene mutations: *TSC1*, located on chromosome 9q34, and *TSC2*, identified on chromosome 16p13.[4,5] The majority (80–90%) of patients with tuberous sclerosis complex carry the *TSC2* mutation, with the remainder affected by *TSC1*.[6] The protein product associated with *TSC1* is a 130 kDa protein named hamartin, which has a postulated role in regulating cell adhesion via cross-linking intracellular structural proteins and plasma membrane via ezrin-radixin-moesin.[7,8] In *TSC2*, the associated protein product tuberin is a 180 kDa protein that exerts effects on cell differentiation and cell proliferation via the phosphatidylinositol-3 kinase (PI3K)–Akt–mammalian target of rapamycin (mTOR)–S6 kinase signaling pathway. Each gene functions as a tumor suppressor gene and ultimately serves as a negative growth regulator.[9,10] The hamartin and tuberin proteins form a functional complex with guanosine triphosphatase (GTPase) activity, which further regulates cell-cell signaling pathways and neuronal cell migration.[11,12]

BRAIN LESIONS

With the advent of modern neuroimaging, brain anatomy, neurophysiology, and brain function activation have all become possible to quantify and better understand. A survey of the literature can identify a number of brain lesions associated with tuberous sclerosis complex. Just as there is a genetic heterogeneity in tuberous sclerosis complex (*TSC1* and *TSC2*), so, too, is there phenotypic heterogeneity with no clearly distinctive clinical expression. Therefore, the brain lesions discussed in the following sections are pertinent to both *TSC1* and *TSC2* and are not unique to either locus.

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Table 1. Supratentorial and Infratentorial Brain Lesions

Supratentorial
Cortical/subcortical tubers
Subependymal nodules
Subependymal giant cell astrocytoma
White matter (~ 30%): linear migration lines, "cystlike" lesions
Corpus callosum agenesis/dysplasia
Transmantle cortical dysplasia
Association with
Hemimegalencephaly
Schizencephaly
Intracranial arterial aneurysms (60%)
Intracranial moyamoya vasculopathy
Infratentorial
Linear and gyriform cerebellar folia calcification
Cerebellar nodular white-matter calcifications
Cerebellar hemisphere/vermis agenesis and hypoplasia
Cerebellar hemisphere enlargement
Brain stem/fourth ventricle subependymal nodules and tubers

If one divides the brain lesions of tuberous sclerosis complex into those found in the supratentorial compartment and those confined to the infratentorial compartment, the predominant lesions are found in the supratentorial regions. More than 95% of patients with tuberous sclerosis complex will demonstrate at least one of the lesions listed in Table 1.

SUPRATENTORIAL BRAIN LESIONS

The most commonly identified brain lesions in tuberous sclerosis complex are cortical tubers, subependymal nodules, and subependymal giant cell astrocytomas (Figure 1).[13] Cortical tubers are variable in size, occur most often at the gray-white junction, and can be multiple in the same individual. They are identified as low-signal lesions on magnetic resonance imaging (MRI) T_1-weighted sequences and as high-signal lesions on T_2-weighted and fluid-attenuated inversion recovery sequences.[14] Tubers are typically seen in the cerebrum but can be noted in the cerebellum as well.[14] Less frequently, tubers can degenerate into cystic lesions associated with a subcortical component adjacent to the cortical lesion (Figure 2).[13–15] Bourneville first identified these in 1880.[16] Cystic change in tubers does not imply malignant transformation. There is no particular lobar predilection or clear clinical phenotypic association with isolated tubers. However, there is a clear correlation that the greater the number of cortical tubers, the greater the negative impact on cognitive functioning.[17,18] Tubers themselves can be limited to the cortex or the subcortical white matter and occasionally will have a more "wedge-shaped" appearance extending from the ventricular margin out toward the broader surface of the tuber itself.[14,19,20] Not infrequently, they can have focal areas of calcification.[14,19,20] Often the overlying cortex of a tuber will also be dysplastic and suggestive of pachygyria or polymicrogyria.[14,19,20] On gross histologic section, tubers can be firm to the touch, which leads to the name "tuberous sclerosis" ("potato-like" firmness). Histologically, they are composed of a proliferation of glial cells arranged in perpendicular orientation to the pia mater.[12,13] There are, in addition, giant cells or "balloon cells," which are cytomegalic

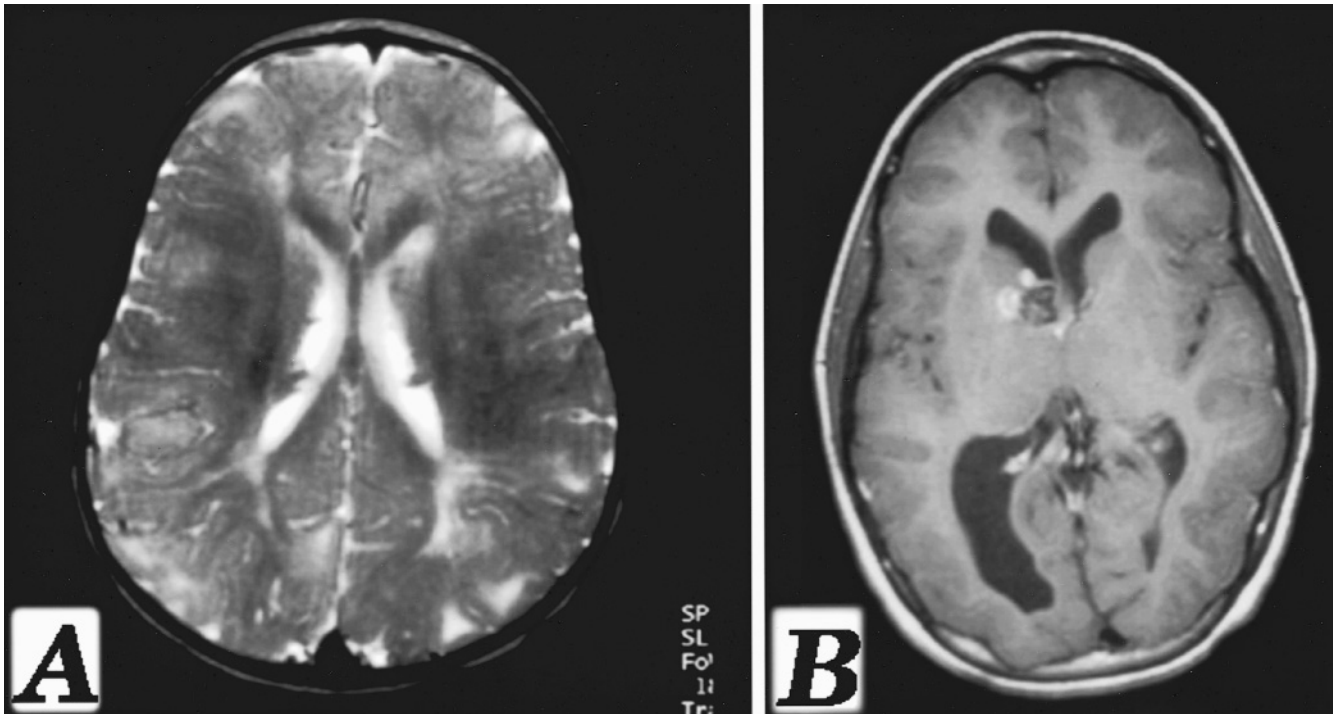

Figure 1. *A,* Axial T_2-weighted magnetic resonance image (MRI). Subependymal nodules seen along the periventricular margin as low-signal nodules. Tubers are seen as subcortical, cortical, and high-signal lesions. Migration lines extend from the posterior atria toward the parietal cortex. *B,* Axial T_1-weighted MRI with contrast enhancement. Subependymal giant cell astrocytoma seen at the right foramen of Monro.

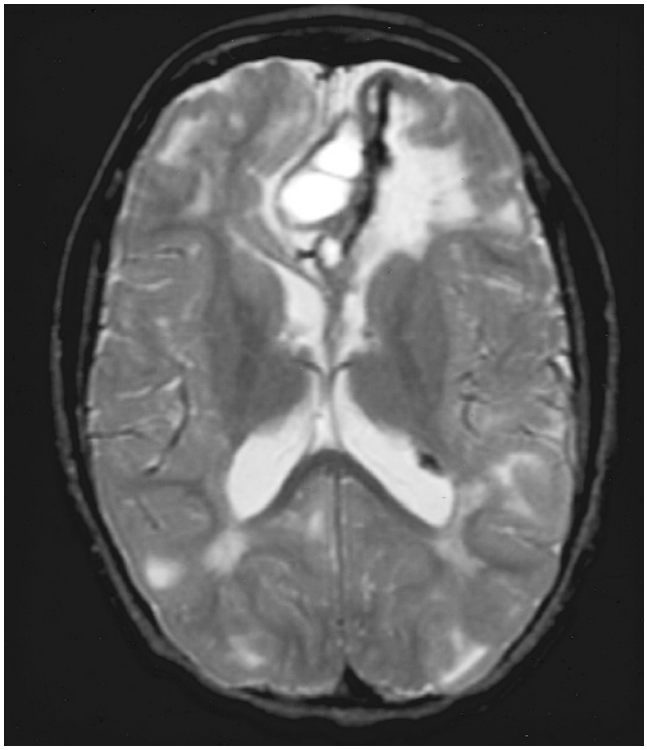

Figure 2. Tuber with cystic degeneration on axial T_2-weighted magnetic resonance image, seen as a high-intensity subcortical lesion. Courtesy of D. N. Franz, Cincinnati, OH.

cells of mixed astrocytic and neuronal lineage.[12,21] These are all intertwined in an irregular lamination pattern and aberrant columnar orientation.

Subependymal nodules are notable adjacent to the ventricular walls. They are most prominent adjacent to the lateral ventricles but can be present at all ventricular levels.[14,17,18] Subependymal nodules appear as small protrusions into the cerebrospinal fluid cavity, often with associated calcification. Calcification can be present in infancy or evolve more gradually over childhood into puberty. This characteristic to calcify is more readily identifiable with computed tomography (CT) as a high-density lesion but can also be identified with MRI as a low-intensity nodule on T_1- or T_2-weighted sequences (see Figure 1A).[19] On gross histology, they can give the appearance of "candle guttering" because they can line the ventricular surface. Subependymal nodules, too, are composed predominantly of dysplastic astrocytes and mixed-lineage astrocytic or neuronal cell components.[12,21] Although subependymal nodules can occur anywhere along the ventricular margins, they have a particular predilection for the region around the foramen of Monro. The biologic behavior of subependymal nodules in this region is to enlarge. Thus, by virtue of size and location, they are designated as subependymal giant cell astrocytomas (see Figure 1B).[13,14,19,20] These occur in as many as 10 to 15% of patients with tuberous sclerosis complex. Subependymal giant cell astrocytomas enlarge over a long period of time and are typically identifiable by the end of the first decade of life. Enlargement can produce obstruction of cerebrospinal fluid pathways and invasion into the underlying hypothalamic and chiasmatic region, producing endocrinopathy and visual impairment. The prediction of growth rate and impending need for neurosurgical intervention is not possible on static neuroimaging alone. Follow-up evaluations and clinical judgment are required.

Other brain lesions in patients with tuberous sclerosis complex are also identifiable on neuroimaging. These include a number of white-matter linear lesions, referred to as migration lines (see Figure 1A).[14,18,22] These can be notable in upward of 20 to 30% of patients when examined very carefully on T_2-weighted MRI sequences. Linear migration streaks can be found extending from the subependymal surface of a subependymal nodule outward to a cortical tuber or to the cortex without a tuber identified.[22] They can also extend from a smooth ventricular surface to a cortical tuber lesion as well.[22]

More varied evidence of brain dysplasia can be identified on neuroimaging. Partial agenesis of the corpus callosum has been reported but not complete agenesis.[19,20,23] Cortical dysplasia with focal gyriform abnormalities and heterotopias to more extensive lobar migration abnormalities has been identified. A more dramatic expression of cortical dysplasia can include large segments of cortex, referred to as transmantle cortical dysplasia (Figure 3).[19,20,23] These can encompass an entire lobar surface or comprise an entire hemisphere, consistent with hemimegalencephaly[4]

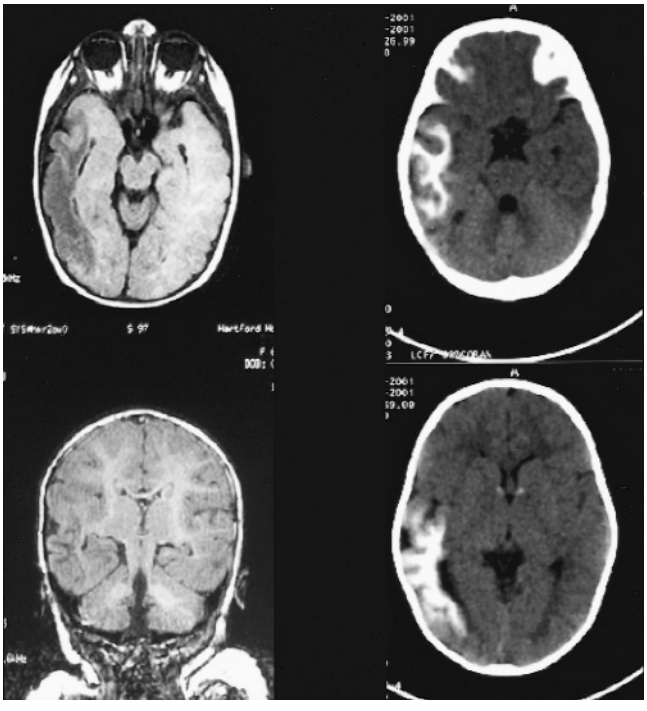

Figure 3. Transmantle cortical dysplasia. The upper and lower panels on the right show axial noncontrast computed tomographic scans displaying extensive calcification of the entire right temporoparietal lobe. The upper panel on the left shows the corresponding T_1-weighted magnetic resonance image (MRI), whereas the lower panel on the left shows the corresponding coronal T_1-weighted MRI.

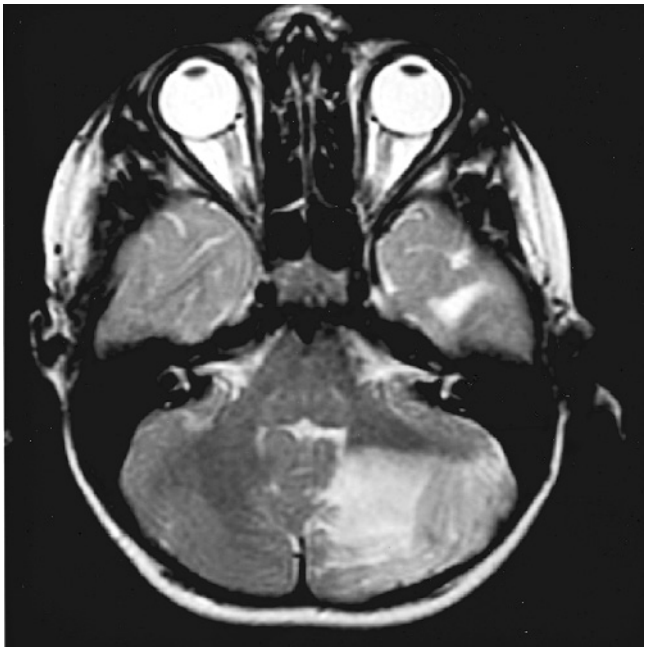

Figure 4. Cerebellar tuber with associated left cerebellar hemisphere hyperplasia on an axial T_2-weighted magnetic resonance image. Courtesy of D. N. Franz, Cincinnati, OH.

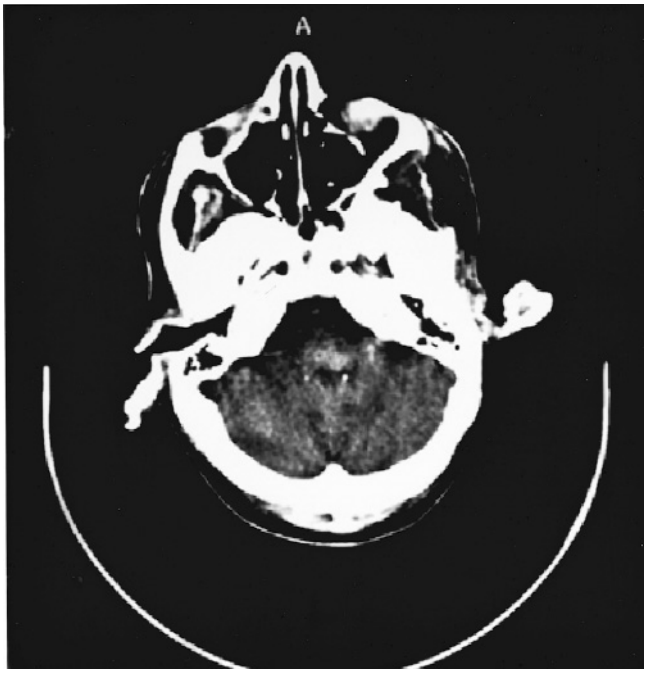

Figure 5. Brainstem tuber with distortion of the floor of the fourth ventricle as seen on an axial noncontrast computed tomographic scan. Two small subependymal nodule calcifications are evident. Reproduced with permission from DiMario FJ.[29]

(see Figure 3).[24,25] Although the neuroradiologic and histologic appearances are similar, transmantle cortical dysplasia associated with tuberous sclerosis complex is marked by the striking calcification found within it. This feature distinguishes it from non–tuberous sclerosis complex–associated dysplasia. Isolated reports of schizencephaly have also been published.[20] Apart from parenchymal dysplasia, vascular abnormalities have been described as well. Intracranial aneurysms have been reported in a small number of patients (8 patients less than 17 years of age; 15 total cases worldwide).[26] The internal carotid artery was affected in 60% of those cases reported.[26] This is an increased rate compared with the general population, in whom the incidence of internal carotid artery aneurysm is approximately 30%. Why there might be an apparent predilection of the carotid artery is unknown. Intracranial moyamoya vasculopathy as a consequence of carotid artery disease has also been noted in isolated instances.[27]

INFRATENTORIAL BRAIN LESIONS

A smaller fraction of patients with tuberous sclerosis complex (< 30%) will exhibit lesions within the infratentorial compartment (see Table 1). Similar to those lesions identifiable in the supratentorial compartment, multifocal lesion locations, especially within the cerebellar white matter, can be identifiable. These incorporate both linear migration streaks and gyriform and cerebellar folia tubers and calcifications.[14,19,20] Rarely have there been reports of nodular white-matter calcifications within the cerebellum.[28] Although cerebellar hemisphere hyperplasia exists more frequently (similar to cerebral hemimegalencephaly; Figure 4), vermis agenesis has been identified in isolated cases.[27] In a series of five patients with TSC and hemimegalencephaly, there was a coexisting cerebellar hemisphere megalencephaly.[25]

The brainstem region in patients with tuberous sclerosis complex is second only to the spinal cord for having had the least involvement identifiable with neuroimaging or on necroscopy.[13] Isolated cases of fourth ventricular calcification have been identified on pathologic specimens. These have been microscopic in most accounts and not recognized premortem. Nonetheless, significant functional impairment can be associated with brainstem involvement. Rarely, tubers have involved the floor of the fourth ventricle, producing distortion of chemosensitive cells and brainstem nuclei involved in respiratory drive centers (Figure 5).[29] An isolated case report of a brainstem tuber distorting the IVth ventricle has resulted in central hypoventilation syndrome with central apnea. This was partially amenable to treatment.[29]

NEURONAL MIGRATION DISORDER

Tuberous sclerosis complex should be thought of not merely as collections of isolated central nervous system lesions but more cohesively as a neuronal migration disorder. This is justified because in tuberous sclerosis complex, histologic features exist that are characteristically identified in neuronal migration disorders in general. The essential features and characteristics of a neuronal migration disorder include (1) abnormal number, size, and thickness of cortical gyri; (2) heterotopic neuronal aggregates; and (3) variable degrees

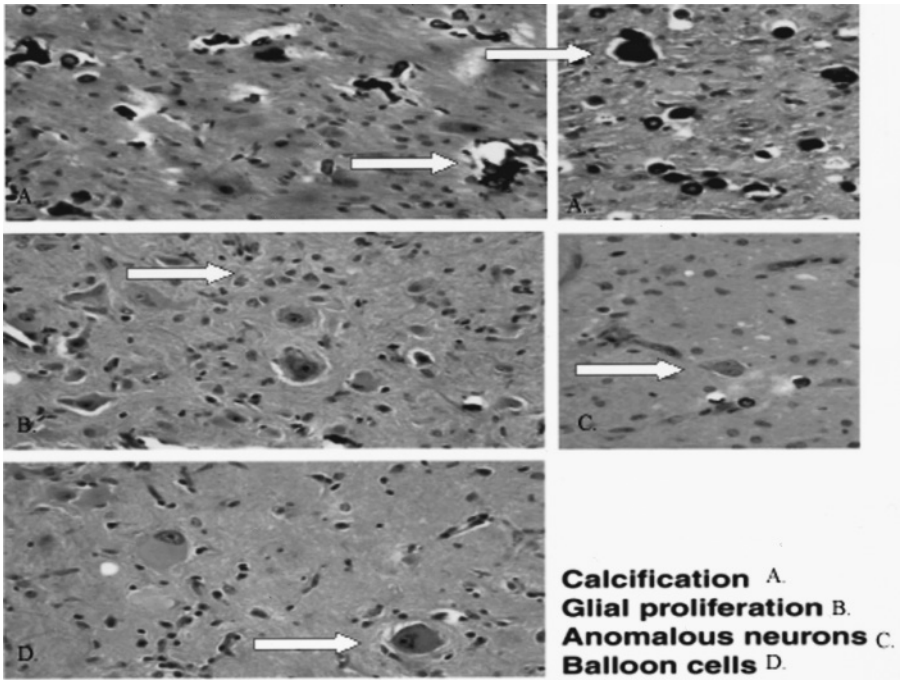

Figure 6. Histologic section, light microscopy, ×200 original magnification from the resected section of transmantle cortical dysplasia seen in Figure 3. Labeled panels show calcification, glial proliferation, anomalous neurons, and balloon cells. Courtesy of E. Novotny, New Haven, CT.

of cortical cytoarchitectural disorganization and aberrant columnar and laminar arrangement.[30]

The fundamental pathogenesis of tuberous sclerosis complex must incorporate a logical explanation for the lesions identifiable both outside and within the central nervous system. The brain lesions seen in tuberous sclerosis complex are, histologically, tissue hamartomas. These are focal "tumorlike" malformations composed of abnormal proportions of cellular elements normally present in the brain. These elements have an abnormal cellular morphology, are in abnormal quantities and locations, and are in a disorganized cytoarchitectural arrangement (dysplasia) (Figure 6).[13,31] Tubers, subependymal nodules, and subependymal giant cell astrocytomas represent focal tissue dysplasia. Transmantle cortical dysplasia, hemimegalencephaly, and cerebellar hemisphere megalencephaly represent more regional tissue dysplasia. Linear migration streaks represent trails of abnormal cell migration (Figure 7).

When one examines the histology of otherwise normal-appearing cortical brain regions in patients with tuberous sclerosis complex, abnormalities are still identifiable. These abnormalities incorporate large dysplastic neurons in addition to subtler laminar and columnar malalignment.[31] The entity of focal cortical dysplasia also has relevance in the current discussion.[24] Patients with focal cortical dysplasia are brought to clinical attention owing to focal and often intractable epilepsy. The neuroimaging characteristics of focal cortical dysplasias are similar to those identified with cortical tubers (Figure 8). The neuropathologic features are also nearly identical to those found in the cortical tubers of tuberous sclerosis complex.[24,31] These incorporate thick gyri with abnormal columnar and laminar architecture, cytomegaly, and heterotopic neuronal aggregates.[24,31] Antigenic markers of cell lineage identify both mixed-lineage neuronal and astrocytic cell markers in individual cells. However, the fundamental distinction between focal cortical dysplasia and tuberous sclerosis complex is based in part on not merely the extent of the cytoarchitectural abnormality but, more importantly, on whether there exists the presence or absence of additional clinical signs (ie, multiple organ system hamartoma) consistent with a diagnosis of tuberous sclerosis complex.[24,32] The clinical examination, therefore, determines the diagnosis of tuberous sclerosis complex versus the isolated histologic evidence of focal cortical dysplasia.[32,33]

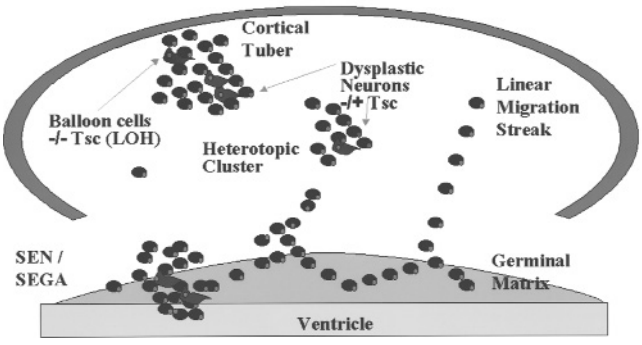

Figure 7. Schematic of migration and hamartomatous lesions of tuberous sclerosis complex. LOH = loss of heterozygosity; SEGA = subependymal giant cell astrocytoma; SEN = subependymal nodules.

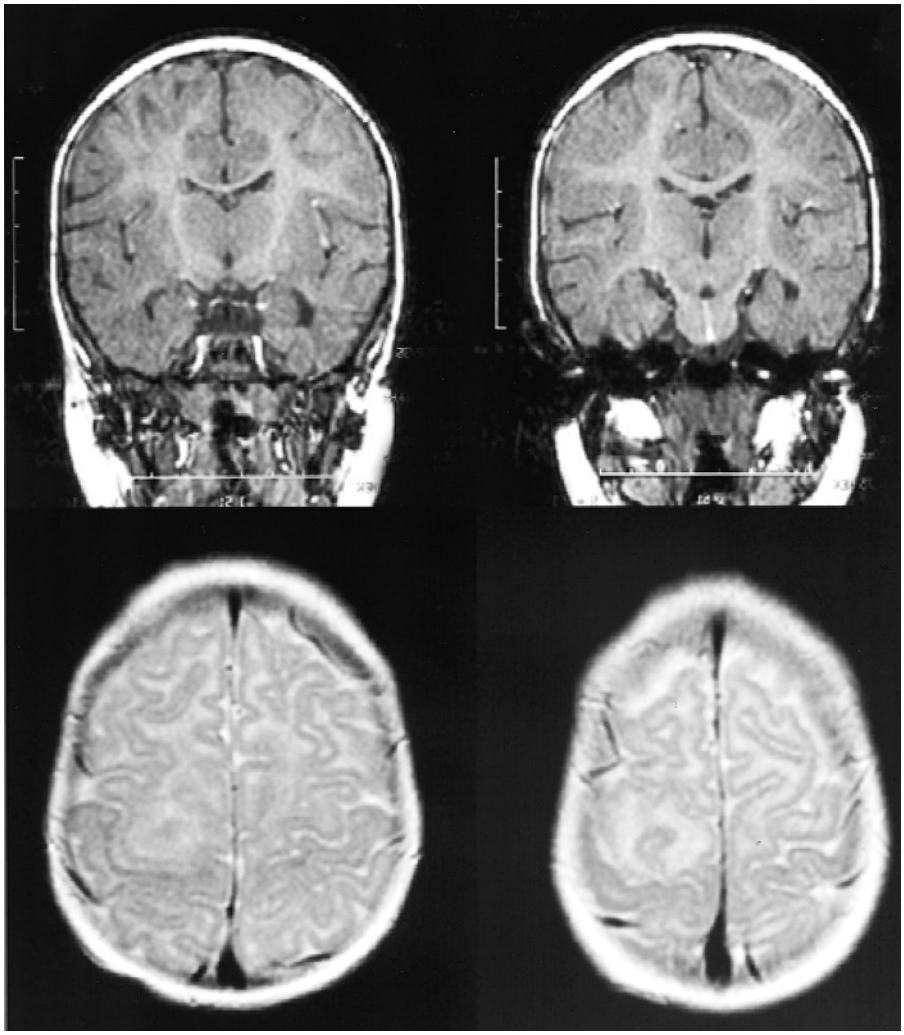

Figure 8. Focal cortical dysplasia. Upper panels show coronal T_1-weighted magnetic resonance image with the corresponding, lower panel, axial T_2-weighted sequence showing a right frontoparietal focal cortical dysplasia lesion.

TUBEROUS SCLEROSIS COMPLEX MORPHOMETRIC STUDIES: WIDESPREAD BRAIN ABNORMALITIES EXIST

A widely held hypothesis now based on sound basic science evidence supports the "two-hit" hypothesis as the underlying pathogenetic mechanism for the abnormalities of tuberous sclerosis complex.[34,35] The application of this hypothesis to the development of the brain in patients with tuberous sclerosis complex begins with an individual cell along the formative germinal matrix. These progenitor cells carry within them an inherited germline mutation at one of the tuberous sclerosis complex loci. These cells then go on to populate the germinal matrix with additional progeny carrying the same mutation. Ultimately, these daughter cells migrate away from the ventricular zone and localize within the brain parenchyma. If an additional somatic mutation (or "second hit") results in a loss of heterozygosity, this would then render both copies of the gene nonfunctional in that cell and all of its subsequent progeny. These daughter cells with loss of heterozygosity exhibit the resultant effects on cell function of a loss of heterozygosity for the tuberous sclerosis complex genes. The hamartin-tuberin protein complex is thus changed and exerts an altered effect on the regulation of cell differentiation, proliferation, and cell-cell interaction focally and regionally (see Figure 7). Focal lesions and regional brain abnormalities as identified on neuroimaging and histologic specimens can certainly be credited to this faulty intrinsic cellular signaling, which results in these migrational abnormalities. A question can then be posed: What about nonlesion tuberous sclerosis complex brain regions? How can we account for the more profound brain effects found in patients with tuberous sclerosis complex if only focal or no lesions are identifiable with neuroimaging?

As alluded to earlier, subtle abnormalities on histologic specimens of a "nonaffected" tuberous sclerosis complex cortex can be identified. More compelling evidence, however, exists along two lines: histologic and morphometric. Knockout mice experiments using *TSC1*$^{+/-}$ and *TSC2*$^{+/-}$ cell cultures demonstrate increased astrocytic proliferation, suggesting that even heterozygous tuberous sclerosis complex cells exhibit at least some disrupted cellular mechanisms.[36,37] More recently, loss of heterozygosity has been identified in the "balloon cells" of tuberous sclerosis

Table 2. Morphometric Measures

	TSC Subjects (n = *22)*		*Controls* (n = *48)*		
Measure	*Raw Means**	*Adjusted Means**	*Raw Means**	*Adjusted Means**	P *Value*
Bifrontal width	34.2 ± 1.4	37.2	29.8 ± 0.49	12.4	.003
Bicaudate width	15.1 ± 0.95	17.0	8.7 ± 0.26	3.4	.001
Biatrial width	59.8 ± 2.1	82.0	52.8 ±1.0	52.3	.023
Fourth ventricle width	14.2 ± 0.62	14.8	11.6 ± 0.22	12.6	.001
Frontal lobe depth	45.1 ± 1.6	25.9	151.2 ± 5.0	136	.001

*Measurements in mm.

complex histologic specimens.[38] It can be inferred that the observed cytomegaly is, in fact, a direct result of the intrinsic faulty cellular signaling responsible for this poorly regulated individual cell growth. This strengthens the "two-hit" hypothesis and resultant brain disruption as a multifocal cytopathologic mechanism.

Brain MRI morphometric evidence has demonstrated decreased cerebral gray- and white-matter volumes and increased cerebellar white-matter volume.[39,40] In a study of brain morphometry using a manual measurement technique of 10 predetermined morphometric measures, significant regional abnormalities were identified in the MRI of 22 subjects with tuberous sclerosis complex compared with 48 controls (Table 2).[39] Significant differences were identified between subjects with tuberous sclerosis complex and control subjects with an increase in ventricular size and smaller brain (gray and white matter) volumes in the periventricular regions. This was especially pronounced in the frontal lobes and in the region of the foramen of Monro (Figure 9). The higher number of subependymal nodules correlated with increased posterior ventricular size, whereas an increased number of tubers was correlated with an increased anterior ventricular size. The presence of calcifications within lesions strengthened these effects. As a corollary, a greater number of identified tubers and subependymal nodules was correlated with higher seizure frequency, greater cognitive impairment, and fewer self-help skills.[39]

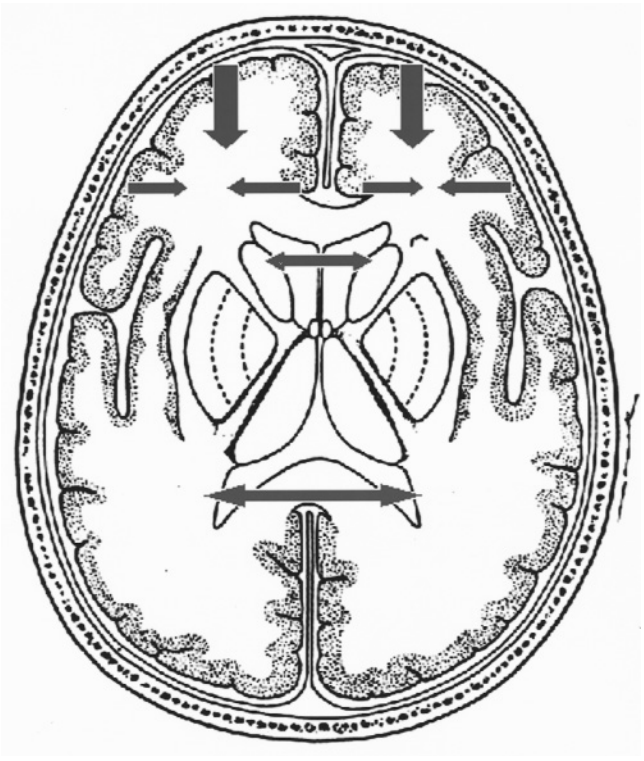

Figure 9. Axial brain slice drawing with unidirection arrows depicting regions and the direction of parenchymal size change and bidirectional arrows depicting regions and the direction of ventricular enlargement.

An additional brain MRI morphometric study using an automated segmentation algorithm has identified similar morphometric disturbances. In a study of 10 subjects with tuberous sclerosis complex with normal intelligence compared with 8 age- and cognition-matched control subjects, there were no differences in whole-brain volume, total gray-matter volume, total white-matter volume, or cerebrospinal fluid distribution.[40] There were, however, localized gray-matter reductions in volume in subjects with tuberous sclerosis complex in medial temporal lobes, posterior cingulate gyri, ventral basal ganglia, hypothalamus, and cerebellum, among other areas.[40] There was also a reduction in localized white-matter volume in subjects with tuberous sclerosis complex within the cingulum, occipitofrontal fasciculi, and superior longitudinal fasciculi.[40] An increase in white-matter volume of the cerebellum was also noted.

CONCLUSIONS

Tuberous sclerosis complex is a neuronal migration disorder. There are both focal and widespread anatomic and histologic abnormalities identifiable within the brains of patients with tuberous sclerosis complex. These migrational disturbances are a direct result of disturbed cellular control owing to a loss of the functional interaction of the hamartin-tuberin protein complex with its downstream nuclear signaling components. As a consequence, regional morphometric brain disturbances exist, which occur in conjunction with and separate from migration lesions. There is a positive correlation between the degree of clinical dysfunction and the degree of morphometric disturbance. Yet even in subjects with tuberous sclerosis complex with evidence of significant morphometric disturbances of both gray and white matter, there can be preservation of normal intelligence. Nonetheless, subtler cognitive and behavioral problems can still

exist. The presence of calcification within the more focal lesions of tuberous sclerosis complex correlates with an augmentation of both the morphometric and clinical disturbances observed. The observation that mineralization augments these effects might deserve further scrutiny.

Acknowledgment

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Special Article

Standardized Whole Brain Mapping of Tubers and Subependymal Nodules in Tuberous Sclerosis Complex

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ABSTRACT

Tuberous sclerosis complex is associated with radiologically visible abnormalities of brain structure, principally tubers and subependymal nodules. We reviewed the literature on neuroimaging of tubers and subependymal nodules and found qualitative evidence of bilateral, predominantly frontal distribution of tubers and bilateral, predominantly subcortical distribution of subependymal nodules in prior studies of pediatric samples. We studied 25 high-functioning adults with tuberous sclerosis complex and normal IQ, acquiring both dual spin-echo and fluid-attenuated inversion recovery magnetic resonance imaging sequences to optimize radiologic diagnosis of tubers and nodules. Individual lesion maps were then coregistered in a standard stereotactic space to facilitate construction of lesion density maps and estimation of lesion density in cortical and subcortical regions reliably defined by a parcellated template image. We found the highest frequency of tubers in frontal lobes and the highest density of tubers in parietal regions. There was significant regional variation in tuber density but no significant lateralization of frequently bilateral tubers. Nodules were located predominantly in the caudate nucleus and were not significantly lateralized. Tuber and nodule volumes were significantly positively correlated. Tuber volume was larger, on average, in patients with a lifetime history of epilepsy, but there was no correlation between IQ and these measures of lesion load. Contemporary image processing tools can be used to enhance quantitative, whole brain analysis of lesion load in patients with tuberous sclerosis complex. (*J Child Neurol* 2004;19:658–665).

Tuberous sclerosis complex is an autosomal dominant cortical dysgenesis syndrome for which two causative genes are known (*TSC1* and *TSC2*). The basic central nervous system pathology of tuberous sclerosis complex includes cortical and subcortical tubers, subependymal nodules, subependymal giant cell astrocytomas, heterotopic dysplastic neurons in white matter, and some ventricular enlargement,[1] although, in most cases, overall brain size is normal.[2] Possible neurologic outcomes of tuberous sclerosis complex include epilepsy, mental retardation, attention-deficit hyperactivity disorder (ADHD), and autism. The number and size of central nervous system lesions, like the behavioral and cognitive presentation, vary greatly for different individuals. Thus, tuberous sclerosis complex is an important genetic model to lead to wider understanding of the neuropathologic correlates of intelligence, ADHD, and autism.

Many studies have attempted to define the neuropathologic correlates of clinical phenotypes by comparing neuroradiologic "lesion load" across subjects. Despite the differing psychopathologic criteria for inclusion in such studies, we were interested in possible commonalities across subjects, perhaps indicating a typical lesion topography of tuberous sclerosis complex. Table 1 presents a summary of studies that reported information on lesion topography in

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Table 1. Prior Neuroimaging Studies of Frequency and Density of Tubers in Patients With Tuberous Sclerosis Complex

	Study												
	Cusmai et al (1990)[3]	*Jambaque et al (1991)*[4]	*Webb et al (1991)*[5]	*Braffman et al (1992)*[1]	*Shepherd et al (1995)*[6]	*Takanashi et al (1995)*[7]	*Bolton and Griffiths (1997)*[8]	*Griffiths et al (1998)*[9]	*Baron and Barkovich (1999)*[10]	*Seri et al (1999)*[11]	*Marti-Bonmati et al (2000)*[12]	*Weber et al (2000)*[13]	*Walz et al (2002)*[14]
Scan type	T_2	T_2	T_1	Short TR	T_1 and T_2	T_2 and FLAIR	12 MRI and 6 CT	T_1 and T_2	T_1 and T_2	FLAIR/T_1/T_2	T_1 and T_2	T_1 and T_2	T_2
N	34	23	11	42	75	5	18	20	7	14	34	29	50
Age mean	6	8	—	7.9	—	18	—	9	—	9	9	8	Pediatric
(range)	(0.5–18)	(3–16)	(5–60+)	(0.1–27)	(5–60+)	(13–25)	(3–23)	(2–17)	< 0.25	(7–10)	(2–14)	(0.5–26)	
Subjects with lesion (%)													
Tuber	94	96	82	95	100	100	89	95		100	94		92
SEN			64	95				100					
WM				93				100	100				
Bilateral lesion (%)													
Tuber	76	65	73	92						86			
SEN				85									
WM				92									
Average lesion number													
Tuber	—	—	4	11.7	14.4	17.6	—	4.1	10.3	5.5	14.3	14.3	—
(range)		(0–8+)	(0–9)	(0–52)	(1–46)	(10–33)	(0–17)	(1–15)	(0–30)	(2–9)	(0–30)	(0–23)	
SEN			2.1	6.4					13.7				
(range)			(0–5)	(0–15)				(2–15+)	(6–24)				
WM				6.1					35.7				
(range)				(0–15)					(4–69)				
Subjects with tuber in each lobe (%)													
Frontal	91	43	64		81	100	67			93		60	92
Parietal	88	74	64		87	100				93		60	90
Occipital	—	65	36		27	80				43		50	64
Temporal	74	35	18		63	80	44			50		50	76
Cerebellar				12			22	15			44		
Subjects with tuber in lobe bilaterally (%)													
Frontal		50	43							23			
Parietal		18	57							23			
Occipital		40	25							17			
Temporal		0	50							29			
Total tuber number in group in each lobe (%)													
Frontal			34	58		47	46		50				
Parietal			41	27		24	18		26				
Occipital			16	13		16	16		9				
Temporal			7	4		14	15		15				
Cerebellar				1			5						

CT = computed tomography; FLAIR = fluid-attenuated inversion recovery; MRI = magnetic resonance imaging; SEN = subependymal nodule; WM = white matter lesions.
This table represents a summary of studies of tuberous sclerosis complex that included tuber distribution information. Tuber distribution statistics were given in two forms: either as a percentage of subjects in the group or as a percentage of the tubers for the group. Once a tuber location was identified (by lobe), several studies also calculated the percentage of those subjects who had bilateral lesions within that lobe (rather than the percentage of the entire group).

subjects with tuberous sclerosis complex.[1,3–14] To obtain the sample sizes needed for these mainly retrospective clinical studies, samples included a wide range of ages (mostly pediatric) and levels of intellectual function. We also note that some prior studies of lesion load, for example, Bolton et al[15] and Asano et al,[16] are not tabulated here because the results were not reported in a form that allowed direct comparison with the rest of the literature.

Another immediate observation from this summary of previous reports of lesion topography in tuberous sclerosis complex was that a variety of imaging modalities had been used. In adults and older children with tuberous sclerosis complex, fluid-attenuated inversion recovery sequences are currently the most sensitive way to identify tubers[7,17]; however, only two studies in the current literature used fluid-attenuated inversion recovery for tuber identification. Although T_2-weighted magnetic resonance images (MRIs) identify many intermediate-sized tubers, the tubers are usually identified by a signal change in the subjacent white matter, and on T_2-weighted images, the gray matter of tubers is rarely highlighted.[7] Fluid-attenuated inversion recovery images show both the gray and the white matter of a tuber very clearly, which seemed essential when attempting a volumetric analysis of tuber topography.

Despite this heterogeneity in terms of sample characteristics and imaging methodology, several important findings were consistent across the studies summarized in Table 1. First, all three classic, neuroradiologic lesions of tuberous sclerosis complex (tubers, subependymal nodules, and white-matter abnormalities) occurred in approximately 90% or more of the patients studied. Second, when a subject presented with any lesion, there would be more than one lesion in a majority of cases, and the brain would often be affected bilaterally (65–92% of cases).[1,3–5,11] Third, there was a considerable variance in the number of lesions reported in different studies and different individuals (see Table 1). This likely reflected not only clinical heterogeneity, for example, owing to variable disease severity, but also differences in both scanning sensitivity (owing to scanner field strength, slice thickness, and MRI protocol) and lesion identification protocols.

To measure the lobar distribution of tubers, studies have, on the whole, employed two reporting styles. The first method reported the percentage of subjects with a tuber affecting each lobe, which provides an estimate of the frequency of lobar involvement by tubers. The second method reported the percentage of the group's tubers present in each lobe, which provided an estimate of tuber density. Tubers occurred most frequently in the frontal (43–100% frequency) and parietal lobes (60–100% frequency); they were less common and had more variable incidence in the occipital (36–80% frequency) and temporal (18–80% frequency) lobes. Tubers were least likely to be present in the cerebellum (12–44% frequency), and there were no reports in the literature of cerebellar tubers in the absence of cortical tubers.[1,12] Whether tubers were bilateral at the lobar level did not appear to be dependent on their location and was variable for different studies (0–57% of tubers affected lobes bilaterally; see Table 1 for details). Several studies reported the percentage of the group's tubers identified in each lobe, and all of these studies produced a similar profile.[1,5,7,8,10] The highest density of tubers occurred in the frontal lobe (34–58% of all tubers); then, in order of tuber density, came the parietal, occipital, and temporal lobes; finally, the lowest density of tubers was in the cerebellum (only 1–5% of total tubers affected the cerebellum). Previously, no study had considered whether lobe size or lesion volume affected the lobar density of lesions in tuberous sclerosis complex.

Baron and Barkovich explored correlations between the different types of lesions in tuberous sclerosis complex in a pediatric sample of seven patients (all aged less than 3 months).[10] A positive correlation was found between the number of subependymal nodules, white-matter abnormalities, and tubers. Baron and Barkovich also observed that lesion size can be correlated for the three types of lesions; however, this was not formally tested owing to the small size of the sample.

When we focused solely on the results from studies using fluid-attenuated inversion recovery images, it was interesting to note that the percentage of subjects with a lesion in any lobe was typically somewhat higher than for studies using conventional MRI sequences.[7,11] Walz and colleagues found no significant relationship between tuber location and autism and noted the high incidence of lesions in each lobe for all individuals.[14] They called for more sophisticated measures of lesion load and more careful assessment of the relationship between neuroradiologic lesions and other neuroimaging markers of tuberous sclerosis complex.[14] Two studies have considered whole brain lesion load and compared it with other neuroimaging variables. The first of these studies used positron emission tomography and MRI to analyze the relationship of autism and epilepsy to structural and functional brain abnormalities in children with tuberous sclerosis complex.[16] There was no significant association between lesion topography and autism; however, there was evidence of functional imbalance in subcortical circuits (including areas without lesions), which could be linked to the severity of autistic symptom scores in tuberous sclerosis complex.[16] The second study concluded that irrespective of tuber number, temporal lobe lesions played a crucial role in the pathogenesis of autistic behavior of some children with tuberous sclerosis complex.[11] Seri and colleagues found that disruption in auditory sensory processing and transient auditory memory storage could play an important role in the pathogenesis of autistic behavior in tuberous sclerosis complex and commented that tubers might constitute a hallmark of a disturbance of cerebral function that is more extensive than suggested by morphologic imaging.[11] As Walz and colleagues suggested, more refined methods of morphometry and/or the combination of multiple imaging modalities might help to resolve inconsistencies in the literature concerning the neurophysiologic correlates of autism in tuberous sclerosis complex.[14]

However, we note also that there might be relevant variation in the instruments used to diagnose autism. For example, both the Walz et al and Asano et al studies focused their attention on the presence or absence of autism (diagnosed clinically or with the aid of the Autism Diagnostic Interview),[14,16] whereas the study by Bolton et al used both the Autism Diagnostic Interview and the Autism Diagnostic Observation Schedule to assess and diagnose the behavioral syndrome and examined tuber distribution according to the presence or absence of an autism spectrum disorder, which encompasses a broader range of impairments.[15]

Having thus reviewed the literature on the topography of lesions in tuberous sclerosis complex, it seemed that more sensitive and standardized methods of lesion load measurement might be useful to provide a more comprehensive profile of neuropathologic abnormality and to address several questions about lesion topography in tuberous sclerosis complex: (1) Is the reportedly higher frequency of tubers in the frontal lobe just a reflection of the greater size of frontal lobes, that is, what is the lobar distribution of tubers when lobar volumes are appropriately controlled? (2) Is the anatomic distribution of tubers among sublobar brain regions significantly nonrandom? and (3) Is there any evidence of significant lateralization of lesion load in tuberous sclerosis complex?

METHODS

Subjects

Twenty-five high-functioning adults with a diagnosis of tuberous sclerosis complex and a normal IQ were included in the study (17 male, 8 female). All patients satisfied standard operationalized diagnostic criteria for tuberous sclerosis complex.[18] The mean age of the patient group was 39 years (range 16–57 years), and the mean IQ[19] was 117 (range 84–131).

Two of the adults with tuberous sclerosis complex were currently being treated for depression. Subjects with a clinical diagnosis of any other psychopathology were excluded from the study. A structured questionnaire was used to retrospectively ascertain the characteristics of epilepsy. Sixteen (65%) patients had a history of seizures, and for these individuals, the mean age at seizure onset was 8.67 years and the mean duration was 14.13 years; only eight patients had a history of infantile spasms. At the time of scanning, five patients suffered currently from seizures (one or more seizures in the last 12 months) and a further six patients were seizure free but continued to take anticonvulsant prophylaxis. Therefore, 11 patients were taking anticonvulsant medication at the time of the scanning.

Structural MRI Acquisition

All subjects were scanned using a GE Signa 1.5 Tesla system (General Electric, Milwaukee WI) located at the Magnetic Resonance Imaging and Spectroscopy Unit, Addenbrooke's Hospital, Cambridge, UK. A preliminary localizing scan in the sagittal plane was used to identify anterior and posterior commissures and to prescribe acquisition of a dual-echo fast spin-echo dataset in a near-axial plane parallel to the intercommissural line. Forty contiguous, interleaved, 4 mm thick proton density– and T_2-weighted images were acquired, providing whole brain coverage. The repetition time was 5625 milliseconds; echo times were 20 milliseconds and 102 milliseconds with an 8-echo train length. The matrix size was 256×256 and the field of view was 22 cm, giving an in-plane resolution of 0.859 mm. The total acquisition time was 12 minutes and 10 seconds. Cerebrospinal fluid-attenuated inversion recovery images were also acquired from subjects in the same (near-axial) orientation as that of the fast spin-echo images. For the fluid-attenuated inversion recovery data, repetition time was 10,002 milliseconds, echo time was 112.5 milliseconds with two excitations, inversion time was 2250 milliseconds, matrix size was 256×256, slice thickness was 4 mm, and total acquisition time was 14 minutes and 40 seconds.

Neuroradiologic Lesion Analysis

The fluid-attenuated inversion recovery MRI data were used to identify tubers and white-matter hyperintensities, whereas T_2-weighted MRI data were used to identify subependymal nodules. Tubers and nodules were visually diagnosed by an experienced neuroradiologist (N.H.) according to the following criteria.

A tuber was defined as a lesion affecting the cortical gray matter and adjacent white matter with an inner core hyperintense to gray and white matter on fluid-attenuated inversion recovery images.[7] White-matter anomalies were defined as areas of abnormal signal intensity in the white matter immediately subjacent to a tuber or nodule,[7] linear or wedge-shaped lesions extending from the ventricular surface to a cortical tuber (hyperintense to white matter on fluid-attenuated inversion recovery images),[1] and deep white-matter cysts (with the appearance of cerebrospinal fluid on all sequences).[20] Subependymal nodules were defined as small, nonobstructing nodular lesions at any subependymal location of any ventricle.[1]

The locations of all tubers, nodules, and white-matter lesions were marked on each individual image in native space using a mouse-controlled cursor on the computer display. These maps of lesion location were then coregistered with the proton density–weighted image from the same individual in native space and then with the proton density–weighted template image in the standard space of Talairach and Tournoux,[21] using the same 12-parameter affine transformation (implemented using the Fletcher-Davidon-Powell algorithm[22,23]) for both steps of this registration. Finally, a linear transformation was used to coregister the data in the Talairach and Tournoux space with an anatomically parcellated image[24] in the space of the Montreal Neurological Institute template image. Once each individual's lesion maps had been coregistered in the standard space of the anatomically parcellated template image, it was then straightforward to construct lesion density maps, illustrating the anatomic distribution of lesions over all patients with tuberous sclerosis complex, and to estimate the proportion of each cortical or subcortical region defined by the parcellated template image that was occupied by lesions.

To test the significance of regional and lateralization effects on lesion distribution, repeated measures analysis of variance (ANOVA) models were fitted with the proportion of regional volume occupied by tubers or nodules as the dependent variables and region nested within side (right or left) as within-subject explanatory factors.

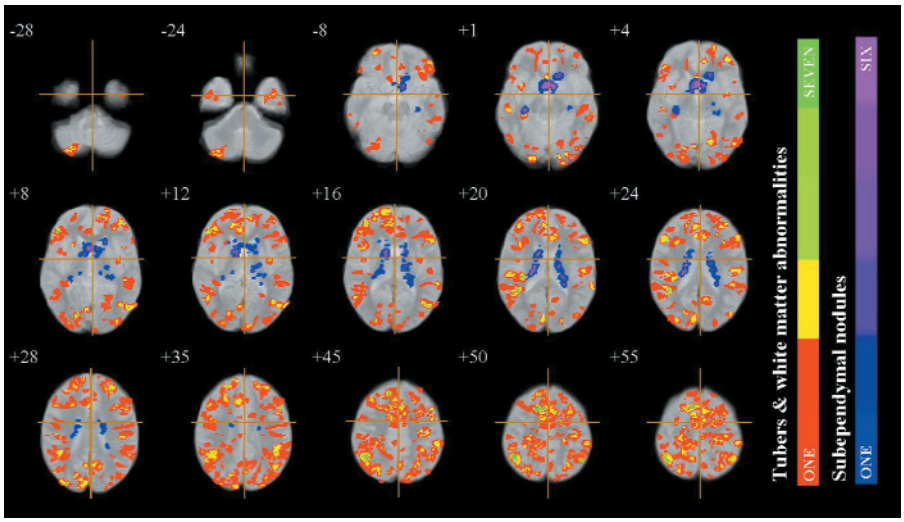

Figure 1. Whole brain lesion density map showing the frequency of tubers (red and green color scale) and subependymal nodules (blue and purple color scale) in a group of 25 high-functioning adults with tuberous sclerosis complex. The lesion density map was developed by superimposing all of the subjects' lesion maps in the standard space. The left side of each panel represents the right side of the brain; the z-coordinate for each axial slice in the standard space of Talairach and Tournoux[21] is given in millimeters. The scale for tubers and white-matter abnormalities ranges from one subject having a lesion in the same voxel (*red*) to seven subjects (the maximum overlap) having a lesion in the same voxel (*green*). The scale for nodules ranges from one subject having a lesion in the same voxel (*blue*) to six subjects (the maximum overlap) having a lesion in the same voxel (*pink*).

RESULTS

Tubers

Almost all patients (23/25) had radiologic evidence of at least one classic central nervous system lesion. The mean tuber count was 13.04 (SD = 10.36; range = 0–36).

Whole brain tuber density mapping (Figure 1[21]) demonstrated that tubers were widely but not uniformly distributed throughout the cerebral gray matter. Neocortical gray matter had the highest density of tubers; tuber density was relatively reduced in the cerebellum, medial temporal lobe, and subcortical nuclei.

Tuber frequency was quantified in each neocortical lobe, cingulate gyrus, and medial temporal lobe and the major subcortical structures (Figure 2A). The highest frequency of tubers was in the frontal lobe, followed by the parietal, temporal, occipital, and cingulate cortices. Bilateral occurrence of tubers was frequent, especially in the frontal lobe. The high frequency of tubers in the cingulate gyrus was notable in the absence of any prior reports of cingulate tubers.

To account for different lobar sizes, we used the percentage occupancy of each lobe by tubers to estimate tuber density (Figure 2B). This analysis revealed a slightly different rank order of lesion load in that the greatest percentage occupancy by tubers was found in the parietal lobe. We also estimated the percentage occupancy by tubers at a finer-grained level of anatomic resolution defined by sublobar regions corresponding approximately to major cortical gyri and subcortical nuclei (Figure 3A[24]).

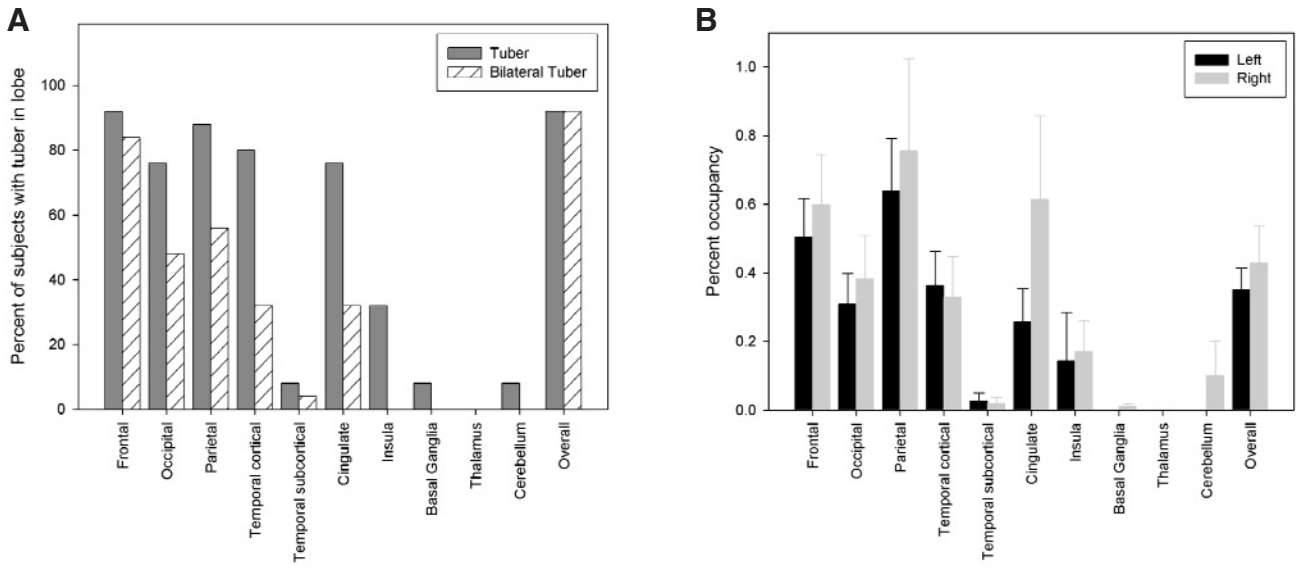

Figure 2. *A,* Tuber frequency for lobes and major subcortical structures. *B,* Tuber density for lobes and major subcortical structures. The error bars represent one standard error from the group mean. Frequency was given in the percentage of subjects with an affected lobe or major subcortical structure. Tuber density was measured by the average percentage of each lobe occupied by lesions across the group.

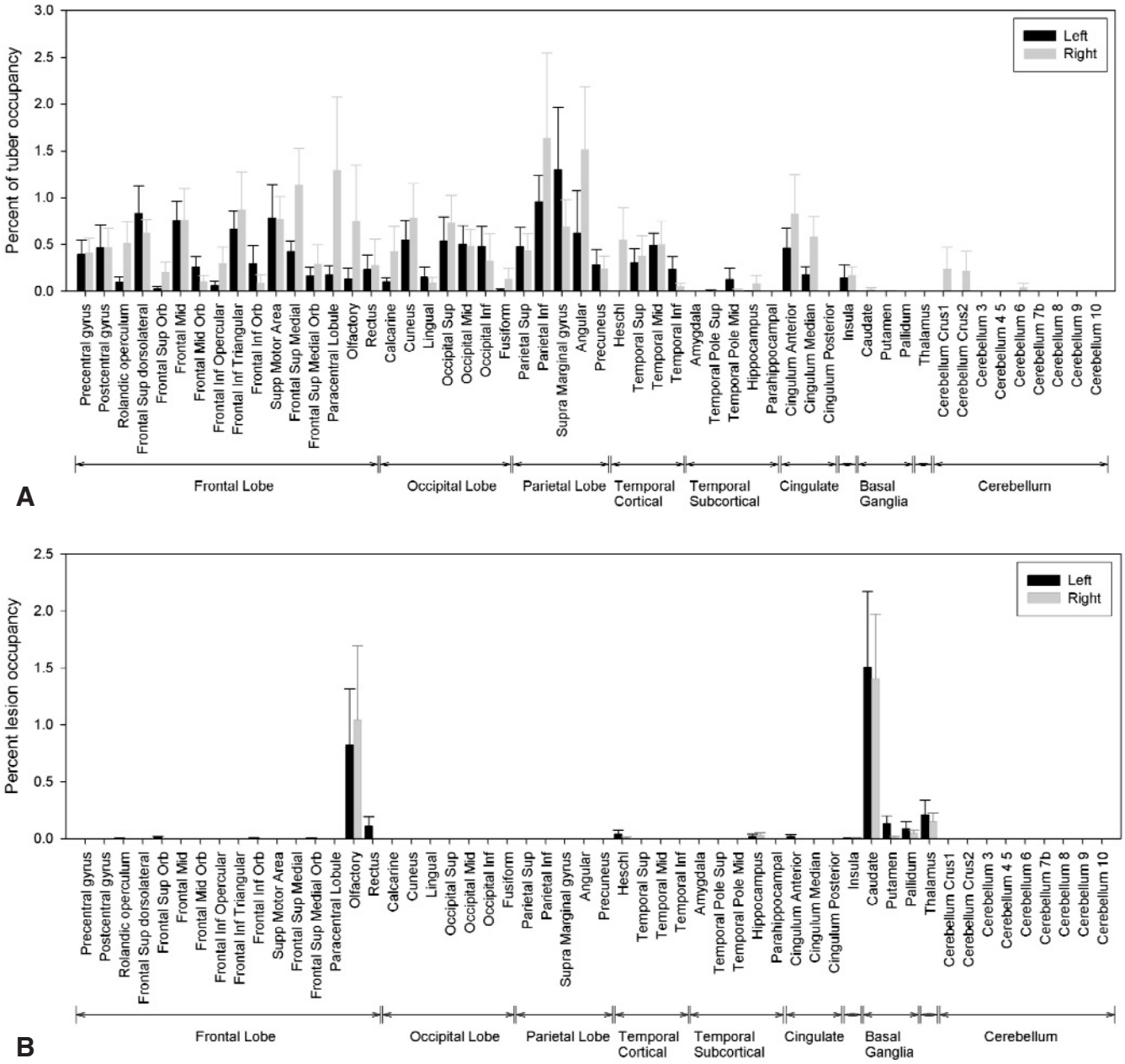

Figure 3. *A,* Tuber density for cortical regions and major subcortical nuclei. *B,* Subependymal nodule density for cortical regions and major subcortical nuclei. These panels represent the density of lesions, which was measured as the average percentage occupancy of lesions for each region on each side of the brain, where regions were the Montreal Neurological Institute template regions.[24] The error bars represent one standard error from the mean. As expected, subependymal nodules encroached from the ventricles mainly into the caudate. The lobar and major subcortical structure groupings of these regional parcellations are presented across the bottom of the figure. Inf = inferior; Mid = middle; Orb = orbital; Sup = superior.

This showed that the inferior parietal lobule had the highest mean density of tubers (1.15% regional volume) and the angular gyrus had the maximum density of tubers (14.1% of regional volume was replaced by tubers in one individual).

Repeated measures ANOVA of the regional tuber density data demonstrated a significant main effect of region ($F_{(53,24)}$ = 3.87; P = .001) but no significant effect of side (right versus left: $F_{(1,24)}$ = 2.82; P = .11) and no significant interaction between region and side ($F_{(1,53)}$ = 0.98; P = .51).

Subependymal Nodules

Whole brain subependymal nodule density mapping (see Figure 1) demonstrated that nodules were concentrated in subcortical nuclei bilaterally. The caudothalamic sulcus in the walls of the lateral ventricles was the site of greatest nodule frequency; 56% of patients had one or more nodules in this region.

Nodule density was estimated for each sublobar cortical region and major subcortical nuclei (Figure 3B). The highest density of nodules was in the caudate nucleus (the mean

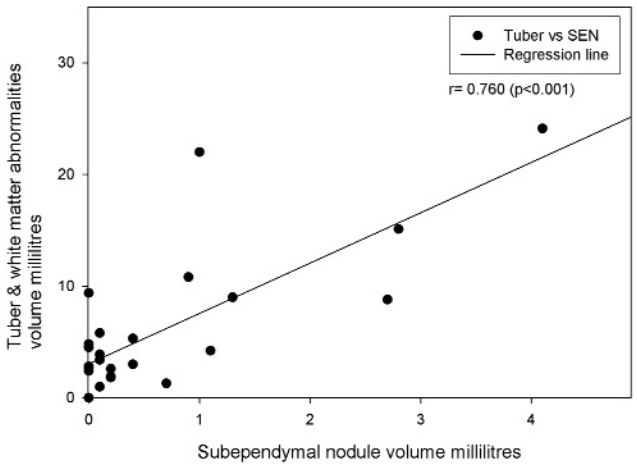

Figure 4. Positive correlation between whole brain tuber and white-matter abnormality volume and whole brain subependymal nodule (SEN) volume in 25 high-functioning adults with tuberous sclerosis complex. $r = .76$; $P < .001$.

percentage occupancy by nodules was 1.45%); the maximum density of nodules was also located in the caudate nucleus (11.0% of caudate volume was occupied by nodule in one individual). Caudate nodules were almost always bilateral. The putamen, pallidum, thalamus, and olfactory and anterior cingulate cortices were the other sites of notable nodule density. The thalamus was frequently involved (48% of patients had one or more nodules in the thalamus), but the density of nodules in the thalamus was typically low (the mean percentage occupancy by nodules was 0.18%).

Repeated measures ANOVA of the regional nodule density data demonstrated a significant main effect of region ($F_{(53,24)} = 6.39$; $P < .001$) but no significant effect of side (right versus left: $F_{(1,24)} = 0.04$; $P = .85$) and no significant interaction between region and side ($F_{(1,53)} = 0.09$; $P = .85$).

Tuber and Nodule Correlations

There was a strong positive correlation between whole brain tuber volume and both whole brain white-matter lesion volume ($r = .962$; $P < .001$) and whole brain subependymal nodule volume ($r = .760$; $P < .001$) (Figure 4). There was also a significant positive correlation between whole brain subependymal nodule volume and whole brain white-matter lesion volume ($r = .813$; $P < .001$).

At a regional level of analysis, subependymal nodule volume in the basal ganglia was significantly positively correlated with tuber volume in frontal ($r = .639$; $P < .001$), occipital ($r = .797$; $P < .001$), temporal ($r = .715$; $P < .001$), and parietal lobes ($r = .601$; $P < .001$). Tuber volume in the frontal, occipital, and parietal lobes was also significantly positively correlated with subependymal nodule volume in the thalamus ($P < .05$ for all correlations). However, there were no significant correlations between tuber volume in the cingulate cortex and subependymal nodule volume in the basal ganglia or thalamus.

Intelligence, measured by the Wechsler Abbreviated Scale of Intelligence (WASI), was not significantly correlated with whole brain tuber count, tuber volume, white-matter abnormality volume, or subependymal nodule volume ($P > .05$ for all correlations).

When the sample was subdivided into those patients ($n = 9$) who had never had an epileptic seizure and those patients ($n = 16$) who had a lifetime history of at least one seizure, we found significantly greater whole brain tuber and associated white-matter abnormality volume in the patients with a history of epilepsy (Table 2).

DISCUSSION

The main innovation reported in this article is the use of computational tools for image registration in standard neuroanatomic space to locate radiologic lesion maps, defined individually for each patient by expert interactive diagnosis, in a consistent frame of reference. This approach has allowed us directly to produce whole brain lesion density maps (see Figure 1), representing the frequency of tubers and nodules at voxel-level resolution, and to estimate the frequency and density of lesions in lobar and regional brain volumes defined by a prior cerebral parcellation template image (see Figures 2 and 3).[24]

Our results corroborate and extend the results of previous radiologic studies of lesion load in tuberous sclerosis complex. We have replicated the observation that the frontal lobes are most frequently affected by (commonly bilateral) tubers, but when differential lobar size is appropriately controlled, we found that the highest density of tubers was in the parietal cortex. Our whole brain analysis also highlighted the frequency and density of tubers in the cingulate gyrus, which has not previously been reported. Formal statistical modeling confirmed that regional differences in tuber density were highly significant, but there was no evidence of significant lateralization of lesion load. Similarly, our maps and regional analysis of subependymal nodule distribution confirmed that nodules are concentrated bilaterally in the basal ganglia, especially the caudate nucleus, with no evidence of significant lateralization.

Table 2. Cognitive and Neuroimaging Differences Between Subgroups of Patients With Tuberous Sclerosis Complex Categorized According to Presence or Absence of a Lifetime History of Epilepsy

Measure	*Seizure Free (n = 9)*	*Seizure Positive (n = 16)*	*t (df = 23)*	P	*Mean Difference*	*95% CI of Mean Difference*
WASI IQ (SD)	121.67 (7.4)	114.19 (14)	−1.738	.096	−7.479	−16.38 1.43
SEN volume (SD)	0.23 (0.2)	0.89 (1.3)	2.033	.058	0.660	−0.026 1.35
Tuber count (SD)	10.28 (8.9)	13.78 (10.4)	0.888	.356	3.503	−4.76 11.76
Tuber and white-matter lesion volume (SD)	2.42 (1.6)	8.05 (7.0)	3.064	.007	5.628	1.76 9.49

SEN = subependymal nodule; WASI = Wechsler Abbreviated Scale of Intelligence.

Generally, different measures of lesion load were positively correlated, that is, patients with large whole brain tuber volume tended also to have large whole brain nodule volume, suggesting that these two aspects of central nervous system pathology in tuberous sclerosis complex might share common, presumably genetic, causes. The pluripotentiality of central nervous system stem cells might also be relevant, in so far as the early second-hit mutational events that are postulated to give rise to the clonal abnormalities in central nervous system cell growth and differentiation that typify tuberous sclerosis could produce quite widespread effects and involvement of different cell types and central nervous system structures. It will be interesting in future studies to explore systematically the effects of variable genetic mutations in *TSC1* and *TSC2* genes on the frequency, density, and anatomic distribution of tubers and nodules in tuberous sclerosis complex because several reports have indicated that *TSC2* mutations are associated with more marked and severe phenotypic abnormalities. It will also be important to pursue the hypothesis suggested by Ridler and colleagues that classic lesion load is correlated with the extent of morphometric deficits in radiologically normal-looking gray- and white-matter regions.[25] In other words, it remains plausible that tubers and nodules represent only the most visible aspect of a more distributed neuropathologic phenotype.

The overall consistency between our results and those reported previously (see Table 1) is quite striking in light of the age and IQ differences between our sample of high-functioning adults and the generally more impaired pediatric samples reported in the literature. This suggests perhaps that lesion load is not dynamically variable in the course of the life cycle and that lesion load might not be a strong predictor of intelligence. Indeed, we could find no evidence of a significant correlation between tuber or nodule density and IQ in this sample, although this might also reflect the modest sample size and the fact that only subjects of normal intelligence were investigated. Our data on epilepsy were retrospectively ascertained, but it seemed that a lifetime history of one or more seizures was significantly associated with greater whole brain tuber volume. It will be important in future work to explore the relationships between these novel measures of radiologic lesion load and a broader range of prospectively measured clinical phenotypes.

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Special Article

Behavioral and Cognitive Aspects of Tuberous Sclerosis Complex

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ABSTRACT

Tuberous sclerosis complex is a multisystem genetic disorder. Of all the possible manifestations of this complex disorder, the cognitive and behavioral problems represent the area of greatest concern to parents and caregivers. This review outlines the current evidence regarding global intellectual abilities, behavioral problems, psychiatric diagnoses, learning disorders, and specific neuropsychologic deficits for which individuals with tuberous sclerosis complex are at particularly increased risk, and outlines approaches to intervention. Approximately half of individuals diagnosed with tuberous sclerosis complex present with global intellectual impairment and developmental psychopathologies. Those with normal intellectual abilities are also at high risk of specific neuropsychologic deficits and behavioral, learning, and other psychiatric disorders. There is no evidence for an inevitable decline in cognition or behavior, and any such changes should be investigated. The evolving neurocognitive literature suggests that frontal brain systems might be most consistently disrupted by tuberous sclerosis complex–related neuropathology, thus leading to abnormalities in regulatory and goal-directed behaviors. (*J Child Neurol* 2004;19:666–674).

Tuberous sclerosis complex is a multisystem genetic disorder associated with the formation of multiple hamartias and hamartomas throughout the body but particularly in the brain.[1] There is a wide range of structural central nervous system manifestations, including radiologically detectable abnormalities (such as cortical tubers and subependymal nodules)[2] and more subtle fine-grain aberrations of gray and white matter.[3] In addition, there is an extremely high rate of functional central nervous system manifestations, such as the full spectrum of epilepsies,[4] learning difficulties, and behavioral problems,[5] which can, to a greater or lesser extent, be associated with the structural central nervous system features. Of all of the possible manifestations of this complex disorder, however, the cognitive and behavioral problems represent the area of greatest concern to parents and caregivers.[5]

The first cases of tuberous sclerosis complex described by Bourneville in 1880 and 1881 had severe mental retardation, intractable epilepsy, and "sclerose tubereuse" (white potato-like brain growths) on postmortem.[6,7] In 1908, Vogt introduced the classic diagnostic triad for tuberous sclerosis complex: mental retardation, epilepsy, and so-called adenoma sebaceum.[8] These studies predated refinement of diagnostic criteria[1] based on large population-based and clinic samples free of ascertainment biases and predated the incorporation of better imaging and other diagnostic tools.[1–3] The identification of the two genes associated with the disorder further aided to broaden the diagnostic boundaries of tuberous sclerosis complex.[9] These historic changes in the conceptualization of the disorder have led to the identification of individuals who might not necessarily have presented clinically in the past, for example, family members without any overt functional or medical impairments but who are screened for the purpose of genetic counseling. Neither mental retardation nor epilepsy has remained as a diagnostic feature[1] owing to their lack of specificity for the disorder. Contemporary studies report that no more than 30% of individuals with tuberous sclerosis complex seen in clinics today have Vogt's classic triad,[1,10] although, unfortunately, that triad is still presented in many medical textbooks

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Table 1. Common Cognitive and Behavioral Problems in Tuberous Sclerosis Complex: Typical Ages at Presentation and Appropriate Interventions

Age (yr)	*Most Likely Cognitive or Behavioral Problem Presenting at This Age*	*Appropriate Intervention*
0–3	Global intellectual impairment Autism spectrum disorders	Individual educational plan Early intervention programs for autism
3–8	Impulsivity ADD/ADHD	Provide structure/coaching ± psychopharmacology
8–12	Organizational impairment ADD/ADHD	Provide structure/coaching ± psychopharmacology
12–18	Independent living skills Affect-regulation and judgment Anxiety disorders and depressive disorders	Coaching Coaching Cognitive behavioral therapy ± psychopharmacology
18+	Anxiety disorders and depressive disorders	Cognitive behavioral therapy ± psychopharmacology

ADD = attention-deficit disorder without hyperactivity; ADHD = attention-deficit hyperactivity disorder.

and remembered by aspiring clinicians as the hallmark of tuberous sclerosis complex.[11]

As detection, diagnosis, and systematic study of tuberous sclerosis complex has progressed, the results have been remarkably consistent in finding that almost 100% of individuals with tuberous sclerosis complex have structural brain abnormalities and that approximately 90% have a history of seizures, but, in marked contrast to initial clinical reports, no more than 50 to 60% of all individuals with tuberous sclerosis complex have global cognitive impairment.[1–4,12] Although these relatively recent studies contradict the previously espoused doctrine of inevitable mental retardation and epilepsy in tuberous sclerosis complex,[13] as yet, little is known about the neurocognitive and behavioral profiles of individuals with tuberous sclerosis complex who do not have global cognitive impairment. Until recently, studies tended to categorize individuals as either "impaired" or "managing independently" based on functional criteria. As more studies started to incorporate standardized clinical testing, findings have begun to confirm that tuberous sclerosis complex renders affected individuals at high risk of a range of specific behavioral and cognitive difficulties that include but are not limited to global mental retardation. Good clinical practice should therefore take these risks into account in diagnostic evaluations across the lifespan, with the aim of identifying and managing those cognitive and behavioral risks appropriately if and as they emerge. Early identification and management is fundamental to avoid or, at least, mitigate the short- and long-term effects of developmental disruptions on a child's subsequent intellectual, behavioral, and social or emotional development.

The focus of this review is to outline the specific behavioral and cognitive problems for which children and adults with tuberous sclerosis complex are known to be at particularly increased risk and to do so within a developmental framework. To that end, the timepoints at which specific difficulties are most likely to emerge will be indicated. The intention is not to assume that those difficulties will necessarily emerge or that they will emerge only at that timepoint. Rather, the goal is to provide information that will allow affected individuals, their families, and their caregivers to anticipate and monitor for these difficulties in an attempt to reduce the lag between the emergence of problems and suitable intervention. The review does not set out to be a detailed research document but rather aims to provide a succinct clinical guide of current evidence. The cognitive and behavioral problems that commonly present in clinical practice are summarized in Table 1. Each is briefly elaborated below with regard to diagnostic characteristics, currently documented frequency, and suggested interventions.

GLOBAL INTELLECTUAL ABILITIES

"Global intellectual ability" is formally defined in terms of performance on standardized measures of both general intelligence and independent living skills. "Mental retardation" is defined in the *Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV)*[14] and the *International Classification of Diseases-10*[15] as a standardized IQ that is 2 SD or more below the population mean (eg, a Wechsler Intelligence Scale for Children [WISC]-IV[16] IQ = 70) combined with an impaired ability to function independently in daily living skills, such as self-care, communication, work, or leisure (formally assessed through an interview with a caregiver using a standardized measure such as the Vineland Adaptive Behavior Scales[17]). A range of other terminologies, such as "learning difficulties" or "global developmental delay", is often preferred to the term "mental retardation". For the purpose of this review, the term "global intellectual abilities or impairment" is used.

A number of studies have investigated global intellectual ability in tuberous sclerosis complex and have shown that, as a rule of thumb, about half of individuals with tuberous sclerosis complex are in the normal or near-normal range of overall abilities.[10,18–25] In the most recent population-based study, 55% of those with the disorder had an IQ > 70, whereas 30.5% had an estimated IQ < 21. Individuals with tuberous sclerosis complex in the normal range of global intellectual abilities showed a normal distribution of IQ but with a mean IQ 12 points lower than their unaffected siblings.[12] Even normally

intelligent individuals with tuberous sclerosis complex without a lifetime history of seizures had mean IQs below those of their siblings without tuberous sclerosis complex.[12]

On closer inspection, global intellectual abilities in tuberous sclerosis complex therefore seem to be distributed bimodally.[12] About 70% of individuals fall on a relatively normal distribution of intellectual abilities (akin to a bell-shaped Gausssian curve), with IQ scores ranging from 20 to 130+. The remaining 30% of individuals with tuberous sclerosis complex have IQ scores that cluster in the profoundly impaired range (IQ < 20). This is an important observation for clinical practice. In the first instance, individuals who fall in the profoundly impaired range are often unable to participate in "formal" standardized testing of their global intellectual abilities. Abilities are therefore typically estimated through characterization of their functional abilities based on structured interview with a parent or caregiver. In contrast, the more able individuals are mostly able to participate in a range of standardized tests to assess their cognitive strengths and weaknesses. Second, the most profoundly impaired children with tuberous sclerosis complex often have arrested development. That is, they do not show developmental gain over time. In contrast, the majority of children with tuberous sclerosis complex will progress in their developmental stages over time, albeit often at a slower rate and lower level than their unaffected siblings.

Further work will be required to determine whether tuberous sclerosis complex is, indeed, associated with two (or more) distinct cognitive phenotypes based on global cognitive abilities, which, in turn, can be associated with differential developmental trajectories and underlying causal mechanisms.

BEHAVIORAL AND PSYCHIATRIC MANIFESTATIONS

"Behavioral difficulties" are defined as symptoms or problems identified in daily life by parents, caregivers, teachers, or others. A wide range of such difficulties is seen in tuberous sclerosis complex, including aggressive outbursts, severe and persistent temper tantrums, overactivity, restlessness, impulsivity, poor ability to concentrate, other disruptive behaviors, self-injury, anxiety, and depressed mood.[5,10,13,23–30] More than 50% of children with tuberous sclerosis complex are likely to display some behavioral difficulties during development that can be of sufficient degree for parents to seek professional advice.

It is well recognized that individuals with global intellectual impairment are at higher risk of presenting with a range of behavioral problems and psychiatric disorders than those with normal intellectual abilities,[14,15] and individuals with tuberous sclerosis complex are no exception. Between 60 and 70% of children with tuberous sclerosis complex with global intellectual impairment are likely to present with one or more behavioral problems, whereas about 20 to 30% of children with tuberous sclerosis complex with normal global intellectual abilities can have such behavioral difficulties.[23–30] In contrast, about 5 to 10% of children without tuberous sclerosis complex are likely to present with significant behavioral problems.[14,15] Identifying and acknowledging behavioral problems should lead to determining whether any specific psychiatric disorder might be present to investigate the possible causes of the behavioral difficulty and to implement an appropriate management strategy.

"Psychiatric disorders," as defined in the *DSM-IV*[14] or the *International Classification of Diseases-10*,[15] are based on a phenomenologic nosology. That is, disorders are groupings of symptoms into syndromes or symptom clusters without any necessary assumptions about the underlying etiologies or mechanisms of the syndromes. In spite of much debate about how syndromes should be defined, there is clear pragmatic usefulness in making a diagnosis to describe the behavioral profile and to determine the needs of a child, adolescent, or adult with such a psychiatric diagnosis.

Developmental Disorders: Autism and Attention-Deficit Hyperactivity Disorder

Children and adolescents with tuberous sclerosis complex are at high risk of developmental disorders as defined in the *DSM-IV*[14] and the *International Classification of Diseases-10*.[15] Two disorders in particular have been reported in tuberous sclerosis complex at much higher rates than expected. Population-based studies reported classic infantile autism in 25% of cases,[27,30] autism spectrum disorder (also referred to as pervasive developmental disorder) in about 50%,[24,27,30] and attention-deficit hyperactivity disorder (ADHD) and related disorders in more than 50% of individuals with tuberous sclerosis complex.[24,28] Psychiatric diagnoses are significantly higher among those with global intellectual impairment.[23] More than 60% of children with tuberous sclerosis complex with global intellectual impairment can present with autism, autism spectrum disorder, or autistic features that require clinical intervention, in contrast to about 6% in the normally intelligent group with tuberous sclerosis complex.[28] Even though this represents a significant difference within the tuberous sclerosis complex population, an autism rate of 6% among normally intelligent children with tuberous sclerosis complex is a 10-fold overrepresentation in comparison with the prevalence rates of autism spectrum disorders in the normal population.[31] A diagnosis of ADHD is similarly 10-fold overrepresented in normally intelligent children with tuberous sclerosis complex (over 30%) in contrast to their sex-, age-, and IQ-matched peers without tuberous sclerosis complex (about 3–5%).[32]

Other Psychiatric Disorders

Limited systematic data are available about the rates of other psychiatric disorders in tuberous sclerosis complex. During adolescence and into adulthood, a significant number of individuals with tuberous sclerosis complex develop severe affective symptoms, such as anxiety or depressed mood.[25] In a postal survey of behavioral, psychological, and psychiatric problems in 510 children and adults with tuberous sclerosis complex, 45% reported anxiety symptoms,

whereas 29% had symptoms of depressed mood.[25] Smalley and colleagues suggested prevalence rates of 35% for depressive disorders and 59% for anxiety disorder in a study of adults with tuberous sclerosis complex.[29,33] Mood and anxiety disorders can be extrinsic to tuberous sclerosis complex (eg, secondary to the psychologic impact of having a genetic disorder or as a consequence of seizures or medication). It is, however, not inconceivable that the intrinsic pathophysiologic mechanisms of the disorder can also render individuals at higher risk of mood and anxiety disorders.

Earlier case reports included schizophreniform illnesses or psychosis,[34,35] mania,[36] and Capgras' syndrome,[37] but no clear systematic studies have since been performed. There is, however, an association of temporal lobe epilepsy and psychosis[11] in tuberous sclerosis complex that requires clinical management with appropriate antiepileptic drugs and, potentially, short-term antipsychotic medication.

Taken together, individuals with tuberous sclerosis complex are at high risk of having global intellectual impairment and a range of psychopathologies. The evidence also suggests that even those with normal intellectual development are at a significantly higher risk of behavioral, developmental, and other psychiatric disorders than individuals without tuberous sclerosis complex. Developmental disorders (autism and ADHD) are the most likely diagnoses in childhood and early adolescence, whereas mood and anxiety disorders predominate in late adolescence and adulthood. Behavioral problems (regardless of psychiatric diagnosis) most commonly include symptoms that involve the dysregulation of affect or attention, such as aggressive outbursts, temper tantrums, overactivity, impulsivity, and distractibility. Possible mechanisms for these symptoms and disorders are discussed in subsequent sections.

LEARNING DISORDERS

Learning disorders are diagnosed when an individual's performance on standardized tests of reading, writing, or mathematics shows a discrepancy with the level expected for age, schooling, and global intellectual ability.[14,15] Learning disorders are often referred to as learning difficulties, scholastic difficulties, academic difficulties, or a specific diagnosis, such as dyslexia. Between 2 and 20% of school-aged children can have learning disorders, and the rates are significantly higher in association with specific neurogenetic disorders. These disorders of scholastic skills are often associated with low self-esteem, deficits in social skills, and a general sense of demoralization for the child.

No systematic studies have been performed in tuberous sclerosis complex to date, but anecdotal evidence and feedback from clinicians suggest very high rates of learning disorders in tuberous sclerosis complex, including in children with normal global intellectual abilities. Clinicians are advised to maintain a low threshold for parental concern about a child's scholastic performance and to support appropriate evaluation of and intervention for such potential difficulties.[38,39]

SPECIFIC NEUROPSYCHOLOGIC SKILLS

Neuropsychologic evaluations are used to determine an individual's profile of the strengths and weaknesses of brain-referenced systems.[40,41] Specific neuropsychologic tests and tools are used to measure, for instance, anterior "planning and production" versus posterior "comprehension" systems, left hemisphere "language" versus right hemisphere "spatial" systems, and cognitively "higher-level" cortical versus "lower-level" subcortical functional systems.

Relatively few studies of neuropsychologic profiles in tuberous sclerosis complex have been reported to date. The first study to incorporate specific neuropsychologic assessments examined 23 children in a sequential clinic sample.[42] Among the 7 children with normal global abilities, the authors observed dyspraxia, speech delay, visuospatial problems, memory deficits, and dyscalculia. Among the 10 children with global intellectual impairment without autism, the authors observed linguistic problems and visuospatial difficulties.

More recently, the neuropsychologic profiles of 44 children between the ages of 6 and 18 years were examined as part of a large-scale clinical study.[43] Consistent with reports from population-based studies, half of the children in this clinic sample showed global intellectual impairments and were not able to complete age-appropriate standardized testing. Twenty-two children did, however, complete a standard protocol that included measures of executive control processes (planning, problem solving), language, memory, and spatial abilities. Data for individual test results were summarized as test performance "within the broad average range" (defined as performance above the 16th percentile) or as "deficient" (defined as performance below the 16th percentile). Deficiencies were most frequent in the domain of attention or executive control processes (with 66% of children showing deficient performance in attentional-executive tasks compared with 19% for spatial, 24% for language, and 38% for memory domains). It was of note that although there was a decline in the frequency of spatial and language deficiencies from the younger age group (6–11 years) to the older group (12–18 years) of children, there was no change in the frequency of executive control deficiencies between the two age groups.[43]

A population-based study of specific attentional skills in children with tuberous sclerosis complex who had normal or near-normal intelligence showed very high rates of selective attention, sustained attention, and attentional switching deficits.[28,44] The children in the study showed most consistent impairment during dual-task performance.[28] Importantly, whereas all of the children in the study who fulfilled the criteria for ADHD showed specific attention deficits, almost all of the children without a diagnosis of ADHD also showed very significant specific attentional deficits. Overall, 17 of the 19 children in the study (89%) had neuropsychologic attention deficits (defined as performance below 2 SD of the population mean) in one or more attentional domains.[28]

No detailed studies of language ability and language impairments had been published at the time of this review. In a survey of 510 individuals with tuberous sclerosis complex, only 28% of families reported normal language development in children and adults with tuberous sclerosis complex.[25] Detailed analysis of 34 children with tuberous sclerosis complex showed that only 30% of children had normal language acquisition and development (Baltaxe CAM, unpublished data, 1994). Among those with normal global intellectual abilities, Baltaxe reported an overrepresentation of difficulties in expressive vocabulary, abstract language skills, and expressive semantic-grammatic difficulties. Language deficits in normally intelligent children with tuberous sclerosis complex therefore more specifically suggested significant problems primarily with production and organization of language. These are predominantly attributed to frontal system difficulties.

Studies of neuropsychologic skills in adults with normal intelligence have shown impairments in planning, self-monitoring, and goal-directed attention.[45–47] In contrast, recognition tasks seemed relatively intact. Similar to the salient features of language abnormalities, the specific deficits so far identified in adults with tuberous sclerosis complex also represent executive processing difficulties, thus supporting frontal systems involvement.

Taken together, research evidence suggests that individuals with tuberous sclerosis complex are at high risk of very specific neuropsychologic deficits in attentional-executive skills, even when their global intellectual abilities are judged to be within normal limits and even when they might not present with sufficient features to fulfill criteria for a psychiatric diagnosis.

ASSOCIATION BETWEEN NEUROPSYCHOLOGIC DEFICITS AND THEIR MANIFESTATIONS IN DAILY LIFE

The term "attentional-executive control" processes can be divided into two main components: first, regulatory mechanisms, such as the regulation and modulation of attention, behavior, and affect, including the ability to sustain attention independently, and second, goal-directed executive mechanisms, such as the ability to plan, organize, execute, and monitor goal-directed activities and to anticipate consequences.[40,41] The general term "attentional-executive control processes" is used to reflect a continuum of functions in which, depending on specific task demands, more regulatory abilities or more executive contributions might be required.[41, 48]

Deficits or reduced strengths in attentional-executive control processes can manifest in various ways in daily life.[40,41,49–51] A child who has difficulty in attentional set-shifting, that is, with shifting attention from one stimulus to another, shifting set can appear to be inflexible, which may manifest as difficulty with managing transitions or sudden changes in rules. If pushed to change a routine unexpectedly, such a child might withdraw or present with disruptive or aggressive behaviors that seem out of proportion to the request. Reduced planning ability can contribute to impulsivity and poor choice of behavior, as well as failure to anticipate the consequences of specific behavior secondary to reduced memory for the future. Because executive control processes are associated particularly with management and organization, children with attentional-executive deficits might have the ability to perform all of the individual parts of a complex task with supervision, for example, when an adult is nearby to prompt each step, but become overwhelmed or disorganized when required to combine elements or to monitor task management independently.

It is of great importance to identify such manifestations of specific neuropsychologic deficits in daily life. In the first instance, behaviors can then be viewed as a manifestation of tuberous sclerosis complex rather than as "bad" or "uncooperative" behavior. Furthermore, specific interventions can be implemented under the guidance of, for example, an experienced clinical neuropsychologist. Intervention should aim to anticipate tasks or situations in which a child is likely to struggle and should incorporate structure and appropriate coaching based on the individual's strengths and weaknesses.[49–53]

In summary, the specific neuropsychologic deficits seen in tuberous sclerosis complex can present as significant difficulties in "managing" daily life. These difficulties might not amount to sufficient symptoms to fulfill criteria for a psychiatric diagnosis such as ADHD or autism but should nevertheless be assessed and managed appropriately.

DEVELOPMENTAL TRAJECTORY OF COGNITIVE AND BEHAVIORAL FEATURES IN TUBEROUS SCLEROSIS COMPLEX

Research data about the lifespan progression and outcome in tuberous sclerosis complex were recently reviewed by de Vries and Bolton.[25] The authors commented on the dearth of longitudinal data. A recent study showed that infants with tuberous sclerosis complex who show significant delays in early development relative to their peers without tuberous sclerosis complex remain delayed, and do not catch up later in development.[54] There are suggestions that more able children who do not start off behind their peers early in development can fall behind over time.[28] As highlighted earlier, individuals with profound global intellectual impairment often show developmental arrest and do not show any significant progression from a basic sensory-motor stage. In a follow-up of 23 adults with tuberous sclerosis complex from the age of 5 years to adulthood, little change was observed in the functional abilities of those with severe global intellectual impairment. Behaviorally, whereas symptoms of autism and hyperactivity decreased, the rates of aggressive behavior and self-injury remained unchanged.[55]

In the study of cognition and behavior in tuberous sclerosis complex, it will be important not to make false assump-

tions about the developmental trajectory based solely on studies of the end state, as seen in the study of adults with brain-related disorders. In the context of developmental plasticity, Dennis outlined a model for cumulative cognitive difficulties following early brain insult.[56] She argued that, owing to early disruption, more deficits would emerge over time and that the discrepancy in cognitive and functional abilities between a child with central nervous system abnormalities and their peers would increase. In tuberous sclerosis complex, speculatively, it is possible that the observed tendency for children to fall behind their peers over the course of development could reflect failure in the emergence, development, and establishment of frontal brain systems. Development of these systems is a prerequisite to increased independence in integrating and generalizing skills in the service of novel and complex problem solving.

It is important to note that tuberous sclerosis complex is not associated with inevitable intellectual or behavioral decline (in contrast to slowed gains), as believed at the start of the twentieth century.[13] Any evidence of regression, deterioration, or change should trigger a careful evaluation (see the section on interventions).

POSSIBLE DETERMINANTS OF COGNITIVE AND BEHAVIORAL PROBLEMS IN TUBEROUS SCLEROSIS COMPLEX

There are a number of different levels at which the potential determinants of neurodevelopmental difficulties in tuberous sclerosis complex are investigated. At the genetic level, there are suggestions that individuals with a *TSC2* mutation might be at greater risk of more severe cognitive and behavioral manifestations.[57–60] No specific mutation types have been shown to lead to specific neurodevelopmental manifestations. On the contrary, there is at least one report of nine individuals with the same *TSC* mutation who presented with global intellectual abilities ranging from profoundly impaired to above average.[60]

Progress in the understanding of the cell signaling pathways involving the *TSC* genes[61–64] might shed light on the cellular mechanisms associated with specific learning or behavioral problems in tuberous sclerosis complex. As studies on the psychoneurobiology of tuberous sclerosis complex detail those mechanisms, new avenues might be opened for psychopharmacologic and cognitive enhancers.

The role of structural abnormalities in cognition and behavior has received some focus.[65–69] At present, there is, however, no convincing evidence that tuber count and tuber load are useful markers of global cognitive abilities in tuberous sclerosis complex. Earlier suggestions that a tuber count over 6, for example, suggests severe global cognitive impairment are potentially clinically misleading. It is not uncommon to see children with 20 or more tubers who have normal intellectual abilities. Magnetic resonance imaging is an important assessment tool but should not be considered a substitute for thorough cognitive and behavioral assessment. There are no biomarkers of cognitive abilities or behavioral problems that are currently of predictive value in the clinic. Reports suggesting a complex interaction between temporal lobe tubers and infantile spasms as causal mechanisms of autism[30,70] are discussed in a separate article focusing on autism in tuberous sclerosis complex in this issue.[71]

There is no doubt that epilepsy is an important factor in relation to cognitive and behavioral features[72] and in relation to the developmental trajectory of children and adolescents with tuberous sclerosis complex.[73] Infantile spasms and severe intractable seizures are highly correlated with global intellectual impairment, but not inevitably so.[4,12,30] Although most individuals with profound global intellectual impairment have a history of seizures, many of those with a history of seizures have normal or above-average global intellectual abilities.[12] Absence seizures can impair a child's ability to learn and progress academically.[11,74] Partial seizures can manifest as disruptive, repetitive, or aggressive behaviors.[11] Nighttime behavioral disturbance and sleep disorders should, in particular, raise the possibility of seizures.[10,23] The side effects of antiepileptic drugs can hinder a child's ability to learn or can lead to behavioral disturbances.[11,75] Accurate identification and appropriate treatment of seizure disorders are therefore paramount. The management of epilepsy in tuberous sclerosis complex is discussed elsewhere in this issue.[4]

Taken together, there are a number of pathways to the psychopathologies and cognitive difficulties associated with tuberous sclerosis complex. From a clinical perspective, seizures and seizure control, as well as monitoring for the emergence of a subependymal giant cell astrocytoma,[1,2] require careful attention. It is, however, important to be mindful that neither seizures nor cortical tubers are necessary and/or sufficient to explain the cognitive and behavioral risks associated with tuberous sclerosis complex, nor are they predictive of individual outcomes.

INTERVENTIONS FOR NEURODEVELOPMENTAL DIFFICULTIES IN TUBEROUS SCLEROSIS COMPLEX

There are no specific or unique interventions for the cognitive or behavioral problems associated with tuberous sclerosis complex, and no studies have been performed in tuberous sclerosis complex to investigate the effectiveness or side effects of interventions used to address similar problems in individuals without tuberous sclerosis complex.

Assessment

The first step toward intervention is to perform comprehensive age-, and developmentally appropriate assessments at key timepoints. Consensus clinical guidelines have recently been drawn up by an international panel of psychiatrists, neuropsychologists, clinical psychologists, pediatricians, pediatric neurologists, special education experts, service users, and caregivers.[76] These guidelines included two main recommendations. First, the panel advocated

routine assessments in infancy, preschool, early school years, middle school years, adolescence, and adulthood. The purpose of these periodic assessments is to identify cognitive and behavioral problems as they emerge rather than to wait until severe clinical problems have become established. The second recommendation was to perform urgent assessments whenever evidence of regression in cognitive skills or a change in behavior occurs. A change in a child's behavior can sometimes be the only sign of a developing subependymal giant cell astrocytoma. Assessment should therefore include a comprehensive physical and neurologic review plus appropriate special investigations to identify the likely underlying causes of the cognitive or behavioral change.[76]

In the context of the multisystem nature of tuberous sclerosis complex, it is prudent to adopt a hierarchical approach to evaluating the potential causes of cognitive and behavioral difficulties.[25] Physical factors pertaining to the central nervous system, such as seizure control, change of seizure type, and the possibility of a subependymal giant cell astrocytoma, should be considered first. Non–central nervous system factors should next be examined, for example, renal failure, electrolyte disturbances, or the effect of medication. Next consider the role of an emerging developmental disorder or other psychiatric disorders. Finally, environmental factors, such as change, unpredictability, life events, or inconsistent management, can all play important roles, particularly when behavioral changes are observed.[25,76]

Multi-agency, Multidisciplinary Work

Once the cognitive and behavioral profile of the individual has been assessed, information should be integrated into an overall formulation of the needs of that person.

It will soon become apparent to clinicians who work with children, adolescents, and adults with tuberous sclerosis complex that their complex needs can rarely be assessed and managed by a single professional or discipline. Appropriate management will almost undoubtedly require multi-agency, multidisciplinary work, including educational, medical, therapeutic, and social service agencies.

Interventions as appropriate to non–tuberous sclerosis complex should be implemented. For instance, a child with an autism spectrum disorder should be linked with an appropriate early intervention program designed for children who have primary social communication disorders. Children with global or specific cognitive deficits should receive statutory assessment of their educational needs, and individual educational plans should be implemented. Many children might require regular follow-up by a psychologist, psychiatrist, or behavioral neurologist or pediatrician to monitor their behavior and educational progress. Clinical psychologists and/or learning disability specialists can play an extremely important role in performing functional analysis of behaviors and in the implementation of a behavior management or modification plan. These are often particularly useful for repetitive behaviors, self-injury, eating difficulties, and sleep disturbances. In some instances, psychopharmacologic interventions might be useful. It is, however, essential that psychotropic drugs be used with great care and consideration in the context of the multisystem nature of tuberous sclerosis complex.

Psychoeducation and Support

Cognitive and behavioral problems are often the most worrying of all of the tuberous sclerosis complex manifestations to parents and carers.[5] Clinicians should therefore not underestimate the impact of the disorder on families. Parents need clinicians to listen to their concerns, to identify their child's needs through careful assessment, and to build a therapeutic alliance with them. The needs of individuals with tuberous sclerosis complex will remain lifelong; therefore, families need clinicians who will be able to maintain a long-term relationship that affords consistent support and perspective.

Families with tuberous sclerosis complex are very fortunate to have the support of an excellent network of nonprofit organizations, such as the Tuberous Sclerosis Association (UK) and the Tuberous Sclerosis Alliance (USA). Both the Tuberous Sclerosis Association and the Tuberous Sclerosis Alliance provide excellent information and fact sheets through their Web sites (<www.tuberous-sclerosis.org> and <www.tsalliance.org>). An umbrella organization, Tuberous Sclerosis International, provides a very useful initial source of international contact details for professionals or families (<www.stsn.nl/tsi/tsi.htm>).

FUTURE DIRECTIONS

In contrast to the explosion in research related to the molecular genetics and biochemical pathways associated with tuberous sclerosis complex, very little is known about the cognitive and behavioral manifestations of the disorder. In broad terms, longitudinal studies of developmental trajectories, studies of the mechanisms that underlie cognitive and behavioral deficits, intervention studies, and further searches for biomarkers of cognitive and behavioral disorders are essential. Developmental models to explain the variability of expression and efforts to integrate the behavioral, cognitive, structural, and biochemical levels of investigation hold great promise but will require collaborative work.

Based on the current evidence regarding the patterns of strengths and weaknesses presented here, the findings point with remarkable consistency toward frontal systems involvement in tuberous sclerosis complex. The convergence of attentional-executive neuropsychologic deficits,[24,25,28,43–47] high rates of developmental disorders,[24,27,28,30] and replicated neuroradiologic findings that highlight fine-grain abnormalities in the thalamus, basal ganglia, and frontostriatal white matter[3] raises the possibility that the *TSC* genes, independent of the role of cortical tubers or subependymal nodules, might impair the functional development and integrity of the basal forebrain, thus leading to deficits in the emergence, development, and establishment of attentional-

executive skills, subserved by the frontostriatal networks.[77] These aberrant networks present with specific neuropsychologic deficits[78] associated with impaired development of widespread networks required for complex attention and executive control processes. These deficits, in turn, could be predicted to impede the development of social cognitive and metacognitive skills, thus contributing to the risk of developing a range of psychiatric disorders (including ADHD), behavioral disorders, and social communication disorders.[78]

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Special Article

Autism and Tuberous Sclerosis

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ABSTRACT

The co-occurrence of autism spectrum disorder and tuberous sclerosis complex has been recognized for decades. The prevalence of tuberous sclerosis complex in the autism spectrum disorder population is 1 to 4%, whereas features of autism spectrum disorder are present in 25 to 50% of individuals with tuberous sclerosis complex. The underlying reason for this association might be a nonspecific disruption of brain function owing to tuberous sclerosis complex, including tuber location, seizures and their effect on brain development, cognitive impairment, a disturbance in brain development in regions associated with autism spectrum disorder, or, less likely, a linkage between a *TSC* gene and an autism susceptibility gene. Awareness of the relationship between autism spectrum disorder and tuberous sclerosis complex is important during the evaluation of individuals with either disorder. Better delineation of the association and its causative factors is needed for the development of possible interventions. (*J Child Neurol* 2004;19:675–679).

The autism spectrum disorders encompass a group of developmental disorders with common core features. These include a significant qualitative impairment in socialization, a significant qualitative impairment in communication, and the presence of restricted interests and repetitive behaviors. The label of autistic disorder is given to those children who meet these core criteria and have onset prior to age 36 months. Less severely impaired children can be classified as either Asperger's syndrome when they have normal language development but impairment in the other areas or pervasive developmental disorder not otherwise specified when there is an impairment in socialization plus either an impairment in social communication or the presence of restricted interests and repetitive behaviors. Diagnosis at this time is based on *Diagnostic and Statistical Manual for Mental Disorders-IV* (*DSM-IV*) criteria.[1,2]

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WHAT IS THE RELATIONSHIP BETWEEN AUTISM SPECTRUM DISORDER AND TUBEROUS SCLEROSIS COMPLEX?

The co-occurrence of autism spectrum disorder and tuberous sclerosis complex has been recognized for over 50 years and might predate Kanner's initial description of autism in 1944.[3] Isolated case reports have described the presence of autistic features in children with tuberous sclerosis complex. However, many of these case reports, especially in the 1970s and 1980s, have limited applicability in the determination of the relationship between autism spectrum disorder and tuberous sclerosis complex because of failure to definitively confirm the diagnosis of tuberous sclerosis complex (especially in the absence of neuroimaging studies), the transient presence of features of autism spectrum disorder (which is not the expected natural history for autism spectrum disorder), and inadequate description of the core diagnostic features of either disorder or failure to use recognized diagnostic criteria, such as a validated rating scale or *DSM* criteria.[4–13] Therefore, demonstration of the increased risk of autism spectrum disorder within the tuberous sclerosis complex population awaited investigations targeting larger populations and the use of validated diagnostic tools.

Multiple authors have reported on the prevalence of tuberous sclerosis complex in autism spectrum disorder. An early report in 1963 by Creak described a prevalence of 1 child of 100; in 1967, Lotter reported 1 of 32 children. These findings are limited by failure of an accurate description of

an autistic disorder versus the presence of autistic mannerisms in children with significant mental retardation. However, since that time, reports by Smalley et al, Gillberg, and Fombonne et al have described a small number of individuals with tuberous sclerosis complex in autism spectrum disorder populations (with autism spectrum disorder diagnosis confirmed with validated scales). The reported prevalence is 1 to 4%, depending on the cohort and the presence or absence of associated medical conditions. This is much higher than the described frequency of 0.1 to 0.2% of autism spectrum disorder in earlier years and, more recently, 0.1 to 0.6%.[14–16]

Studies have also looked at the prevalence of autism spectrum disorder in the tuberous sclerosis complex population. Fisher et al reviewed data from the North Dakota pervasive development disorder and clinical genetics registry. They found that 4 of 12 children with tuberous sclerosis complex had autism spectrum disorder features, according to *DSM-III* criteria.[17] Hunt and Dennis used a medical records review and a self-developed questionnaire and found that 40 of 69 individuals in their tuberous sclerosis complex population had autistic behaviors. However, they did not specifically state whether these behaviors were of sufficient severity to meet the criteria of autism spectrum disorder.[18] More recently, studies have addressed this question using specific autism spectrum disorder rating scales or *DSM* criteria. Smalley et al used the Autism Behavior Checklist and *DSM-III-R* criteria and found that 7 of 24 individuals had autism.[19] In a later publication, Smalley et al used the Autism Diagnostic Interview and stated that 7 of 13 met *International Classification of Diseases-10* criteria.[14] Gillberg et al used a psychiatric interview, the Autism Behavior Checklist, and the Childhood Autism Rating Scale and identified 17 of 28 children with an autistic disorder and an additional 6 children with autistic-like features.[20] Baker et al used the Autism Behavior Checklist as a screening tool and the Autism Diagnostic Interview for diagnostic confirmation for a cohort of 20 respondents to the study (with 32 invited participants) and identified 4 of 9 children with autism and 12 children showing some features on the screen.[21] Gutierrez et al, using the Autism Diagnostic Interview and the Autism Diagnostic Observation Scale in 28 individuals, found that 8 met the diagnostic criteria for autism and 4 for pervasive development disorder not otherwise specified.[22]

Other studies have used different tools to identify the populations. Curatolo et al used a tuberous sclerosis complex behavioral questionnaire to identify 6 of 23 individuals with features of autism.[23] Using this questionnaire, Jambaqué et al found 2 of 13 with autistic behavior in a cohort treated with vigabatrin for infantile spasm or partial seizures. This questionnaire was not correlated with *DSM* criteria.[24] Hunt and Shepherd used a questionnaire (telephone or mail) and found that, of 21 children, 5 met *DSM-III-R* criteria for autism and an additional 4 met *DSM-III-R* criteria for pervasive development disorder not otherwise specified.[25] Ferguson et al. used a questionnaire format to identify 71 of 138 respondents showing features of social aloofness (interpreted as being an autistic behavior).[26] None of these studies used clinical examination criteria.

In summary, the frequency of autism in individuals with tuberous sclerosis complex in these and other studies is approximately 25%, with about 40 to 50% meeting criteria within the autism spectrum disorders. However, the information from these studies is limited by the sample population (clinic based or responding to a questionnaire), limitations in the type of diagnostic tool being used (whether or not based on *DSM* criteria), the threshold for the diagnosis of autism spectrum disorder, and a lack of consideration for the confounding effects of mental retardation and uncontrolled epilepsy (because the frequency of autistic features rises with an increasing severity of mental retardation and with the presence of an epileptic encephalopathy or uncontrolled epilepsy with frequent daily seizures). Overall, the limited surveys of populations of individuals with tuberous sclerosis complex find that the prevalence of autism spectrum disorder is increased in comparison with the general population.

When the population with autism spectrum disorder and tuberous sclerosis complex is evaluated, it is found that the male-to-female ratio is approximately equal, in contradistinction to the increased male prevalence in the general autism spectrum disorder population. However, even in the general population with autism spectrum disorder, the male-to-female ratio becomes more equal with lower IQ, especially below 50. In the population with combined autism spectrum disorder and tuberous sclerosis complex, a much larger percentage of these individuals show evidence of cognitive impairment, usually severe to profound in nature, in comparison with the overall tuberous sclerosis complex population. In addition, these individuals also have a history of a seizure disorder, especially infantile spasms in the first year of life, which might contribute to the cognitive impairment.[27]

WHY DO AUTISM SPECTRUM DISORDER AND TUBEROUS SCLEROSIS COMPLEX CO-OCCUR?

Several reasons have been proffered for the association between autism spectrum disorder and tuberous sclerosis complex. First, there can be a direct effect of the abnormal *TSC* gene on the development of brain regions that are dysfunctional in autism spectrum disorder. Second, nonspecific brain dysfunction owing to tuberous sclerosis complex, including cognitive impairment, seizures, and tuber location, can cause some individuals to have autism spectrum disorder. Lastly, there can be a linkage between the *TSC* gene and an autism spectrum disorder susceptibility gene.[27,28] There is no support for an association with events during the birth process.[29]

Evidence in support of potential linkage between a tuberous sclerosis complex gene and an autism spectrum disorder susceptibility gene is the genome scan finding that shows a putative location on chromosome 16 of an autism spectrum disorder susceptibility gene. This is in a region similar to that of the *TSC2* gene at chromosome 16p13.[30]

Although this linkage is possible, the equal male-to-female ratio, level of significant cognitive impairment, and frequent presence of poorly controlled seizures argue that the relationship is not usually, if ever, due to gene linkage.

Neurobiologic investigations have identified abnormalities of the frontal and temporal regions in individuals with autism spectrum disorder. Tuberin, the *TSC2* gene product, is highly expressed in these areas.[30] A study of individuals with tuberous sclerosis complex and autistic features showed abnormalities in the caudate, cerebellar nuclei, and lateral temporal gyri on positron emission tomographic studies. These findings have been interpreted as evidence of the neurodevelopmental deficits inherent in autism spectrum disorder.[31] This might provide an explanation for the presence of autism spectrum disorder in individuals with tuberous sclerosis complex and normal intelligence and no seizure disorder, although more data are required before a direct relationship is established.

The most likely reason for an association between autism spectrum disorder and tuberous sclerosis complex is a nonspecific consequence of the disruption of brain function in individuals with tuberous sclerosis complex. In those with both autism spectrum disorder and tuberous sclerosis complex, 75% have cognitive impairment, the majority in the severe to profound range. However, autism spectrum disorder features are frequently present in individuals with severe to profound mental retardation, irrespective of the etiology of the mental retardation.[27,30]

Similarly, 75 to 100% of individuals with autism spectrum disorder and tuberous sclerosis complex have a history of a seizure disorder. Seizure onset is usually within the first few years of life, including a frequent association with a prior occurrence of infantile spasms. The seizure focus is usually frontal or temporal, similar to findings described in individuals with autism and abnormal electroencephalograms (EEGs). The features of autism spectrum disorder can appear in association with the initial onset of seizures or fluctuate in severity or manifestation in conjunction with the presence or absence of seizure control. Therefore, the presence of features of autism spectrum disorder might be related to a functional disruption of brain processes owing to an underlying epileptic encephalopathy or, possibly, a disturbance of central nervous system organization owing to early and persistent seizure activity.[28,32–38]

Lastly, multiple studies have shown an association between localization of tubers and the presence or absence of autism spectrum disorder features (independent of the presence or absence of mental retardation). Most studies suggest the presence of tubers in the temporal lobe as a predisposing feature, whereas isolated studies have suggested an association with frontal lobe and posterior tubers or with the presence of tubers in the cerebellum. Curatolo et al described two populations: one with onset before age 2 years with parietal and temporal tubers and one with onset from 3 to 5 years with frontal and posterior tubers. More than 8 tubers were found in 5 of 6 children with tuberous sclerosis complex and autism spectrum disorder but in only 6 of 17 children without features of autism spectrum disorder.[23] Bolton and Griffiths localized tubers in individuals with autism spectrum disorder to the temporal lobes, with a greater number of tubers in those with both autism spectrum disorder and tuberous sclerosis complex.[39] In a later report of a larger series, they stated that risk factors for autism spectrum disorder were temporal lobe tubers associated with temporal lobe epileptiform activity, early onset, and ongoing spasms.[37] Seri et al also described temporal lobe localization in those with autism spectrum disorder but found no difference in the total number of tubers.[40] Jambaqué et al examined 23 children with tuberous sclerosis complex and identified 6 with autism spectrum disorder, mental retardation, seizures, and bifrontal and posterior tubers.[41] Weber et al evaluated data on 29 individuals with tuberous sclerosis complex and found a positive correlation between higher scores on the Childhood Autism Rating Scale and the number of cerebellar tubers.[42] Two studies have suggested that there is no relationship with any supratentorial tubers.[31,43] Overall, the evidence suggests a relationship with tuber localization, although interpretation of the data in some studies is limited by the use of computed tomography, which can underidentify the number of tubers, and the effect of intractable seizures on the features of autism spectrum disorder.

In summary, although no definitive reason has been determined for the association between autism spectrum disorder and tuberous sclerosis complex, it is most likely that autism spectrum disorder features in this population are present as a consequence of a nonspecific disruption of brain function related to the complications of tuberous sclerosis complex (including seizures, mental retardation, and tuber localization). Less likely, although possible, is abnormal brain organization owing to the adverse impact of a tuberous sclerosis complex gene, perhaps in conjunction with an autism spectrum disorder susceptibility gene. At present, there are no data to support a linkage between the *TSC* gene and an autism spectrum disorder susceptibility gene.

ASSESSMENT AND INTERVENTION

Because of the co-occurrence of autism spectrum disorder and tuberous sclerosis complex, evaluation of individuals with one disorder requires a heightened awareness for the other condition. All of those with autism spectrum disorder should be examined for clinical features of tuberous sclerosis complex, including the presence of skin lesions, and, if necessary, with the use of a Wood's lamp. Routine magnetic resonance imaging is not warranted unless there are clinical features, such as significant cognitive impairment, seizures, or skin lesions, to support this testing. In individuals with tuberous sclerosis complex, ongoing monitoring for features of autism spectrum disorder is warranted. In young children, one must be careful not to confuse autism spectrum disorder features with those of severe to profound cognitive impairment, because, with age, the latter population will manifest more appropriate social skills and a relative absence of repetitive behavior or restricted interests. In

the tuberous sclerosis complex population, the occurrence of an autistic regression should raise the question of an underlying seizure disorder and lead to an appropriate evaluation, including an EEG that includes sleep. Given that the features of autism spectrum disorder can wax and wane in relationship to the control of seizures, it might be more prudent to use the label of an epileptic encephalopathy rather than true autism spectrum disorder in this circumstance. Evaluators should remember that the clinical features of autism spectrum disorder can have different underpinnings in the tuberous sclerosis complex population in comparison with those individuals with "idiopathic" autism spectrum disorder.

Intervention for this population is dependent on cognitive level and, indirectly, on autism spectrum disorder features. All children require appropriate educational and behavioral programming, although it should be noted that the response to an autism spectrum disorder–based program might not be as robust in this population, especially those with more severe cognitive impairment, in comparison with the idiopathic autism spectrum disorder population. However, this type of programming might lead to an improvement in overall behavioral functioning.[44] Early and aggressive management of seizures can lessen a theoretical adverse impact on central nervous system function and organization and lessen the overall level of seizure-related cognitive impairment or autism spectrum disorder features. The presence of an unexplained regression should lead to an evaluation for a seizure disorder, central nervous system tumor or the occurrence of increased intracranial pressure or hydrocephalus owing to a giant cell astrocytoma.

Lastly, challenging and disruptive behaviors require appropriate medical and behavioral management. This includes identification of the reason for the behavior and implementation of necessary interventions. Evaluation and treatment should use strategies found to be effective in other populations with similar problems.[44, 45]

FUTURE DIRECTIONS

Despite many publications on autism spectrum disorder and tuberous sclerosis complex, our knowledge about the relationship is still limited. Areas for future investigations include the following:

1. Better definition of the neurobiologic basis for autism spectrum disorder in tuberous sclerosis complex, including the use of functional neuroimaging; determination of the exact relationship between tuber localization, disruption of function, and development of autism spectrum disorder; the relationship between autism spectrum disorder and epilepsy in the tuberous sclerosis complex population; the impact of the neurodevelopmental effects of the abnormal *TSC* genes on brain development and autism spectrum disorder; and the relative contributions of the type of *TSC* gene (*TSC1* or *TSC2*) toward an autism spectrum disorder predisposition
2. Epidemiologic studies of tuberous sclerosis complex cohorts more representative of the community tuberous sclerosis complex population, including segregation into *TSC1* and *TSC2* groupings, to provide more meaningful prevalence data
3. Use of well-defined autism spectrum disorder screening and diagnostic instruments for identification of affected individuals to allow comparison of data between different tuberous sclerosis complex populations
4. Differentiation between those with autism spectrum disorder owing to a functional brain impairment caused by difficult to control epilepsy, which can remit with successful seizure management, and those with autism spectrum disorder related to a structural abnormality, which will persist over time, although the intensity of clinical features might lessen with age
5. Determination of the efficacy of recommended interventional strategies for idiopathic autism spectrum disorder in children with tuberous sclerosis complex and autism spectrum disorder and the relationship of treatment response to other factors, such as intelligence and seizure frequency
6. Exploration of the possibility of the existence of subgroups within the combined autism spectrum disorder and tuberous sclerosis complex population that have independent or interrelated causes of autism spectrum disorder, such as the level of cognitive impairment, age at onset and type of seizure, tuber localization, and close proximity of the *TSC* gene to an autism spectrum disorder susceptibility gene

Studies to date have begun to define the relationship between autism spectrum disorder and tuberous sclerosis complex. Future work should better delineate these features and their underpinnings and define the types of intervention that will be most effective.

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Special Article

Managing Epilepsy in Tuberous Sclerosis Complex

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ABSTRACT

Epilepsy is very common in tuberous sclerosis complex and occurs in 80 to 90% of affected individuals during their lifetime. Onset usually occurs during childhood, and up to one third of children with tuberous sclerosis complex will develop infantile spasms. Although not completely understood, the incidence of epilepsy is thought to relate to the neuropathologic features of the disorder, including cortical tubers and other dysgenetic features. Individuals with tuberous sclerosis complex frequently have epileptiform features to their electroencephalograms. Treatment of epilepsy in tuberous sclerosis complex is similar to epilepsy resulting from other causes and includes anticonvulsant medications, the vagus nerve stimulator, and the ketogenic diet. Vigabatrin has been shown to be particularly effective in treating infantile spasms in the setting of tuberous sclerosis complex. Epilepsy surgery has a very important role in the management of children and adults with pharmacoresistant epilepsy in tuberous sclerosis complex. (*J Child Neurol* 2004;19:680–686).

Tuberous sclerosis complex is a multisystem genetic disorder of variable phenotypic expression, with an incidence of about 1 in 5800 live births worldwide.[1] The disorder results from a mutation in the *TSC1* gene in chromosomal region 9q34 or the *TSC2* gene in chromosomal region 16p13 and is inherited in an autosomal dominant fashion, although up to two thirds of cases result from spontaneous genetic mutation.[2,3] The major neurologic manifestations of tuberous sclerosis complex are seizures, autism, developmental delays, including mental retardation, and behavioral and psychiatric disorders.

Epilepsy is the most common presenting symptom in tuberous sclerosis complex and is also the most common medical disorder in tuberous sclerosis complex. Up to 80 to 90% of individuals with tuberous sclerosis complex will develop epilepsy during their lifetime,[4] with onset typically in childhood. The majority of children with tuberous sclerosis complex have onset of seizures during the first year of life, and up to one third of children with tuberous sclerosis complex will develop infantile spasms.

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Almost all seizure types can be seen in a child with tuberous sclerosis complex, including tonic, clonic, tonic-clonic, atonic, myoclonic, atypical absence, partial, and complex partial. Only "pure" absence seizures are not observed. Seizures that appear generalized, both clinically and by electroencephalographic (EEG) characteristics, can have partial onset in tuberous sclerosis complex and therefore might respond to anticonvulsant medications indicated for partial-onset seizures. If such seizures prove difficult to control with anticonvulsant medications and other medical therapies, seizure foci can potentially be identified by neurophysiologic and neuroimaging techniques, making epilepsy surgery a possible treatment.

RELATIONSHIP OF EPILEPSY TO NEUROANATOMIC FEATURES

Epilepsy in tuberous sclerosis complex is thought to relate to the presence of cortical tubers and other neuropathologic features, although the relationship is not well understood. Cortical tubers consist of dysplastic neurons and giant cells, as well as glial components, and it is hypothesized that abnormal activity in these cells leads to epileptogenesis. Although the molecular mechanisms of epileptogenesis are unknown, abnormalities in glutamatergic and γ-aminobutyric acid (GABA) receptor subunits have been identified in cortical tuber samples,[5] and abnormal glutamatergic transport in astrocytes has been observed in mouse models of tuberous sclerosis complex.[6] Several

studies have characterized the neurophysiologic activity of cortical tubers at the time of epilepsy surgery, with some studies finding cortical tubers to be electrically silent but others finding frequent epileptiform activity associated with the tuber or region around the tuber.[7,8]

Given the range of seizure types and severity in individuals with tuberous sclerosis complex, several investigators have tried to relate seizure activity to anatomic correlates. Cusmai et al found a correspondence between topographic magnetic resonance imaging (MRI) and EEG in 26 of 32 patients with tuberous sclerosis complex studied; all patients had large cortical tubers on MRI.[9] EEG foci without corresponding cortical tubers were identified in 4 of the patients; large cortical tubers without corresponding EEG foci were also observed in 11 of the patients, mainly involving the frontal lobes. Curatolo et al found that the age at which seizures and epileptiform activity develop was related to the location of the cortical tubers identified on MRI, with an earlier expression for temporo-occipital regions than for frontal ones.[10] Goodman et al performed a meta-analysis of the published literature to address the question if the tuber number correlated with the severity of the epilepsy or the age at seizure onset.[11] They compared five independent studies, which showed that the cortical tuber count, as identified by MRI, was higher in individuals with tuberous sclerosis complex with severe disease. They found that the MRI-detected tuber count was six times more likely to be above the median count in patients with severe central nervous system involvement (defined as poor seizure control, moderate mental retardation, or both). However, studies have also shown that up to 10% of individuals with tuberous sclerosis complex with intractable epilepsy have normal brain MRIs, with no evidence of cortical tubers or other dysgenetic features.

EEG CHARACTERISTICS OF TUBEROUS SCLEROSIS COMPLEX

Several investigators have characterized the EEG patterns in individuals with tuberous sclerosis complex. Westmoreland examined EEG findings from 361 patients with tuberous sclerosis complex ranging in age from 2 days to 63 years.[12] A high incidence of abnormalities was identified, with 78% of the patients having epileptiform features. Approximately 12% of the population had a normal EEG, and 10% had slow-wave abnormalities. Of those with epileptiform features, 35% had focal spike or sharp-wave discharges, 33% had multifocal epileptiform discharges, 22% had hypsarrhythmia, and 10% had apparent generalized spike-and-wave discharges. A similar study of 60 patients with tuberous sclerosis complex evaluating over 320 EEG recordings found the most common abnormality to be diffuse slowing, seen in 84% of the recordings. Slow spike-and-wave discharges were seen in 42%, focal spikes in 16%, multifocal spikes in 16%, and normal findings in 8%.[13]

TREATMENT OF EPILEPSY IN TUBEROUS SCLEROSIS COMPLEX

Treatment of seizures in tuberous sclerosis complex is similar to that of epilepsy from other causes, and anticonvulsant medications are the mainstay of treatment. Increasing experience with the newer anticonvulsant medications, especially in the pediatric population, is being obtained in the treatment of epilepsy owing to tuberous sclerosis complex and other causes. As discussed below, vigabatrin has been shown to be particularly effective in treating infantile spasms owing to tuberous sclerosis complex. There have also been a few reports suggesting the efficacy of certain anticonvulsant drugs in the treatment of other seizure types related to tuberous sclerosis complex. Topiramate, lamotrigine, oxcarbazepine, and levetiracetam have all been found to be effective and well tolerated in small populations of individuals with tuberous sclerosis complex and epilepsy.[14–17] Owing to the small size of these studies, it is not yet possible to identify certain anticonvulsant drugs as "drugs of choice" in seizures related to tuberous sclerosis complex other than infantile spasms.

Unfortunately, many children and adults with tuberous sclerosis complex develop seizure disorders that prove to be pharmacoresistant, which is not surprising given the hypothesis that epilepsy results from the associated cortical dysgenesis. One mechanism of refractory epilepsy in tuberous sclerosis complex likely relates to cellular mechanisms of drug resistance because both multidrug resistance transporters *MDR1* and multidrug resistance–associated protein 1 have been shown to be expressed in some cortical tubers.[18]

Alternative treatments to anticonvulsant medications should be considered in patients with tuberous sclerosis complex when seizures cannot be effectively controlled. Current nonpharmacologic treatments include the vagus nerve stimulator, the ketogenic diet, and resective epilepsy surgery.

Vagus Nerve Stimulation

The vagus nerve stimulator was approved in 1997 by the US Food and Drug Administration (FDA) for use in individuals 12 years and older with intractable partial-onset epilepsy; subsequently, over 20,000 stimulators have been implanted worldwide. The only published study assessing the efficacy of the vagus nerve stimulator in tuberous sclerosis complex was by Parain et al,[19] who conducted an open-label retrospective multicenter study evaluating the efficacy of the vagus nerve stimulator in patients with tuberous sclerosis complex. The study involved the review of all children with tuberous sclerosis complex who had the vagus nerve stimulator implanted and operational for at least 6 months in five pediatric epilepsy centers and compared the data with controls with epilepsy from other causes obtained from the Cyberonics vagus nerve stimulator patient registry and other published series of epilepsy surgery. They identified 10 patients with tuberous sclerosis

complex with the vagus nerve stimulator; 9 of the patients had a greater than 50% reduction in seizure frequency, and 5 of these experienced a > 90% reduction in seizures. They concluded that the vagus nerve stimulator is effective in promoting seizure control in tuberous sclerosis complex, although the outcomes were not as positive as those obtained in the published series of epilepsy surgery in tuberous sclerosis complex.

Ketogenic Diet

Another very effective nonpharmacologic treatment for pharmacoresistant epilepsy is the ketogenic diet, which has had widespread clinical use over the past 80 years, particularly in pediatric epilepsy. Based on observations that starvation resulted in improved seizure control, the ketogenic diet was developed to mimic starvation and change the body's main energy source from carbohydrates to fats. Over 20 retrospective and prospective studies have been published evaluating the clinical efficacy of the ketogenic diet in children over the past 80 years, including 4 publications in the past 4 years. All of these studies have shown the ketogenic diet to be an effective treatment for medically intractable epilepsy in childhood and for various seizure types and etiologies.[20] Unfortunately, there are no published reports specifically addressing the efficacy of the ketogenic diet in individuals with tuberous sclerosis complex. In our experience, the ketogenic diet can be an effective treatment for intractable epilepsy in the setting of tuberous sclerosis complex, with efficacies similar to those in seizures from other etiologies.

Resective Epilepsy Surgery

The role of resective epilepsy surgery in tuberous sclerosis complex has been somewhat controversial, owing largely to the fact that many patients have multiple cortical tubers, not a single identifiable epileptogenic "lesion." In addition, it can often prove very difficult and not possible to lateralize or localize the seizure onset in many individuals with tuberous sclerosis complex using conventional presurgical evaluative techniques such as continuous video-EEG monitoring or MRI. EEG monitoring often reveals multifocal epileptiform abnormalities; seizure onset is often difficult to correlate with a discrete EEG change or is associated with apparent generalized or bilateral and multifocal abnormalities. However, multiple clinical series have shown that resective epilepsy surgery is often associated with significant improvement in children and adults with tuberous sclerosis complex, including seizure freedom.[8,21–27] Several investigators have been exploring the utility of other functional and metabolic neuroimaging and neurophysiologic modalities, such as diffusion-weighted MRI, positron emission tomography, single-photon emission computed tomography, and magnetoencephalography, to identify the "epileptogenic tuber" or epileptogenic zone in patients with tuberous sclerosis complex.[8,28–35]

EPILEPSY IN TUBEROUS SCLEROSIS COMPLEX: INFANTILE SPASMS

Approximately one third of children with tuberous sclerosis complex will develop infantile spasms, although some reports suggest an incidence as high as 75%.[36–40] Tuberous sclerosis complex is thought to be the most common single cause of infantile spasms, and in some series, 25% of symptomatic infantile spasms are secondary to tuberous sclerosis complex. Similar to other reports,[40] in our experience, there is a strong association between increasing cortical tuber count and the presence of infantile spasms.

The clinical aspects of infantile spasms in tuberous sclerosis complex are similar in many respects to infantile spasms from other causes. The age at onset of infantile spasms in tuberous sclerosis complex peaks between the fourth and sixth month of life, although onset can occur as early as the second month of life.[41] Partial onset seizures precede infantile spasms in approximately one third of patients with tuberous sclerosis complex who develop infantile spasms.[39,42] Clinically, the spasms themselves can appear to be more subtle than classic flexor or extensor spasms and can appear to be asymmetric, similar to infantile spasms seen in other situations with cortical dysgenesis. Infantile spasms in tuberous sclerosis complex can be flexor, extensor, or mixed and can have lateralizing features such as head turning, tonic eye deviation, and asymmetric or unilateral involvement of extremities. Similarly to infantile spasms from other etiologies, the baby often develops an "indifference" to her or his environment and parents and a change in personality, with increased irritability either coincident to or preceding the onset of infantile spasms. Parents often observe a plateau or even regression in developmental abilities correlating to the onset of infantile spasms and the appearance of this "indifference." There is a strong association between the presence of infantile spasms in tuberous sclerosis complex and subsequent developmental impairment, as discussed below, although children with tuberous sclerosis complex and infantile spasms can have a normal cognitive outcome.[43]

The EEG in infantile spasms owing to tuberous sclerosis complex often shows hypsarrhythmia or modified hypsarrhythmia. However, it is important to realize that the EEG, although typically abnormal, frequently does not have the features of hypsarrhythmia, and in some series, up to 70% of children with tuberous sclerosis complex and infantile spasms do not have the characteristics of hypsarrhythmia.[39] In addition, it should be remembered that hypsarrhythmia is an awake interictal pattern and that even in the presence of hypsarrhythmia, the EEG can appear to be close to normal during rapid eye movement (REM) sleep. Therefore, careful attention must be paid in interpreting the clinical features and the EEG characteristics of an infant with paroxysmal movements, particularly if tuberous sclerosis complex is known or suspected. Other features that can be observed

on EEG in the setting of infantile spasms and tuberous sclerosis complex include one or more foci with spikes activated in slow-wave sleep into diffuse discharges. Ictal correlates are characterized at onset by a focal discharge of spikes and polyspikes originating from the temporal, rolandic, or occipital regions followed by a generalized slow wave and then an abrupt generalized desynchronization in EEG background activities that last up to several seconds.[44]

INFANTILE SPASMS IN TUBEROUS SCLEROSIS COMPLEX: ROLE OF VIGABATRIN

There is convincing evidence in the medical literature that vigabatrin, an irreversible GABA transaminase inhibitor, should be the drug of choice in the treatment of infantile spasms owing to tuberous sclerosis complex. Particularly because vigabatrin has not been approved for clinical use by the FDA in the United States, other treatments can also be used if vigabatrin is not available or proves to be ineffective, including corticotropin (ACTH) and the ketogenic diet, as well as other anticonvulsant medications, such as valproate, topiramate, or zonisamide. If infantile spasms in tuberous sclerosis complex prove to be intractable to medical treatment, then epilepsy surgery should also be considered if an epileptogenic region can be identified.

Several reports in the literature have addressed the efficacy of vigabatrin in infantile spasms, many specifically focusing on tuberous sclerosis complex.[45–49] Hancock and Osborne reviewed 10 published studies assessing the efficacy of vigabatrin in infantile spasms.[45] With vigabatrin treatment, the studies showed a 54% cessation in infantile spasms in 313 children without tuberous sclerosis complex, but in 77 children with tuberous sclerosis complex, there was a 95% complete cessation of infantile spasms. They concluded their review by suggesting that vigabatrin should be offered as first-line therapy in infantile spasms. Mackay et al performed an evidence-based analysis of the established medical treatment regimen for infantile spasms.[47] They surmised a natural history of infantile spasms from clinical studies of infantile spasms in the precorticosteroid era and compared different medical regimens by assessing treatment trials of infantile spasms from 1958 to the present. Evidence of the efficacy of various treatment regimens was defined as class I, II, or III evidence according to established criteria. They concluded that class I and class III evidence existed in published studies, which supported the standard of practice recommendation for the use of vigabatrin in the treatment of infantile spasms with tuberous sclerosis complex.

Chiron et al performed a randomized trial comparing vigabatrin and hydrocortisone in infantile spasms owing to tuberous sclerosis complex.[48] The study design involved a prospective randomized multicenter study using both drugs as monotherapy in patients newly diagnosed with infantile spasms. In their study, all 11 vigabatrin-treated patients became seizure free. Vigabatrin as an initial therapy for infantile spasms was investigated by a European retrospective study in evaluating 250 infants diagnosed with infantile spasms.[49] In this group, there was a 96% response rate in infantile spasms owing to tuberous sclerosis complex, which reflected the most positive response.

Unfortunately, although the efficacy of vigabatrin has been well established in the literature, its use has been limited by many practitioners, in part owing to possible ophthalmic toxicity of the medication. Currently, it is appreciated that ophthalmologic toxicity does occur in a population of individuals exposed to vigabatrin; however, the incidence of such toxicity is unclear, and it is unknown if there are certain risk factors for toxicity, including the relationship to the dose and the relationship to the length of exposure. It is also uncertain if such toxicity is reversible or irreversible following discontinuation of vigabatrin. The electroretinogram is the most sensitive measure of vigabatrin toxicity; if toxicity is present, the full-field electroretinogram shows a reduction of the 30 Hz flicker cone b-wave amplitude.[50–53] These findings are thought to suggest that vigabatrin might have an effect on the inner electroretinal function at the level of the Müller cell. Kinetic perimetry can also be performed in individuals able to cooperate in the study and can show concentric visual field defects in the presence of vigabatrin ophthalmologic toxicity. The electro-oculogram studies have also suggested an effect of vigabatrin on outer retinal function, which might be reversible after vigabatrin treatment is discontinued.[51] In the presence of presumed vigabatrin ophthalmologic toxicity, there is typically a normal appearance to the fundus and macula, although there have been some reports of retinal pigmentary changes.

If vigabatrin is used for infantile spasms, dosing is typically between 100 and 200 mg/kg/day, titrated up over several days. The duration of treatment is typically 1 year, although many clinicians are investigating the efficacy and tolerability of shorter treatment periods owing to the risk of ophthalmologic toxicity. Many physicians suggest that children on vigabatrin be followed by electroretinograms every 6 months to 1 year throughout the treatment course. In many patients, partial seizures can persist following control of infantile spasms by vigabatrin; often combination therapy with other anticonvulsant drugs can be effective in controlling other seizure types.

IMPACT OF INFANTILE SPASMS AND EPILEPSY ON COGNITION IN TUBEROUS SCLEROSIS COMPLEX

Epilepsy, particularly with onset during infancy, and infantile spasms are thought to be risk factors for subsequent neurocognitive impairments and mental retardation in children with tuberous sclerosis complex.[54,55] Joinson et al performed detailed psychometric evaluations on 108 patients with tuberous sclerosis complex and found a bimodal distribution of cognition, with 40% of the population having severe or profound learning disabilities.[56] The remainder of the population was found to function within the normal range

of cognition. Risk factors for more significant cognitive impairment included early-onset epilepsy, particularly infantile spasms. Studies have also shown a higher incidence of autism in children with tuberous sclerosis complex who have early-onset intractable epilepsy and infantile spasms.[57,58] Bolton et al found that increased risk of autism was associated with features of the child's epilepsy, including the presence of temporal lobe epileptiform discharges on EEG, younger age at onset of seizure activity, and a history of infantile spasms.[57]

However, several studies have also shown that children with tuberous sclerosis complex who have early-onset epilepsy and infantile spasms can have a normal cognitive outcome if the seizures are effectively controlled.[40,59–61] Vigabatrin treatment has been shown to result in improved cognitive outcome following infantile spasms in patients with tuberous sclerosis complex, likely reflecting a positive impact of effective seizure treatment.[61] Therefore, it is likely that early and effective control of infantile spasms and other seizure activity can improve developmental outcomes in children with tuberous sclerosis complex.

MANAGING EPILEPSY IN TUBEROUS SCLEROSIS COMPLEX: WHAT WE DO NOT KNOW BUT WOULD LIKE TO KNOW

Epilepsy is very common in individuals with tuberous sclerosis complex and, of course, represents a very significant problem because the seizure activity can also profoundly impact neurocognitive development in children with tuberous sclerosis complex. Although substantial efforts have been made to characterize epilepsy in tuberous sclerosis complex, many questions are left unanswered. Why do individuals with tuberous sclerosis complex have seizures? Although the majority of individuals with tuberous sclerosis complex do experience seizures during their lifetime, a significant population of individuals with tuberous sclerosis complex do not. Some individuals with tuberous sclerosis complex become seizure free and are eventually tapered off medications; others develop highly intractable seizure disorders that are not controlled by anticonvulsant medications, nonpharmacologic treatments, or epilepsy surgery.

The factors that influence the incidence and intractability of epilepsy in tuberous sclerosis complex are poorly understood. Although cortical tubers are thought to somehow be involved in epileptogenicity, it is unclear if all tubers are potentially epileptogenic because many individuals with tuberous sclerosis complex will have multiple cortical tubers but only one region of epileptogenicity. In addition, it is unclear if seizures are produced by the dysgenetic cells of the tuber or if instead (or in addition) these cells are somehow "irritating" to their neighboring neurons. And given that some individuals with tuberous sclerosis complex never experience seizures but have multiple cortical tubers, are there factors that put certain individuals with tuberous sclerosis complex at higher risk of seizures? In addition, do individuals with tuberous sclerosis complex have one or many epileptogenic tubers, and do all of the tubers have epileptogenic potential?

Clinically, there are also several unanswered questions regarding epilepsy in tuberous sclerosis complex. Is it possible to predict pharmacoresistance or intractability sooner in the course of the disease to allow earlier implementation of nonpharmacologic treatment or epilepsy surgery? Is there a role for prophylactic management? Epilepsy occurs in up to 90% of individuals with tuberous sclerosis complex; would it therefore be beneficial to treat individuals once diagnosed with tuberous sclerosis complex, regardless of whether they have a history of clinical seizure activity? Would it be beneficial to treat all infants diagnosed with tuberous sclerosis complex with vigabatrin because roughly one third of them will develop infantile spasms? Why are infantile spasms so common in this population, and why do some children with tuberous sclerosis complex and infantile spasms have a good neurocognitive outcome?

In summary, epilepsy is the most common medical disorder in tuberous sclerosis complex. It is particularly a major issue in childhood because the majority of individuals with tuberous sclerosis complex have seizure onset during the first year of life. Infantile spasms are extremely common in tuberous sclerosis complex; therefore, it could be argued that every child with tuberous sclerosis complex who has onset of paroxysmal movements during infancy has infantile spasms until proven otherwise. In addition, it could be argued that every child with infantile spasms has tuberous sclerosis complex until proven otherwise, given that it is the most common etiology of infantile spasms. Importantly, it should be realized that children with tuberous sclerosis complex can have a good cognitive outcome regardless of epilepsy, even in the presence of infantile spasms.

There are probably several different mechanisms of epileptogenesis in tuberous sclerosis complex, some related to tubers and cortical dysgenesis and possibly others that are not. Efforts to understand epilepsy in tuberous sclerosis complex will broaden and enhance our understanding of the pathophysiology of epilepsy in general.

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Special Article

Epilepsy Surgery for Children With Tuberous Sclerosis Complex

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ABSTRACT

Tuberous sclerosis complex is associated with medically refractory seizures and developmental delay in children. These epilepsies are often resistant to antiepileptic drugs, can be quite severe, and usually have a negative impact on the child's neurologic and cognitive development. It is believed that functional outcome is improved if seizures can be controlled at an early age. The surgical treatment of intractable epilepsy in children and adults with tuberous sclerosis complex has gained significant interest in recent years. Previously published studies have shown a potential benefit from resection of single tubers, with most of the results noted in relatively older children. All of these reports support the idea that if a single primary epileptogenic tuber or region can be identified, then a surgical approach is appropriate. However, most children with tuberous sclerosis complex have multiple potentially epileptogenic tubers, rendering localization challenging, and they are therefore rejected as possible surgical candidates. We have used a novel surgical approach using invasive intracranial monitoring, which is typically multistaged and bilateral. This multistage surgical approach has been useful in identifying both primary and secondary epileptogenic zones in patients with tuberous sclerosis complex with multiple tubers. Multiple or bilateral seizure foci are not necessarily a contraindication to surgery in selected patients. Long-term follow-up will determine whether this approach has durable effects. We await better methods for identifying the epileptogenic zone, both noninvasive and invasive. (*J Child Neurol* 2004;19:687–689).

Tuberous sclerosis complex is associated with medically refractory seizures and developmental delay in children. These seizures are often resistant to antiepileptic drugs, can be quite severe, and usually have a negative impact on the child's neurologic and cognitive development. Over the past few years, it has become apparent that some of these patients might be candidates for epilepsy surgery if it is possible to determine a single main epileptogenic tuber.[1–10] However, most of the epilepsies associated with tuberous sclerosis complex are multicentric.[8] Nevertheless, the surgical treatment of intractable epilepsy in children and adults with tuberous sclerosis complex has gained significant interest in recent years. Several international clinical centers have reported their individual results of operating on patients with tuberous sclerosis complex with medically refractory seizures. However, the current literature is limited by small sample sizes and variability of data collection methodology and analysis. The overall findings from these studies suggest that patients benefit from excisional surgery if seizures can be localized to a single tuber, which conceptually concurs with the epilepsy surgery literature in general.

The first report of successful epilepsy surgery in a patient with tuberous sclerosis complex came from the Montreal Neurological Institute in 1966.[11] A number of subsequent reports demonstrated good short- and long-term seizure outcome in approximately 50 to 60% of drug-resistant patients selected for surgical management.[8] Approximately 58 patients have been reported in the literature. In general, these studies have included relatively older children, adolescents, and adults. There has been no discussion in the literature on the management of multifocal partial seizures owing to multiple tubers. Lastly, there has been little reliance on invasive

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monitoring, with most patients undergoing surgery based on scalp electroencephalogram (EEG) recordings.

All of the reported series demonstrate that when a single seizure focus, generally corresponding to a single tuber, can be identified, the results are good. Bye et al reported the resection of right frontal cortex and two associated tubers in a 5-year-old child with 10 to 20 seizures per day, based on scalp EEG. In the year following surgery, the child experienced two brief seizures.[1] Bebin et al identified the focus of seizure activity in nine patients (mean age 11 years) to correspond with the prominent neuroimaging abnormality in all cases, despite the fact that one third had multifocal interictal scalp epileptiform EEG activity.[2] Two patients underwent cortical resection and seven underwent stereotactic lesionectomy. Six patients were seizure free at follow-up, and only one patient eventually failed surgery.[2] Baumgartner et al were the first to use subdural monitoring, in two of their four patients (mean age 9.3 years).[3] Clinical and EEG data suggested an epileptic focus near a prominent lesion in each case. Two patients were seizure free at follow-up. Avellino et al reported 11 patients with tuberous sclerosis complex (mean age 19.6 years), in whom they altered their surgical approach based on whether EEG abnormalities were focal versus multifocal or generalized.[4] The latter group was evaluated with subdural grid or strip monitoring, whereas the others were studied with scalp EEG and intraoperative electrocorticography. Five patients underwent awake craniotomy. Six of the 11 patients were seizure free at follow-up. Guerreiro et al stratified patients to resection if they had a well-localized epileptogenic lesion or to corpus callosotomy if they did not.[5] Twelve patients (mean age 16.9 years) underwent resection, with excellent results in seven patients. They observed the best outcome in patients who had focal seizures and good imaging and EEG correlation, although they might have multiple seizure types, other imaging abnormalities, and multifocal or generalized EEG findings.[5] Koh et al evaluated the role of noninvasive imaging to localize the epileptogenic tuber or region and the outcome of focal resection.[6] Thirteen patients underwent resection, six guided by subdural monitoring. The epileptogenic tuber or region corresponded to a large discrete tuber in eight patients and a calcified tuber in 13 patients. Nine of the 13 were seizure free at the 26-month follow-up. Karenfort et al identified a main epileptogenic tuber in all eight patients who underwent surgical resection, with good correlation between neuroimaging and EEG.[8] Three of the eight were seizure free at follow-up. They observed that seizure outcome in their group was good but not excellent. They postulated that seizures persisting after surgery can be due to epileptogenicity from other nonresected tubers or nontuber epileptogenic areas.[8] They cited our strategy of multifocal, staged resection as an alternative approach but nevertheless felt that invasive monitoring would not have improved their outcome beyond that achieved with scalp EEG.[8] In concordance with this body of literature, unifocal onset seizures and mild to no developmental delay at the time of surgery were recently found to be predictive of excellent long-term outcome by Jarrar and colleagues.[12]

It is believed that functional outcome is improved if seizures can be controlled at an early age.[1–10] The previously published studies have shown a potential benefit from resection of single tubers, with most of the results noted in relatively older children. All of these reports support the idea that if a single primary epileptogenic tuber or region can be identified, then a surgical approach is appropriate.[1–6,8,11] However, the major dilemma in this field is that most children with tuberous sclerosis complex have multiple potentially epileptogenic tubers, rendering localization challenging, and they are therefore rejected as possible surgical candidates.

In fact, patients with tuberous sclerosis complex have the worst theoretical prognosis for successful epilepsy surgery owing to several factors. The epileptogenic tubers are often extratemporal, multifocal, and bilateral and can overlap with eloquent cortex. Intracranial electrode monitoring is also limited by the presence of secondary epileptogenic foci, secondary epileptogenic foci that can be unmasked by resection of the primary focus, and the fact that the margins of the primary epileptogenic zone can overlap with functional cortex.

We have used a novel surgical approach using invasive intracranial monitoring, which is typically multistaged and bilateral.[7,9,10,13] Unlike the strategies noted in the previously reported surgical series, in which patients without clear focal EEG abnormalities are usually offered a more palliative intervention, our approach is uniform. All patients are considered for invasive monitoring, based on the rationale that patients with tuberous sclerosis complex have partial epilepsy. Despite the fact that this partial epilepsy can be multifocal, if the primary and secondary foci can be identified with a multistage invasive approach, then surgery can be quite effective, even in the face of multifocal partial epilepsy.

In the typical epilepsy surgery approach, the initial operative stage, which consists of electrode placement to map seizure onset and function, is followed by surgical resection of the seizure focus at the second stage. In multistage surgery, electrodes are replaced at the second stage, following resection of the primary seizure focus, to determine whether a second epileptogenic region will need to be resected at the third and final operative stage. Multistage invasive monitoring can detect residual adjacent or distal epileptogenesis and is especially useful when the presurgical data suggest eloquent cortex or bihemispheric involvement.

We reported successful three-stage bilateral resection in a 2-year-old boy with bilateral epileptogenic tubers with excellent seizure and developmental outcome.[7] A child presented with tuberous sclerosis complex and severe epilepsy beginning in the first month of life and delayed development in the first year of life.[7] Video-EEG monitoring at the age of 1 year revealed a left temporal seizure focus associated with one of several tubers. Repeat video-EEG at age 2 years revealed a right posterior quadrant seizure focus associated with a second tuber. His seizures were poorly controlled on several antiepileptic medications. Because of the significant

seizure burden, as well as marked developmental delay, he was evaluated for epilepsy surgery at the New York University Comprehensive Epilepsy Center. The presurgical evaluation revealed multifocal, bilateral seizure foci, which were more significant on the right side than on the left. After an extensive consideration of the risk-benefit profile and discussions with the family, an operative approach was undertaken at age 2 years.

Bilateral subdural electrodes were placed with a right temporoparietal craniotomy for grid, strip, and depth electrode placement and a left temporal bur hole for strip placement. Intracranial recording confirmed independent seizure onsets from the right parietal tuber region and prominent interictal activity from the left anterior tuber region. The right parietal focus was resected, and electrodes were maintained over the left temporal focus. After right parietal resection, ictal discharges were recorded over the left temporal region. A resection was performed 2 days later. At 5-year follow-up, both complex partial and tonic-clonic seizures have been eliminated, with simple partial seizures eliminated by more than 80%. He has also experienced marked cognitive and developmental improvement.

In selected patients with bilateral tuber seizure foci, aggressive bilateral surgery can be safe and effective. This multistage surgical approach has been useful in identifying both primary and secondary epileptogenic zones in patients with tuberous sclerosis complex with multiple tubers.[9] To date, we have used this approach on 21 young children with tuberous sclerosis complex, nearly 70% of whom were seizure free at follow-up, with nearly all significantly improved. Multiple or bilateral seizure foci are not necessarily a contraindication to surgery in selected patients with tuberous sclerosis complex. Long-term follow-up will determine whether this approach has durable effects. Nevertheless, a significant reduction in seizure burden during a critical developmental epoch is likely to have significant benefits, even if seizures recur. We await better methods for identifying the epileptogenic zone, both noninvasive and invasive.

The long-term benefits of surgery with respect to seizure control; cognitive, social, and behavioral development; academic or employment status; and quality of life are not known. These must be weighed against the risks of epilepsy surgery and the natural history of tuberous sclerosis complex. For this reason, we have undertaken coordinating a multicenter, retrospective analysis of surgery outcome in patients with tuberous sclerosis complex and medically refractory seizures. Several international centers will provide data that will be analyzed retrospectively to determine any significant predictors of surgical outcome and to assess the overall therapeutic efficacy of surgery. This study will serve as the basis for a prospective study, which will test the hypothesis that early surgery for medically refractory epilepsy improves long-term seizure control and developmental outcome in children with tuberous sclerosis complex.

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Special Article

Non-Neurologic Manifestations of Tuberous Sclerosis Complex

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ABSTRACT

Tuberous sclerosis complex is a heterogeneous genetic disorder affecting multiple organ systems at various points throughout the lifespan. Increasingly, neurologically asymptomatic adults are being diagnosed with tuberous sclerosis complex through renal or pulmonary presentations. Although chiefly recognized for its cerebral manifestations, understanding of the non-neurologic aspects of the disorder has advanced greatly in recent years, and they are reviewed here. (*J Child Neurol* 2004;19:690–698).

As awareness and appreciation of the divergent manifestations of tuberous sclerosis complex grow, so does the awareness of the non-neurologic manifestations of this disorder. Even in 2004, tuberous sclerosis complex is still, to the majority of physicians, Vogt's classic triad of intractable epilepsy, mental retardation, and facial angiofibromas. Nonetheless, most physicians are chiefly aware of the neurologic manifestations of tuberous sclerosis complex, if they are aware of the disease at all. As appreciation of this complex and heterogeneous disorder grows and its molecular basis is increasingly understood, the need for recognition and appropriate management of other non-neurologic aspects of the disease becomes even more important. Of all of the medical specialties, the pediatric neurologist is the most likely to have knowledge of and experience with diagnosis and treatment of individuals with tuberous sclerosis complex. The non-neurologic manifestations of the disease often present in adulthood. By definition, they are also beyond the usual scope of practice of the pediatric neurologist. Nonetheless, as the medical specialist most likely to be familiar with the disorder, it is important for the neurologist to have an appreciation of the manifold aspects of tuberous sclerosis complex because he or she is increasingly likely to be involved in their recognition and management. The goal of this article is to review crucial aspects of the non-neurologic manifestations of tuberous sclerosis complex and to provide the clinician with a working knowledge of their characteristics and management. Dermatologic manifestations are not discussed.

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RENAL MANIFESTATIONS

After neurologic manifestations, renal lesions are the most common cause of morbidity and mortality in tuberous sclerosis complex. The kidneys are also one of the most frequently involved organs. Two types of renal involvement are recognized. Polycystic kidney disease occurs in 3 to 5% of individuals with tuberous sclerosis complex. When present, this reflects a continuous gene syndrome because the adult polycystic kidney disease gene is adjacent to the *TSC2*-tuberin gene on chromosome 16. A genetic abnormality that affects both *TSC2* and *APKD* gene function will result in patients having a phenotype of both of these diseases.[1] Individuals with tuberous sclerosis complex who have polycystic kidney disease might therefore be assumed to have a genetic deficit of the *TSC2* gene/tuberin. Polycystic kidney disease is distinct from single or multiple renal cysts, which occur much more commonly in tuberous sclerosis complex (Figure 1). In polycystic kidney disease, innumerable cysts are present, which largely replace the renal parenchyma. Patients develop progressive renal insufficiency and hypertension and, ultimately, require renal transplant. Because persons with polycystic kidney disease have so few functioning renal parenchyma, they are highly susceptible to intercurrent processes, such as kidney stones or infections. An acute urinary tract infection or renal stone can

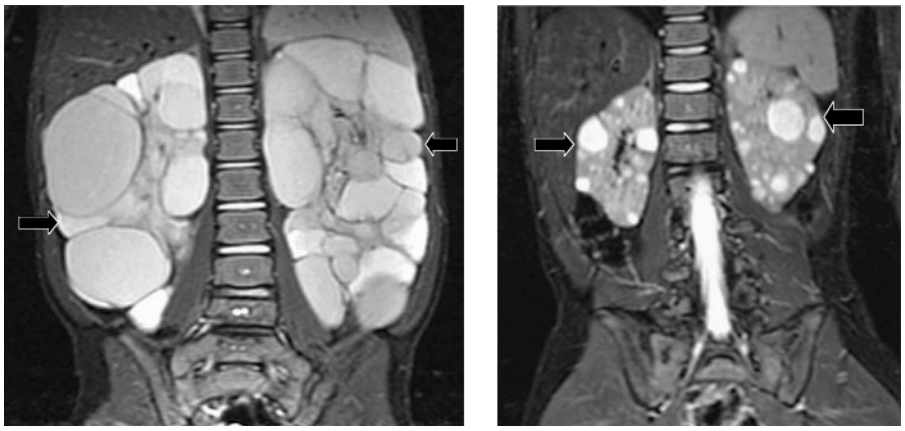

Figure 1. Polycystic kidney disease versus multiple simple renal cysts. T_2-weighted magnetic resonance images from patients with tuberous sclerosis complex. The image on the left shows massively enlarged kidneys with virtually complete replacement of renal parenchyma by cysts; the image on the right shows that multiple but much smaller cysts are present, with intervening normal parenchyma.

provoke a sudden deterioration in renal function or even kidney failure. This should be borne in mind, for example, when using medication that can increase the risk of renal stones, such as topiramate. Isolated renal cysts can occur in tuberous sclerosis complex. These can be associated with angiomyolipomas. They can be single or multiple, but they do not completely replace functioning renal parenchyma or result in renal failure. Polycystic kidney disease tends to present in infancy and childhood, whereas simple renal cysts tend to develop with increasing patient age. Simple renal cysts are typically asymptomatic, although, rarely, they can be painful or have associated symptoms, usually mild hemorrhage.

Angiomyolipomas are present in up to 80% of individuals with tuberous sclerosis complex.[2] Although most commonly seen in the kidney, angiomyolipomas can also occur in other abdominal organs, such as the liver and pancreas, and sometimes within the lymphatic system. Angiomyolipomas have even been reported within the oral cavity.[3] Extrarenal angiomyolipomas tend to be less vascular and less prone to hemorrhage. They typically do not produce clinical symptoms unless there is true obstruction of the biliary tract or adjacent organs or vascular structures. The prevalence in the general population is uncertain. Fujji et al reported a prevalence of 24 in 18,000 individuals who did not have other signs of tuberous sclerosis complex.[4] Renal angiomyolipomas are therefore highly correlated with the diagnosis of tuberous sclerosis complex. Among individuals with tuberous sclerosis complex, the lesions appear to affect male and female patients with equal frequency. They are most commonly bilateral. They typically present in childhood, adolescence, or early adult life. However, there have been reports of angiomyolipomas at the time of premature birth and in young infants. As the name suggests, they consist of variable composition of dysplastic blood vessels, smooth muscle cells, and fat. These tumors are felt to arise from a common renal progenitor cell, which has inherited a somatic mutation of one of the tuberous sclerosis complex genes. This progenitor cell then suffers a "second hit" at the unaffected allele, causing loss of heterozygosity and dysfunction of both alleles of the tuberous sclerosis complex gene. This cell then gives rise to daughter cells, which result in the angiomyolipoma.[5–7] Angiomyolipomas are highly variable with regard to the amount of blood vessels, smooth muscles, and fat cells of which they are composed. This variability is observed from patient to patient and even within different lesions within the same patient. Angiomyolipomas can occur in any location within the kidney. They are sometimes seen as multiple discrete lesions; in other cases, they can be poorly defined and diffusely infiltrate the renal parenchyma (Figure 2). They produce symptoms in a variety of ways. As the lesions become progressively larger, particularly those with a diameter greater than 6 cm, they are associated with a potentially fatal retroperitoneal hemorrhage (Wunderlich's syndrome) (Figure 3). This occurs when dysplastic aneurysmal blood vessels associated with an angiomyolipoma rupture.[8–10] Diffusely infiltrating angiomyolipomas can, over time, gradually replace normal renal parenchyma and cause hypertension and chronic renal insufficiency. Angiomyolipomas located near the renal

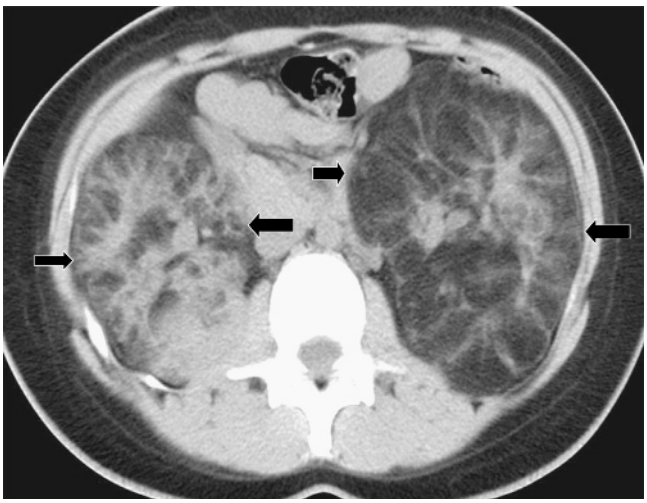

Figure 2. Bilateral diffusely infiltrating angiomyolipomas (*arrows*) identified incidentally following presentation with a pneumothorax from lymphangioleiomyomatosis. The patient had normal renal function and no symptoms from these lesions.

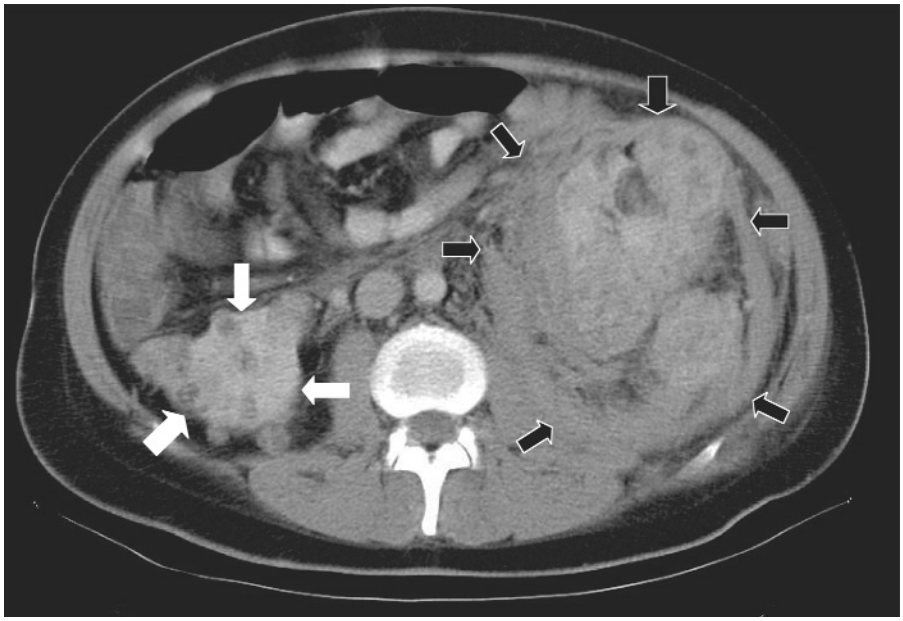

Figure 3. Retroperitoneal hemorrhage from angiomyolipoma, abdominal computed tomographic scan. *Black arrows* show the left kidney with infiltrating angiomyolipoma and surrounded by blood delimited by Gerota's fascia. *White arrows* show the right kidney with multiple small angiomyolipomas.

medulla can result in ureteropelvic junction obstruction and obstructive nephropathy. Finally, large angiomyolipomas can compress or impinge on other abdominal structures (Figure 4).

National Institutes of Health consensus conference guidelines call for periodic screening for renal lesions in individuals with tuberous sclerosis complex.[11,12] This is to identify the presence of angiomyolipomas or polycystic kidney disease to allow for intervention prior to the development of serious complications, such as renal failure or retroperitoneal hemorrhage.

When an angiomyolipoma is identified on renal ultrasonography, the following management strategy is recommended: If a single lesion or multiple small lesions are noted (ie, maximum lesion dimension less than 4 cm), patients are followed with serial renal ultrasonography. If lesions are identified that are greater than 4 to 6 cm in maximum dimension or are felt possibly to have abnormal vasculature on Doppler ultrasonography, magnetic resonance imaging (MRI) with magnetic resonance angiography is undertaken.[13,14] Magnetic resonance angiography allows screening for and identification of aneurysmal blood vessels. The presence of dysplastic vasculature indicates a greater risk of hemorrhage, particularly with progressive increases in the size of the angiomyolipoma. Magnetic resonance angiography also allows a preliminary assessment of suitability for embolization. Following MRI, a determination is made, based on a specific patient's lesion size; the presence or absence of dysplastic vasculature, symptoms, and renal function; whether to proceed to standard angiography; and embolization. Our own practice has been to embolize lesions greater than 4 to 6 cm in maximum dimension with suitable vascular dysplasia, based on series than such lesions are more likely to continue to grow and become symptomatic. When feasible, selective embolization is the preferred method of treatment. Embolization, when performed by skilled practitioners, is typically safe and well tolerated (Figures 5 and 6). Postembolization complications can occur as the result of necrosis of angiomyolipoma tissue. These symptoms include fever, malaise, chills, nausea and vomiting, and flank pain. They can be effectively managed through corticosteroid pretreatment.[15] In addition to avoidance of operative morbidity, embolization often better allows for preservation of normal intervening renal parenchyma. "Routine" postoperative care can be highly complex and fraught with difficulty in individuals who are autistic or cognitively impaired. In patients in whom aneurysmal dilatation or dysplastic vessels cannot be identified, the angiomyolipomas and renal function are followed clinically. Open

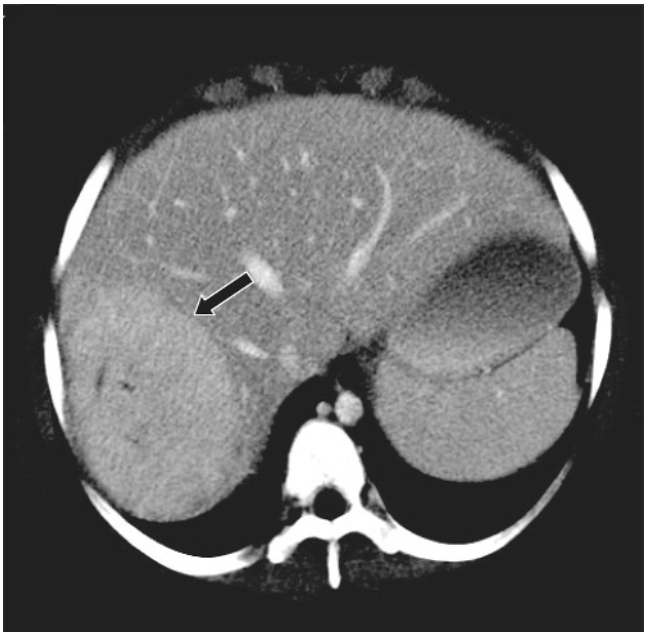

Figure 4. Hepatic angiomyolipoma (*arrow*). These lesions are typically asymptomatic.

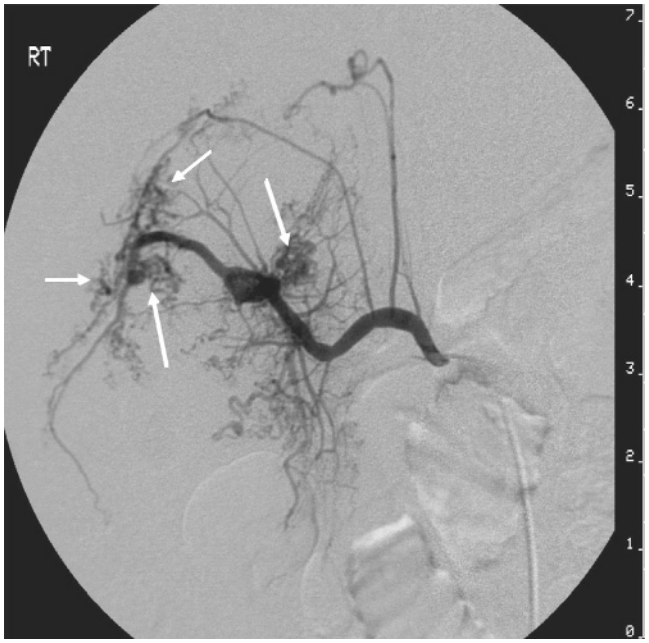

Figure 5. Pre-embolization renal artery angiogram: multiple dysplastic vessels within an angiomyolipoma (*arrows*).

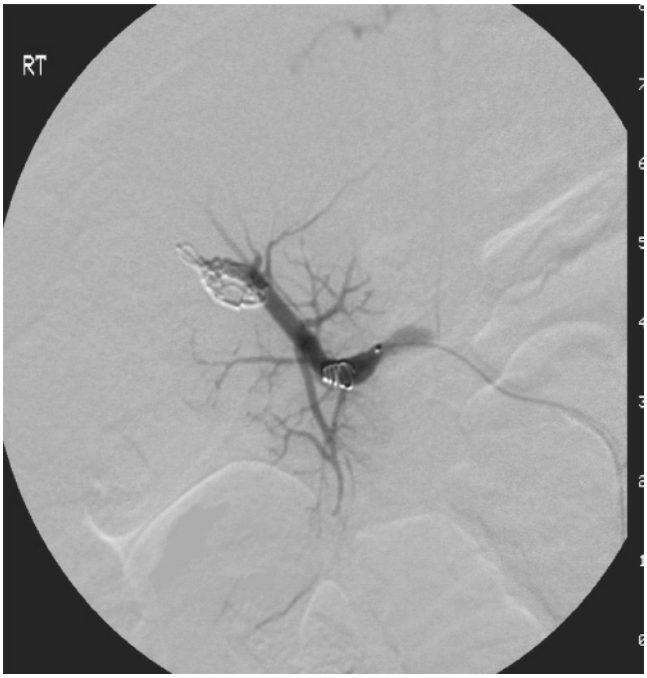

Figure 6. Renal artery angiogram following placement of detachable coils demonstrating occlusion of dysplastic vessels.

surgical intervention might be required for cases of ureteropelvic junction or if other obstructive symptoms are present or in rare instances in which malignant transformation is suspected. Clinical trials are ongoing at our center to assess the effectiveness of the drug rapamycin for therapy of renal angiomyolipomas. Experimental evidence suggests that rapamycin and other agents which inhibit its target (the mammalian target of rapamycin or mTOR) might be able to stabilize or induce regression of these lesions. These agents offer significant promise for therapy of these lesions.[16]

When particularly large renal lesions or those that contain relatively little fat are identified, concern is often raised for the possibility of underlying malignant transformation. Newer computed tomographic (CT) techniques might be helpful in distinguishing angiomyolipomas with little fat from more aggressive lesions.[17] The exact incidence of malignant transformation of renal angiomyolipoma in tuberous sclerosis complex is uncertain. A variety of malignant renal tumors have been described in patients with tuberous sclerosis complex, including epithelial angiomyolipomas, leiomyosarcoma, and renal cell carcinoma. Although there have been multiple case reports of malignant renal tumors in tuberous sclerosis complex, Tello et al, in 1998, could not identify an increased risk of renal cell carcinoma by meta-analysis of reports in the literature.[18] Although the risks of renal malignancy could well be increased in tuberous sclerosis complex, overall, the risk is felt to be very low. This is yet another indication for periodic renal imaging in patients with tuberous sclerosis complex with renal lesions. Given that angiomyolipomas are frequently bilateral and can be very large and impressive on imaging, and many clinicians are only beginning to implement National Institutes of Health clinical consensus guidelines for renal imaging, many patients with tuberous sclerosis complex are only now having their renal lesions identified. The identification of large, often very impressive renal lesions does not necessarily warrant immediate surgical or other intervention. The clinician must bear in mind that the overwhelming majority of tuberous sclerosis complex lesions do not become malignant and that the primary morbidity results from retroperitoneal hemorrhage, hypertension, or chronic renal insufficiency rather than malignant transformation. My colleagues and I have encountered many individuals with tuberous sclerosis complex who, at an advanced age, were receiving their first renal imaging since infancy or childhood. Angiomyolipomas can be quite difficult to resect and often result in significant hemorrhage and loss of the kidney. When renal lesions are identified, it is preferable to perform serial follow-up imaging with ultrasonography or MRI and magnetic resonance angiography to fully characterize an angiomyolipoma and the rate at which it might be growing rather than perform what might be an unnecessary surgical procedure. Patients with larger angiomyolipomas also have a significantly higher incidence of pulmonary involvement or lymphangioleiomyomatosis. Screening high-resolution chest CT should be performed if not already undertaken. Conversely, increasing numbers of adults with normal intelligence and infrequent seizures are presenting later in life with angiomyolipomas as the primary manifestation of their tuberous sclerosis complex. Tuberous sclerosis complex should be strongly considered in any individual who presents with renal lesions consistent with angiomyolipomas regardless of previous neurologic symptoms or a lack thereof.

PULMONARY MANIFESTATIONS

Three types of lung involvement are noted in tuberous sclerosis complex. Lymphangioleiomyomatosis (LAM) is a progressive and often fatal disease that occurs almost exclusively in young women. It is characterized by smooth muscle cell infiltration and cystic destruction of lung tissue, resulting in progressive pulmonary insufficiency (Figures 7 and 8). Although there have been case reports of men with tuberous sclerosis complex who have lymphangioleiomyomatosis, the overwhelming majority of affected individuals are women. The reason for this is not known but has been presumed to reflect hormonal factors and has led to the empiric use of therapies such as progesterone.[19–22]

Individuals with tuberous sclerosis complex are also subject to a condition called multifocal micronodular pneumocyte hyperplasia. This is a benign hypertrophy of type 2 pneumocytes into multiple focal nodules. It is not associated with pulmonary symptoms but can appear radiographically as multifocal nodules on chest CT scans. Individuals with tuberous sclerosis complex can also develop clear cell or "sugar" tumors of the lung. These are typically benign lesions, which do not produce symptoms except through focal bronchial obstruction. Micronodular pneumocyte hyperplasia occurs with equal frequency in men and women with tuberous sclerosis complex. These conditions are chiefly noteworthy to allow their recognition as a cause of multifocal nodules or other abnormalities on chest imaging in individuals with tuberous sclerosis complex, which might otherwise be interpreted as evidence of pulmonary malignancy or another disorder.

Lymphangioleiomyomatosis occurs most commonly in patients who have tuberous sclerosis complex. It also occurs much less commonly in individuals who do not have tuberous sclerosis complex (sporadic lymphangioleiomyomatosis). Approximately 40% of women with tuberous sclerosis complex (birth incidence of 1 in 6000) develop radiographic changes consistent with lymphangioleiomyomatosis.[23,24] Interestingly, for reasons that are unclear, not all of these women develop progressive pulmonary disease. By contrast, the incidence of sporadic lymphangioleiomyomatosis is estimated at approximately 5 per million.[23,24] Women with sporadic lymphangioleiomyomatosis can have other types of tumors, such as lymphangiomyomas, which can also serve as a source of metastatic cells to the lung. Full-blown lymphangioleiomyomatosis is associated with cystic pulmonary change in 100% of cases, 70% of affected individuals suffer pneumothoraces, and approximately one third suffer chylous complications, such as chylothorax.[25] In tuberous sclerosis complex, lymphangioleiomyomatosis is invariably associated with angiomyolipomas, typically of the kidney, although potentially of other locations. In women with tuberous sclerosis complex, lymphangioleiomyomatosis angiomyolipomas tend to be significantly larger than in women with sporadic lymphangioleiomyomatosis. Interestingly, approximately 50 to 60% of women with sporadic lymphangioleiomyomatosis also have angiomyolipomas.[25] Again, these are most commonly located in the kidney but can be present in the liver or elsewhere in the abdomen. Tuberous sclerosis complex mutations have been identified in women with sporadic lymphangioleiomyomatosis, suggesting that at least some of these individuals might be somatic mosaics for tuberous sclerosis complex and that these disorders likely share a common pathophysiology.[26] Women with lymphangioleiomyomatosis typically present with clinical symptoms in their early to midthirties. The rate of progression is variable, with 5- to 9-year survival rates being quoted in various studies as between 40 and 80%.[19,25]

The observation that lymphangioleiomyomatosis can recur following pulmonary transplant for the disorder led to the hypothesis that the disease might result from a circulating factor or metastasis of foreign cells into the lung. It has subsequently been shown that, at least in some women with lymphangioleiomyomatosis, cells from the individual's

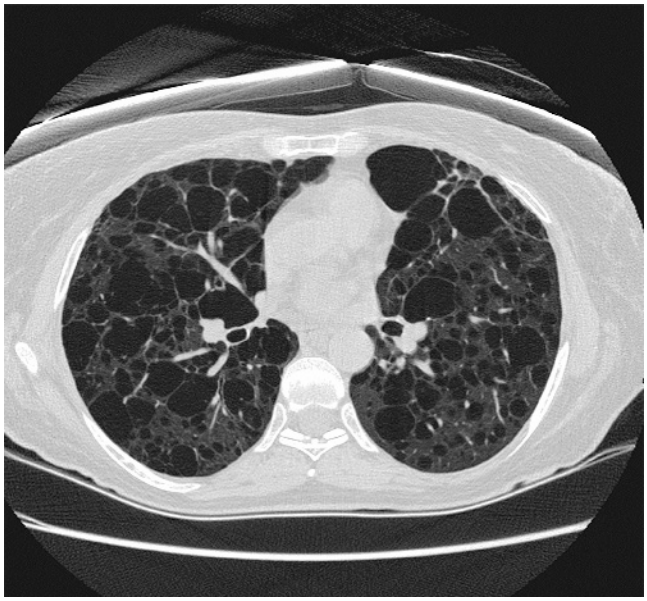

Figure 7. End-stage cystic lung disease in a woman with tuberous sclerosis complex and lymphangioleiomyomatosis.

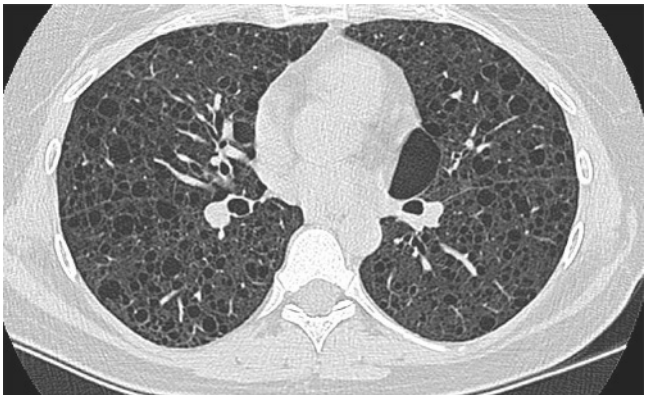

Figure 8. Chest computed tomographic scan showing diffuse cystic change in a patient with lymphangioleiomyomatosis.

angiomyolipomas take up residence in the lung and cause smooth muscle hyperplasia and cystic degeneration. This is consonant with the observation that women with the largest angiomyolipomas tend to have the highest likelihood of having pulmonary involvement, suggesting that larger lesions are more biologically active and more likely to metastasize.[27–29]

Current National Institutes of Health consensus conference guidelines recommend performance of screening high-resolution chest CT in all adult women with tuberous sclerosis complex. Clinicians should have a higher index of suspicion for the disorder in women with large angiomyolipomas.[10,11,23] There is currently no specific therapy for lymphangioleiomyomatosis. Once it is identified, women should be counseled to avoid risk factors such as estrogen exposure and cigarette smoking. They should also be counseled as to the symptoms of chyloptysis, pneumothorax, and progressive dyspnea and instructed to seek medical attention should these develop. General supportive measures such as good pulmonary toilet, immunization, and avoidance of infection are important. Patients should be referred to a pulmonologist knowledgeable in lymphangioleiomyomatosis or interstitial lung disease. A significant number of women with lymphangioleiomyomatosis have a component of bronchial reactivity and might benefit from β-adrenergic agonists; a trial of one of these agents is often warranted. Based on the observation that lymphangioleiomyomatosis occurs almost exclusively in women, antiestrogen therapies and high-dose progesterone have been employed on an anecdotal basis. There is no convincing medical evidence to suggest that hormonal treatments are effective. Again, a trial of rapamycin for angiomyolipomas associated with both sporadic and tuberous sclerosis complex lymphangioleiomyomatosis is ongoing at our center. As more is understood about this disorder, there is considerable hope that agents, if they can be demonstrated to have an inhibitory effect on angiomyolipomas, might theoretically also be of therapeutic benefit for lymphangioleiomyomatosis.

CARDIAC MANIFESTATIONS

Cardiac lesions are present in approximately 50% of all individuals with tuberous sclerosis complex.[30,31] In contrast to lymphangioleiomyomatosis and angiomyolipomas, which typically become symptomatic later in life, cardiac rhabdomyomas are of maximal size and clinical symptomatology during intrauterine life or early infancy (Figures 9 and 10). Although rhabdomyomas can occur in the absence of tuberous sclerosis complex, this is uncommon. Approximately 70 to 80% of individuals with cardiac rhabdomyomas will subsequently be identified as having the full clinical phenotype of tuberous sclerosis complex.[31] Rhabdomyomas are the most commonly identified cardiac tumor at any age. They typically appear between 22 and 28 weeks' gestational age. Histologically, the lesions consist of abnormal cardiac myocytes, which contain large amounts of glycogen and on hematoxylin and eosin staining have a

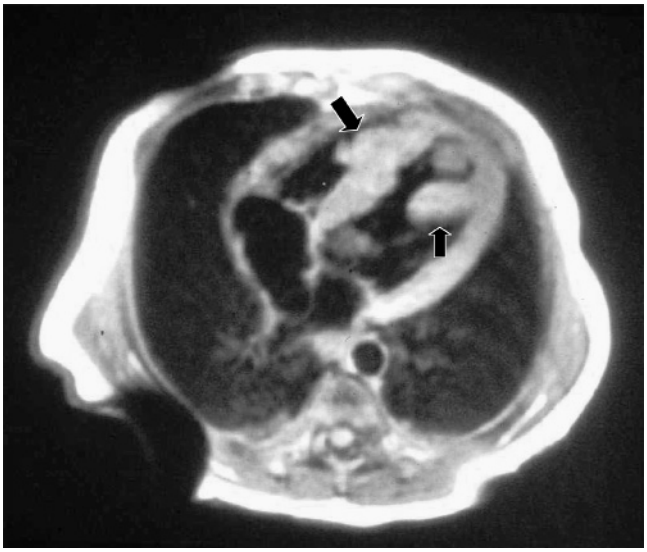

Figure 9. Magnetic resonance image showing cardiac rhabdomyomas infiltrating the ventricular septum and protruding into the right ventricle (*arrows*).

"chickenwire" appearance. These lesions can produce symptoms in a variety of ways. They can be associated with cardiac arrhythmias, most commonly supraventricular tachycardias. Ventricular rhabdomyomas can potentially increase the risk of ventricular tachyarrhythmias, which can be much more immediately life threatening than supraventricular tachyarrhythmias. This could be the explanation for a sudden unexplained death in individuals with tuberous sclerosis complex, as well as in individuals in whom no other cause can be found, in cases not felt to be attributable to intercurrent epilepsy, and in cases in which other causes are not found. For this reason, it is recommended that individuals with tuberous sclerosis complex who have cardiac rhabdomyomas undergo a screening electrocardiogram (ECG) to identify defects and ventricular

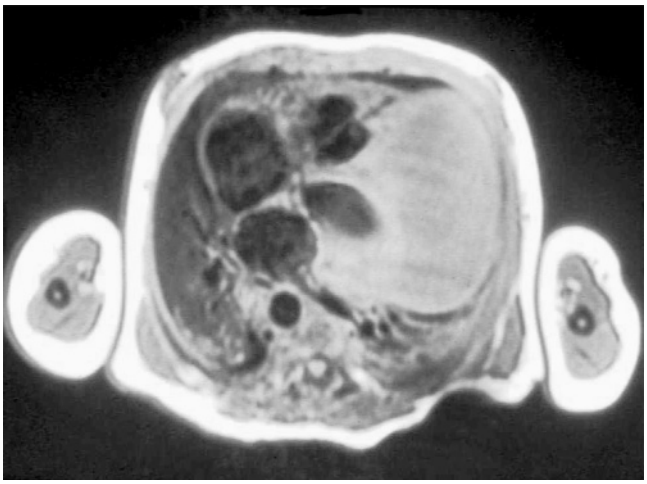

Figure 10. Magnetic resonance image showing a massive cardiac rhabdomyoma diffusely infiltrating both ventricles. The patient was born with fetal hydrops but now has normal cardiac function.

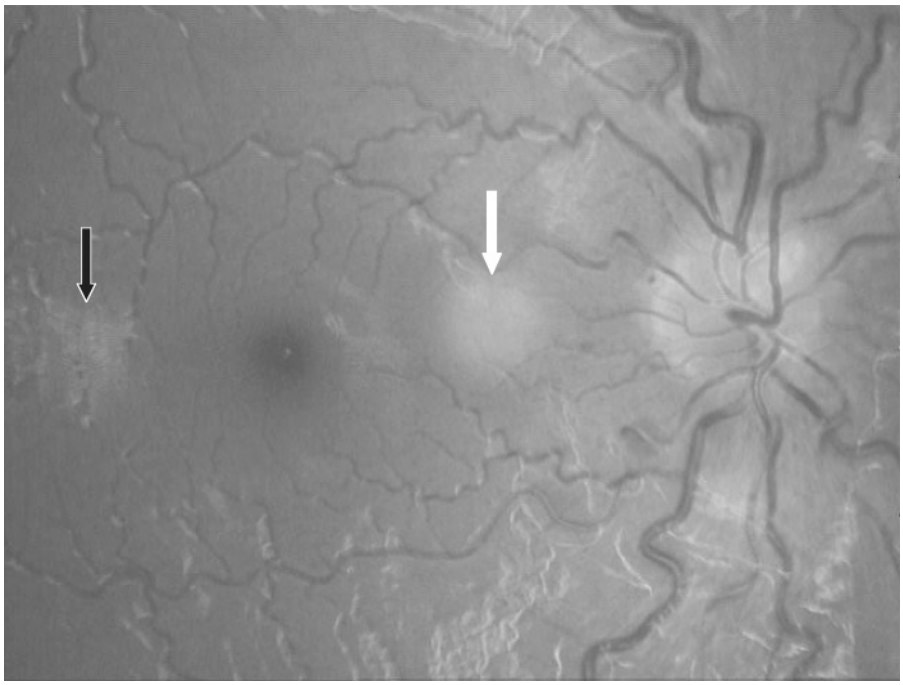

Figure 11. Retinal achromic patch (*white arrow*) and astrocytic hamartoma (*black arrow*) in a patient with tuberous sclerosis complex.

repolarization or other abnormalities. Signal averaged ECG might be better suited to the identification of ventricular repolarization defects than standard ECG techniques. The tendency for cardiac rhabdomyomas to spontaneously resolve should be emphasized. Patients with rhabdomyomas should not be exposed to inappropriate surgery or endomyocardial biopsy, even in the case of extremely dramatic lesions. Intrauterine supraventricular tachycardia can result in cardiac failure and nonimmune hydrops. Alternatively, rhabdomyomas can extend into the ventricle, where they can interfere with valvular function or block the ventricular outflow tracts, again producing obstruction and cardiac failure. Finally, rhabdomyomas can diffusely infiltrate the myocardium, resulting in a dilated cardiomyopathy, again potentially resulting in nonimmune fetal hydrops. Uncommonly, intraventricular rhabdomyomas can serve as a nidus for embolism with a resultant infarction. Although their appearance can be quite dramatic radiographically, cardiac rhabdomyomas invariably regress spontaneously. The nature of treatment for the lesions is primarily supportive. In rare cases, surgical intervention might be necessary, chiefly where critical valvular obstruction is present. Because of their frequent association with tuberous sclerosis complex, identification of cardiac rhabdomyoma mandates a thorough search for other signs of tuberous sclerosis complex, both in the affected individual and in first-degree relatives.[30,32,33]

OPHTHALMIC MANIFESTATIONS

Retinal hamartomas are present in approximately 40 to 50% of individuals with tuberous sclerosis complex.[34] These lesions are typically asymptomatic and are useful primarily in confirming the diagnosis. Three types of lesions have been described: multinodule "mulberry" lesions, punched-out areas of retinal hypopigmentation analogous to ash-leaf macules of the skin, and angiofibromas of the eyelids (Figure 11). Unless they involve the macula or optic nerve, these lesions are typically asymptomatic. Baseline evaluation by an ophthalmologist is recommended, with follow-up as clinically indicated.[34, 35]

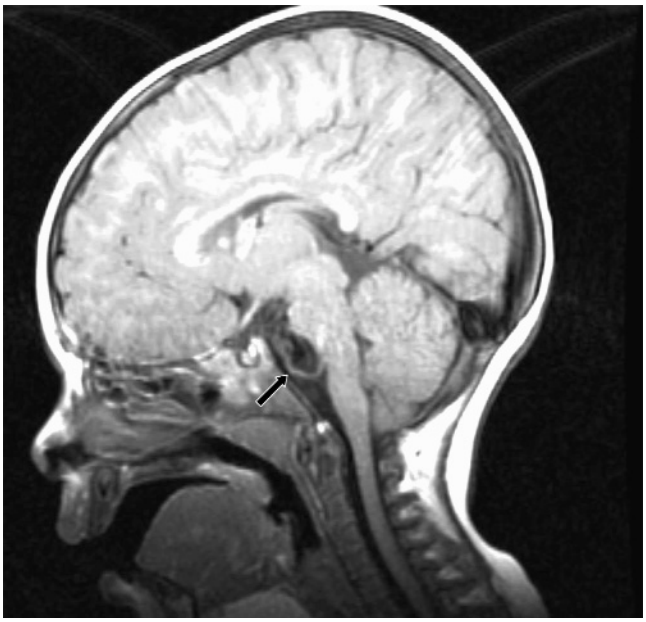

Figure 12. Giant midbasilar artery aneurysm (*arrow*) in an 18-month-old child with tuberous sclerosis complex. The lesion was not present on a magnetic resonance image at 11 months of age.

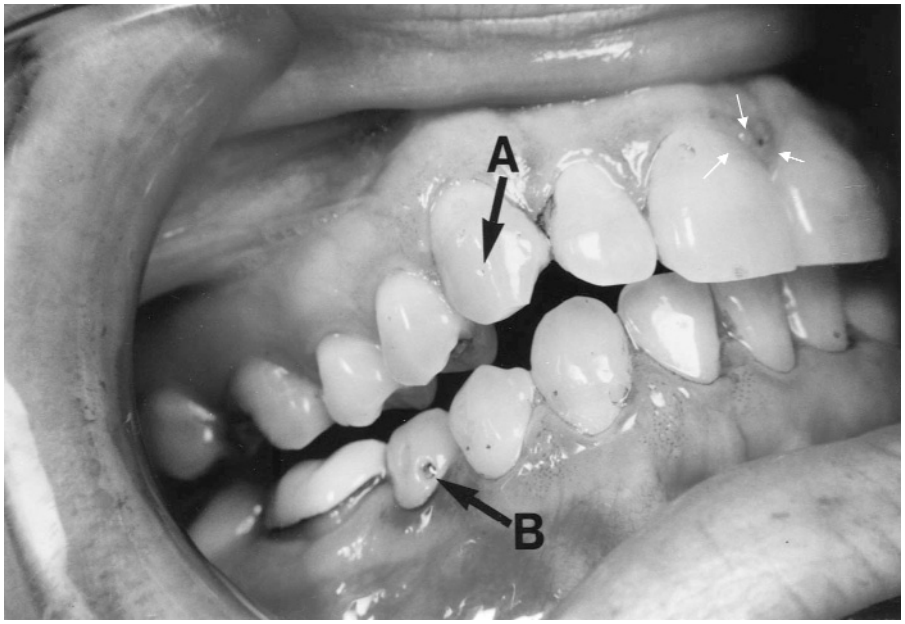

Figure 13. *A,* Dental pit; *B,* dental crater; gingival fibroma (*white arrows*).

VASCULAR MANIFESTATIONS

Dysplastic blood vessels are most commonly seen in association with renal angiomyolipomas. Arterial vascular dysplasia has also been described in individuals with tuberous sclerosis complex. Arterial aneurysms in various locations, as well as arterial ectasia and fusiform dilatation, have been described. The consequences of these lesions can be dramatic and severe, as in the case of abdominal aortic aneurysms or basilar arterial aneurysms (Figure 12). Although the exact incidence of these nontumoral arterial aneurysms in vascular dysplasia is felt to be uncommon in tuberous sclerosis complex, the exact incidence is not known.[33,36,37] Aneurysms are described of the abdominal aorta, axillary artery, basilar artery, and pulmonary artery. The clinician should be aware of this possible manifestation of the disease, in view of its potentially devastating consequences. Awareness of this potential complication of the disorder might also allow for appropriate management strategies and avoidance of unnecessary biopsy or inappropriate management.

DENTAL MANIFESTATIONS

Tuberous sclerosis complex is associated with a particular oral pathology. All individuals with tuberous sclerosis complex have dental pitting in their permanent teeth. Gingival fibromas are present in up to 50% of adults with tuberous sclerosis complex.[38] Enamel pits rarely cause significant symptoms and do not seem to result in an increased incidence of dental decay. Gingival fibromas can exert local effects on dentition, resulting in malocclusion or abnormal eruption. These issues are managed with standard orthodontic techniques. I have found that the primary utility of the oral manifestations of tuberous sclerosis complex is as an adjunct to diagnosis. An oral examination of the first-degree relatives of an affected individual with tuberous sclerosis complex, including staining for dental pitting, is an inexpensive and useful screening technique for the disorder. I have found this to be at least as valuable as Wood's lamp examination. Although dental pits can occur in individuals who do not have tuberous sclerosis complex, persons with tuberous sclerosis complex typically have multiple (ie, greater than 15) dental pits. Dental pits in tuberous sclerosis complex are often quite large (so-called "craters"). The association of multiple dental pits with gingival fibromas is highly suggestive of a diagnosis of tuberous sclerosis complex (Figure 13), particularly when present in a first-degree relative.[39]

CONCLUSION

Tuberous sclerosis complex is truly a disorder that affects virtually every organ system of the body. It is hoped that this review will provide practical information for clinicians to more appropriately manage those patients who must deal with this disease on a daily basis.

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Special Article

Molecular Genetic Basis of Tuberous Sclerosis Complex: From Bench to Bedside

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ABSTRACT

Tuberous sclerosis complex is an autosomal dominant disease of benign tumors occurring in multiple organ systems of the body. Either of two genes, *TSC1* or *TSC2*, can be mutated, resulting in the tuberous sclerosis complex phenotype. The protein products of the tuberous sclerosis complex genes, hamartin (*TSC1*) and tuberin (*TSC2*), have been discovered to play important roles in several cell-signaling pathways. Knowledge regarding the function of the tuberin-hamartin complex has led to therapeutic intervention trials. Numerous pathogenic mutations have been elucidated in individuals affected with tuberous sclerosis complex. Information on the type and distribution of nearly 1000 mutations in the two genes is discussed. Mosaicism for tuberous sclerosis complex mutations has been documented, complicating provision of genetic counseling to families. Emerging genotype-phenotype correlations should provide guidance for better medical care of individuals with tuberous sclerosis complex. (*J Child Neurol* 2004;19:699–709).

Tuberous sclerosis complex is a disease characterized by the growth of tumors, which are usually benign in nature but are occasionally malignant, in multiple organ systems of the body. It has long been recognized that tuberous sclerosis complex is a genetic disease. After the initial descriptions of tuberous sclerosis complex, information began appearing in the literature indicating the genetic underpinnings. As early as 1910, Kirpicznik described twins (identical and fraternal) affected by tuberous sclerosis complex and a family with members affected in three successive generations.[1] Earlier studies had noted that the facial lesions of tuberous sclerosis complex, erroneously called "adenoma sebaceum," were inherited in families.[2,3] Berg described tuberous sclerosis complex as a "hereditary disorder" in 1913.[4] Gunther and Penrose first brought attention to the dominant inheritance pattern and simultaneously suggested that a high mutation rate helps maintain the disease in the population.[5] From that time, a number of reports have appeared in the literature describing familial cases in multiple generations.[6,7]

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The incidence of sporadic versus familial cases of tuberous sclerosis complex has been addressed in various studies. In the sample of 20 cases described by Gunther and Penrose, 50% of the patients had sporadic tuberous sclerosis complex, whereas the other 50% had a positive family history. Variations in the percentage of cases of sporadic tuberous sclerosis complex from 50 to 86% have been reported in different studies.[8–17] It is now generally accepted that roughly two thirds of patients who have tuberous sclerosis are affected secondary to new mutation, whereas the other one third have inherited a mutated tuberous sclerosis complex gene from one of their parents.

MOLECULAR BASIS OF TUBEROUS SCLEROSIS COMPLEX

In 1987, the first clue of the chromosomal location for a gene causing tuberous sclerosis complex was published.[18] Fryer and colleagues tested 19 multigenerational families affected with tuberous sclerosis complex with 26 protein markers. In eight of the families, ABO blood group markers were informative and revealed a positive linkage. The ABO blood group gene mapped to chromosome 9q34.3. Fairly quickly, initial evidence indicating genetic heterogeneity was published by other groups.[19,20] Many groups followed up by testing larger numbers of families, thus confirming that genetic heterogeneity was present for tuberous sclerosis complex.[15,21,22] As the search for the *TSC1* gene continued, various groups began to search the genome for other possible

chromosomal locations of additional genes causing tuberous sclerosis complex. By studying five large multigenerational families affected with tuberous sclerosis complex, a consortium led by Kandt discovered linkage for a gene causing tuberous sclerosis complex on chromosome 16p13.3 near the mapped location of the adult polycystic kidney disease gene (*APKD1*).[23] Because this region had been so well described and mapped by groups searching for the *APKD1* gene, the *TSC2* gene was discovered fairly quickly.[24,25]

The *TSC2* gene codes for a protein named tuberin after the disease tuberous sclerosis complex. The *TSC2* gene has 41 exons distributed over approximately 40 kb of genomic DNA, with 2 exons (25 and 31) alternatively spliced.[26] Tuberin has a calculated molecular weight of approximately 200 kDa, and the transcript is roughly 5.5 kb. In the 10 years since the discovery of the *TSC2* gene, an amazing amount of data concerning the mutations in affected individuals and gene function has been discovered. The *TSC1* gene was finally discovered a decade after the initial report of linkage.[27,28] In contrast to the *TSC2* gene, the *TSC1* gene, with only 23 exons, extends over roughly 55 kb of genomic DNA. The *TSC1* gene contains exons 3 through 23, which are coding, and exons 1 and 2, which are untranslated, and possesses a 4.5 kb 3´ untranslated region. The protein product of the *TSC1* gene is named hamartin, which has an estimated molecular weight of 130 kDa and an 8.6 kb messenger ribonucleic acid (RNA) transcript. With both genes now in hand, research has been fruitful in determining the function of these genes.

The similarity in phenotypes produced by mutations in the *TSC1* and *TSC2* genes suggests that the genes somehow function together; however, little similarity can be identified between the primary amino acid sequences of tuberin and hamartin, suggesting that they do not belong to the same family of proteins. An alternative explanation is that tuberin and hamartin work intimately together within the same or similar biologic pathways that regulate cell growth and cell division. Indeed, direct interaction of tuberin and hamartin has been demonstrated through yeast two-hybrid experiments. Further, coimmunoprecipitation experiments with tuberin constructed to mimic several pathogenic in-frame deletion mutations revealed that mutated tuberin failed to interact with hamartin.[29,30] More importantly, the stability of tuberin and hamartin appeared to be interdependent, and immunohistochemical studies have demonstrated absent or diminishing amounts of both proteins in tuberous sclerosis–related tumor cells.[29,31]

MODEL ORGANISMS

Homologs of the tuberous sclerosis complex genes in model organisms (rat, mouse, *Drosophila*, *Fugu*, and, more distantly, fission yeast) were identified and found to be highly conserved, suggesting similar, if not identical, evolutionary functions. These model organisms serve as valuable experimental tools in facilitating insight into the function of tuberin and hamartin and the molecular pathogenesis of tuberous sclerosis complex. Similar to many evolutionarily conserved genes, the engineered homozygous tuberous sclerosis complex gene mutants are embryonic lethals in these models.

The Eker rat, a spontaneous mutant predisposed to autosomal dominant renal carcinomas, was the first animal model found to contain an insertion mutation in the *Tsc2* gene.[32,33] Various studies with the Eker rat have enlightened our understanding of renal carcinogenesis and contributed to studies of the potency and efficacy of treatment with the drug rapamycin. Several engineered *Tsc1* and *Tsc2* gene disruptions created in the mouse have been shown to have renal pathology similar to that of humans affected with tuberous sclerosis complex but, interestingly, do not have significant brain pathology.[34,35] Also, $Tsc1^{+/-}$ and $Tsc2^{+/-}$ mice have some phenotypic differences, an observation paralleling the human condition.[36] These mice should prove useful in distinguishing phenotypic aspects relating to *TSC1* versus *TSC2* gene mutations in humans. In an effort to investigate brain pathology, a conditional disruption of the *Tsc1* gene was made and specifically deleted from astrocytes. These animals exhibited abnormal neuronal organization and seizures.[37] Such animals are not only useful in understanding pathogenesis, they are also important for testing potential therapies.

Although the mammalian models have been and will continue to be useful in studying tuberous sclerosis complex, genetic studies in *Drosophila* homologs established the role of the *TSC1* and *TSC2* genes in the insulin-signaling pathway in regulating cell size, morphology, and proliferation.[38,39] This discovery led to the placement of the *TSC1* and *TSC2* genes in the phosphatidylinositol-3 kinase/protein kinase B (PI3K/PKB) and mammalian target of rapamycin (mTOR) pathway and has permitted several studies investigating potential therapies exploiting this pathway.[40] As the functions of the *TSC1* and *TSC2* genes expand to include more and more pathways and proteins, existing and new animal models will be important in our understanding of the pathogenesis and development of novel treatments.

TSC1 AND *TSC2* GENE PRODUCTS

At present, more is known about the function of tuberin than hamartin, partly because the *TSC2* gene was identified 4 years prior to the identification of the *TSC1* gene and the *TSC2* gene has more predicted functional motifs (ie, leucine zippers at the amino [N]-terminus, a guanosine triphosphatase activating protein [GAP] 3 motif, and a transcription activation domain at the carboxyl [C]-terminus), as well as many cell kinase–targeting motifs that were identified on the primary amino acid sequence.[24,41] The primary amino acid sequence coding for hamartin suggested two coiled-coil domains, one at the N-terminus and the other in the midsegment of the peptide.[28] Although the mechanism of defective tuberin or hamartin in causing hamartomas in patients with tuberous sclerosis complex remains unresolved 7 years after cloning of the *TSC1* gene, much work

has been performed to characterize the normal functions of these two proteins in cellular processes and the localization of these functional sites, which are summarized in Figure 1.

There are different lines of evidence supporting tuberin-hamartin complex functions in several cell-signaling pathways (Figure 2), including a growth and translation regulatory pathway (PI3K/PKB pathway), a cell adhesion/migration/protein transportation pathway (glycogen synthase kinase 3 [GSK3]/β-catenin/focal adhesion kinase [FAK]/Ras-related homolog [Rho] pathway), and a cell growth and proliferation pathway (mitogen-activated protein kinase [MAPK] pathway).

TUBERIN AND HAMARTIN AND GROWTH AND TRANSLATION REGULATION

Among these three pathways, the function of tuberin and hamartin in the protein translation pathway involving phosphatidylinositol-3 kinase and protein kinase B is the most studied (see Figure 2, light blue pathway). The tuberin-hamartin complex functions to modulate the phosphorylation of p70 ribosomal protein S6 kinase 1, partly through suppressing mTOR kinase activity, as shown by genetic studies of tuberin homologs in *Drosophila*.[38,39,42,43] Follow-up studies revealed that tuberin activity can be suppressed via phosphorylation by protein kinase B (also known as Akt) on insulin stimulation. Phosphorylation of tuberin at amino acids serine 939, 1086, and 1088 or threonine 1422 can disrupt the dimerization of tuberin with hamartin (see Figure 1). Without a functional tuberin-hamartin complex, suppression of S6 kinase 1, mTOR, and translation initiation factor 4E will be released to facilitate assembly of 40S and 60S ribosomal subunits and other translation initiation factors (A, G, and B) on capped messenger RNA to start the protein translation process. Thus, we now understand much better the mechanism by which mutations in the tuberous sclerosis complex genes lead to increased protein translation and, subsequently, cell overgrowth. These discoveries relating to tuberin-hamartin complex function in cells have also led to ideas for treatments to slow the growth of tumors typically observed as part of the tuberous sclerosis complex phenotype. For example, mTOR activity can be suppressed by rapamycin, a drug approved by the US Food and Drug Administration that is commonly used after organ transplant and cancer treatment. Trials are currently under way to assess the efficacy of rapamycin as a therapy for suppressing the growth of renal tumors in the Eker rat, and, recently (fall 2003), a clinical trial with rapamycin was initiated in patients with tuberous sclerosis complex to treat renal angiomyolipomas.[40]

The GAP domain of tuberin has been demonstrated to hydrolyze guanosine triphosphate bound to the small G proteins Ras-related protein (RAP)1 and rabaptin (RAB)5.[44,45] The most recent studies in mammalian cells and *Drosophila* showed that the GAP domain of tuberin can also hydrolyze the guanosine triphosphate bound to small G protein Rheb (Ras homolog enriched in brain, sharing great homology with the RAP family), preventing the activation of mTOR by Rheb–guanosine triphosphatase.[46–50] Rheb activates S6 kinase 1 when insufficient amino acids are present, but the activation is sensitive to rapamycin, suggesting that Rheb mediates nutrient signaling through mTOR. Therefore, a possible role of the tuberin-hamartin complex is to regulate Rheb during nutrient signaling. Astrinidis et al demonstrated that hamartin was phosphorylated by cyclin-dependent kinase 1/cyclin B during the nocodazole-induced G2/M phase in a cultured human embryonic kidney cell line (HEK293) and also in cells overexpressing exogenous hamartin.[51] The phosphorylation of hamartin could regulate phosphorylation of S6 kinase 1 and the translation process during the G2/M phase of the cell cycle. However, Jaeschke et al provided evidence suggesting that the tuberin-hamartin complex can mediate S6 kinase inhibition, bypassing mTOR regulation, suggesting a more complex functional role for the tuberous sclerosis complex proteins.[52]

In addition, other recent findings suggest that the phenotypic findings in tuberous sclerosis complex might occur secondary to a mechanism other than hyperactivation of S6 kinase 1 through mTOR (Au K-S et al, unpublished observation, 2003).[53] Our study of gene expression profiles in renal angiomyolipomas isolated from patients with tuberous sclerosis complex did not show a dramatic increase in the expression of genes in the PI3K/PKB pathway, leading us to speculate that genes in an alternative pathway could be important in causing tumors in patients with tuberous sclerosis complex. Instead, we observed activation of G protein–coupled receptors and their downstream effectors, such as phospholipases and protein kinases, in studies of the kidney angiomyolipoma tissue of a patient with tuberous sclerosis complex, further supporting involvement of other signaling pathways.

TUBERIN AND HAMARTIN AND CELL ADHESION, MIGRATION, AND PROTEIN TRAFFICKING

Other studies have suggested that tuberin and hamartin play a role in cell adhesion, migration, and protein trafficking processes, thus functioning in a second important cell pathway (see Figure 2, green and pink pathways). Lamb et al used a yeast two-hybrid system to show that the C-terminus of hamartin interacts with the N-termini of the ezrin, radixin, and moesin (ERM) family of proteins and that ERM functions to link integral membrane proteins with cytoskeletal proteins such as F-actin through activated Rho–guanosine triphosphate.[54] Perturbation of hamartin-ERM interaction is proposed to cause cells to lose adhesion to the extracellular matrix, leading to abnormal cell migration and hamartoma formation.[54] On the other hand, by introducing exogenous tuberin into different mammalian cell lines, researchers demonstrated that the Rho–guanosine triphosphate protein was elevated. Also observed was a decrease in focal adhesion kinase but an increase in the phosphorylated form of focal adhesion kinase in treated cells. Overexpression of tuberin contributed to an increase of Rho–guanosine triphosphate and phosphorylated focal adhesion kinases, leading to

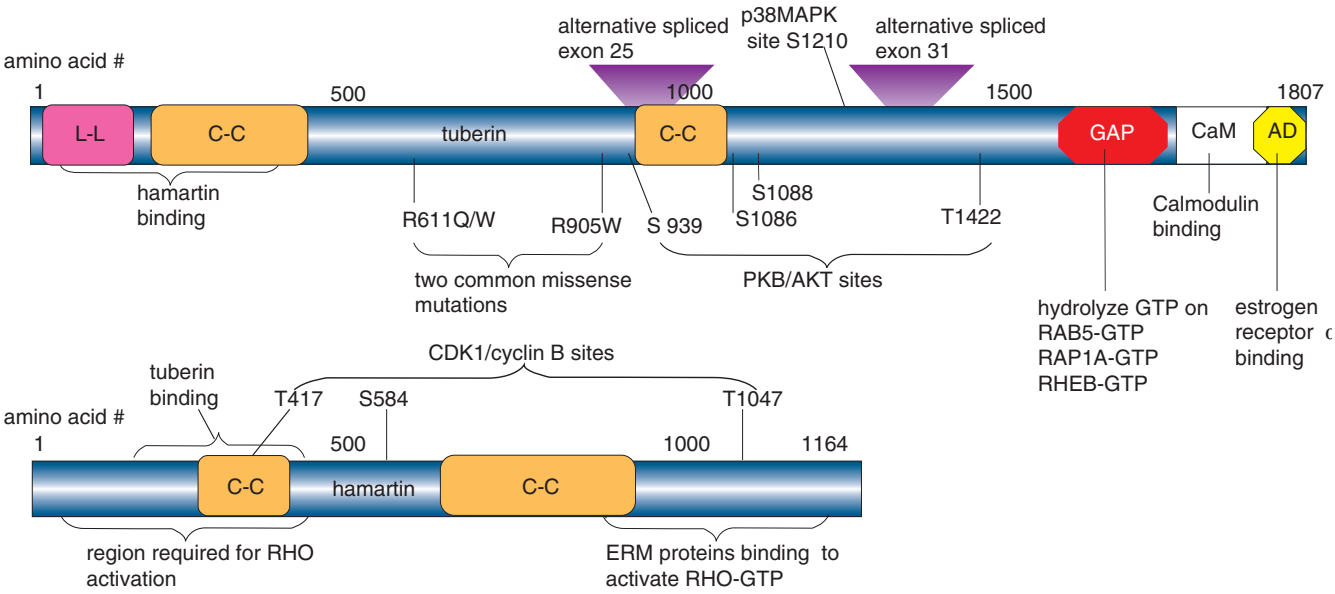

Figure 1. Review of many functional domains within the *TSC1* and *TSC2* gene products. The *TSC1* gene codes for a protein of 1164 amino acids named hamartin, and the *TSC2* gene codes for a protein of 1807 amino acids named tuberin. Since the identification of the *TSC2* gene, many functional domains on tuberin have been reported, including a leucine zipper domain (L-L) and two coiled-coil domains (C-C) for protein-protein interactions with hamartin and other proteins; several cell-signaling kinases (ie, p38MAPK, protein kinase B/Akt), with targets on serine (S939, S1086, S1088, and S1210) and threonine (T1422) to repress tuberin activity; a guanosine triphosphatase activating protein (GAP) domain for activating different small guanosine triphosphatases in cell signaling; a calmodulin binding site; and a transcription activation domain (AD) to activate expression of certain genes. Two of the most common missense mutations (R611Q/W and R905W, ~ 5% and 1.5%, respectively, of all tuberous sclerosis complex mutations) are shown. Two alternatively spliced exons (25 and 31) are depicted. Functional domains of the *TSC1* gene product have been less defined until recently. Hamartin contains two coiled-coil (C-C) domains with the amino-terminal C-C domain interacting with tuberin and constitutes a region required for small guanosine triphosphatase Ras-related homolog (Rho) activation. Like tuberin, the function of the second C-C domain remains to be identified. A cytoskeletal protein (ezrin, radixin, moesin [ERM]) interacting region was localized to the end of hamartin, probably related to functions including actin reorganization, protein or vesicular trafficking, or cell migration. Three serine (S584) and threonine (T417 and T1047) targets of cyclin D kinase 1 (CDK1)/cyclin B were reported recently, suggesting functions directly related to cell cycle progression. CaM = calcium binding protein calmodulin; PKB/Akt = protein kinase B/murine thymoma viral oncogene homolog; p38MAPK = 38 kDa mitogen-activated protein kinase; RAB5-GTP = rabaptin 5–guanosine triphosphatase; RAP1A = Ras-related protein 1A; Rheb = Ras homolog enriched in brain.

increased focal adhesion formation and stress fiber formation via reorganization of actin filaments. These experiments suggested tuberin and hamartin function to promote cell adhesion and correct migration of precursor cells via the activation of focal adhesion kinase and Rho–guanosine triphosphate.[55]

Through overexpression of TSC1 and TSC2 in experiments on HEK293 cells, the tuberin-hamartin complex was shown to change the morphology and adhesive properties of HEK293 cells to more compact clusters. An increase in the level of cellular E-cadherin was found in these cells, suggesting that tuberin and hamartin regulate cell-cell adhesion through the E-cadherin–β-catenin pathway.[56] Cadherins are cell-surface adhesion molecules that help form tight junctions between cell layers. In addition, cadherins also transduce signals into cells through interactions with the catenins. Catenins affect actin cytoskeletal function through interactions with actinin and vinculin and trigger changes in cell shape and motility with signals through the Rho–guanosine triphosphatases. Hamartin and tuberin promote β-catenin degradation involving glycogen synthase kinase 3.[57] It is speculated that loss of tuberin results in increased β-catenin, leading to subsequent activation of cyclin D_1 and the cell proliferation observed in tuberous sclerosis complex–related tumors. Evidence to demonstrate direct interaction of tuberin-hamartin complexes to facilitate hydrolysis of guanosine triphosphate bound to Rho has not yet been reported.

TUBERIN AND HAMARTIN AND GROWTH AND CELL PROLIFERATION

There is also evidence to indicate that tuberin and hamartin regulate the MAPK signal pathway in controlling cell proliferation, implicating the complex in yet a third cellular pathway. Tuberin phosphorylated at amino acid serine 1210 is mediated by p38 mitogen-activated protein (MAP) kinase, and the phosphorylated tuberin-hamartin complex is subsequently sequestered from their functional sites by 14-3-3 protein (see Figure 1).[58,59] Intriguingly, the expression of 14-3-3 protein is regulated by tuberin and hamartin, suggesting a tightly interregulated mechanism.[60] On the other hand, high levels of p42/44 MAP kinase have been detected by anti–MAP kinase antibody in human tuberous sclerosis complex–associated neoplasms and Tsc2Ang1 (a tumor isolated from a heterozygous *Tsc2* mouse lacking functional

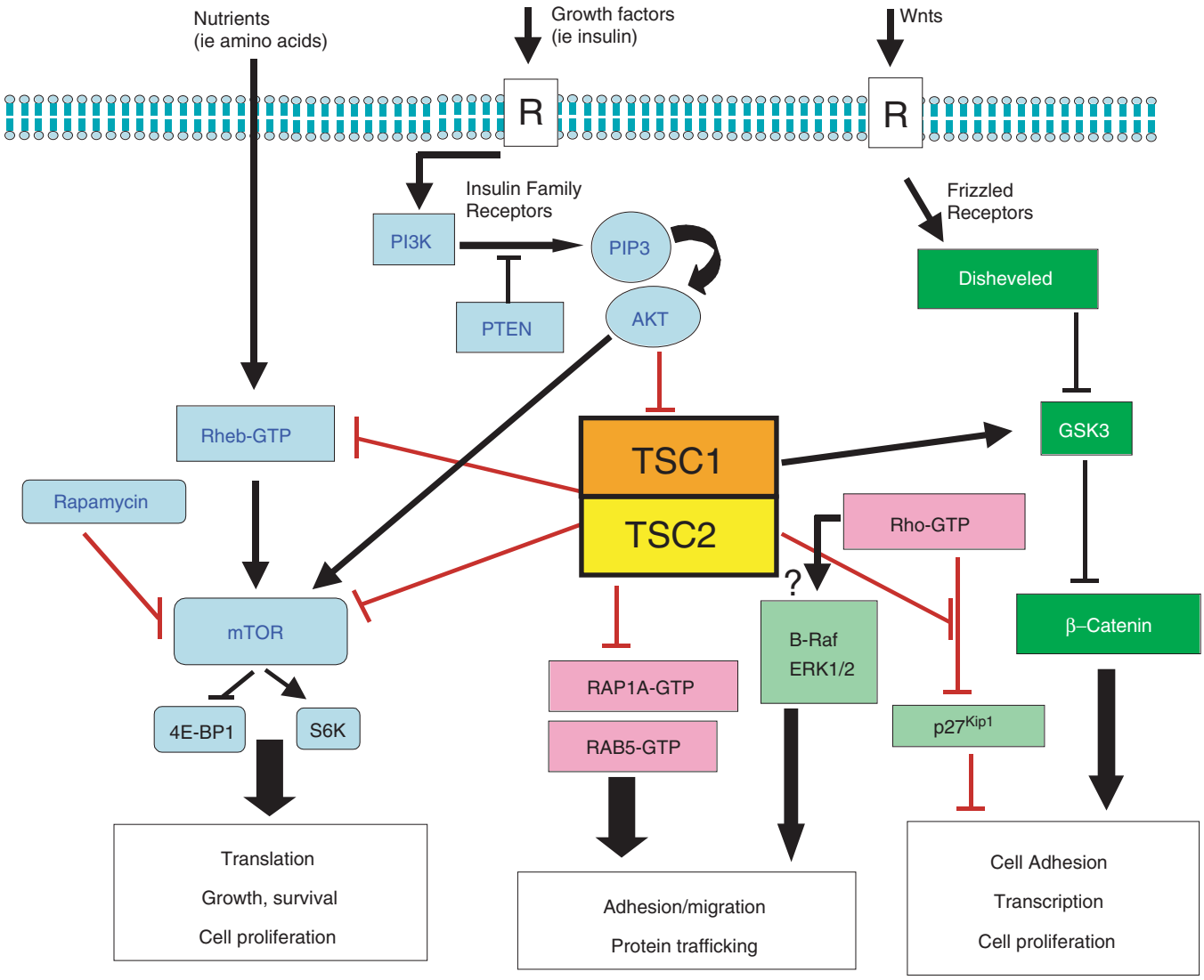

Figure 2. The illustration depicts tuberin-hamartin complex functions in different signaling pathways. The first elucidated role of the tuberin-hamartin complex (*yellow/orange boxes*) involves suppressing protein translation, growth, and cell proliferation through deactivating small guanosine triphosphatase Ras homolog enriched in brain (Rheb) and mammalian target of rapamycin (mTOR) (*light blue boxes*). In the presence of growth factors (ie, insulin) or nutrients (ie, amino acids), tuberin activity will be suppressed by protein kinase B/murine thymoma viral oncogene homolog (PKB/Akt) phosphorylation at several serine and threonine sites to release the suppression on mTOR. Derepressed mTOR activates p70 ribosomal protein S6 kinase (S6K) 1 and suppresses factor 4E binding protein 1 (4E-BP1) to stimulate ribosome assembly and translation. A second pathway is shown in green and pink boxes depicting the tuberin-hamartin complex acting together with glycogen synthase kinase 3 (GSK3) to suppress the WNT signaling pathway through promoting degradation of the WNT pathway effector (β-catenin). Suppressing the WNT pathway leads to dampening of cell-cycle progression. Other signaling guanosine triphosphatases regulating protein and vesicular trafficking include RAP1A = Ras-related protein 1A and rabaptin 5 (RAB5) and are reported to be substrates of tuberin leading to a possible regulatory role of tuberin and hamartin in protein and vesicular trafficking and cell migration. Lastly, tuberin overexpression has been shown to elevate Ras-related homolog (Rho) protein in cultured cells. Rho protein is also a guanosine triphosphatase with multiple roles that affect protein and vesicular trafficking, survival, cell migration, and suppression of 27 kDa cyclin-dependent kinase inhibitor 1B ($p27^{Kip1}$) to activate cell-cycle progression. B-Raf = b-raf murine sarcoma viral oncogene homolog B1; ERK1/2 = p44/42 mitogen-activated protein kinase, extracellular regulated signal kinase 1/2; GAP = guanosine triphosphatase activating protein; GTP = guanosine triphosphate; PI3K = phosphatidylinositol-3 kinase; PIP3 = phosphatidylinositol triphosphate; PTEN = phosphatidylinositol triphosphate phosphatase; R = plasma membrane receptor. *Black arrows* indicate activation and red lines with a bar at the end indicate suppression.

tuberin) cells, suggesting that tuberous sclerosis complex proteins can function to regulate p42/44 MAP kinase activity.[61] Suppressing the phosphorylation of p42/44 MAP kinase resulted in a decrease in vascular endothelial growth factor production and inhibited growth of Tsc2Ang1 tumor cells in vivo. These findings suggest potential therapeutic uses of p42/44 MAP kinase inhibitors to treat tuberous sclerosis complex tumors. In a related finding, expression of ERK2/1(an alias of MAPK1 and MAPK3), murine sarcoma viral oncogene homolog B1 (B-Raf), and RAF-1 was found to be up-regulated in $Tsc2^{-/-}$ renal tumor cells isolated from the Eker rat.[62] Reintroducing tuberin into these cells sub-

Table 1. Brigham and Women's Hospital Tuberous Sclerosis Complex Variation Database as of 2000

Type of Mutation	TSC1 *Gene (%)*	TSC2 *Gene (%)*
Frameshift and protein truncation	82/159 (51.5)	79/323 (24.5)
Nonsense	59/159 (37.1)	58/323 (18.0)
Splicing	13/159 (8.2)	43/323 (13.3)
In-frame	2/159 (1.3)	11/323 (3.4)
Deletion/insertion/duplication	2/159 (1.3)	76/323 (23.5)
Missense	0/159 (0)	55/323 (17.0)
Large deleletion/rearrangement	1/159 (0.6)	1/323 (0.3)

stantially reduced Erk, B-Raf, and Raf1 activity, suggesting tuberin as an upstream regulator of the Erk (Mapk1 and Mapk3) pathway. MAP kinases function to activate RAF and ERK activity through phosphorylation and subsequently activate a battery of transcription factors and proto-oncogenes to stimulate cell growth and proliferation. The tuberin-hamartin complex can therefore act to regulate the activation of guanosine triphosphate binding protein targets (ie, RAF) through the GAP activity and controlling the downstream cell proliferation processes. Direct evidence to prove RAFs to be downstream targets of tuberin and hamartin is not yet available.

Alternatively, cell proliferation owing to a lack of tuberin function could result from a lack of $p27^{Kip1}$ (a cyclin D_1 inhibitor) activity, as seen in Eker rat EEF8 cells ($Tsc2^{-/-}$) and subsequently in Tsc2 null mouse embryonic fibroblasts.[37] Interestingly, Hirai et al showed that membrane-bound geranylgeranylated Rho–guanosine triphosphatase activated degradation of $p27^{Kip1}$ and promoted cdk2 activity in G1/S phase rat FRTL-5 cells to facilitate cell-cycle progression.[63] Activated Rho is required to sustain Erk activation and subsequent cyclin D_1 expression in the mid-G1 phase.[64] This information, coupled with observations by Lamb et al and Astrinidis et al,[54,55] suggests that tuberin activates Rho–guanosine triphosphate to promote cell adhesion and migration. Perhaps a function of tuberin and hamartin is to prevent the membrane localization of Rho–guanosine triphosphate and release the inhibition of $p27^{Kip1}$ during cell growth and migration processes. Loss of tuberin and hamartin will allow Rho–guanosine triphosphate to localize to the membrane for activation and couple with downstream effectors (degradation of $p27^{Kip1}$, to derepress cdk4 and -5/cyclin D_1 on the one hand, and activate cdk2/cyclin E activity on the other) to allow the cell to have G1/S phase progression.

Other factors (ie, platelet-derived growth factor receptor, estrogen receptor, and calmodulin binding) have been suggested to play a role in the function of the tuberin-hamartin complex, suggesting that the tuberin-hamartin complex could be involved in even more cellular functions that have yet to be uncovered (Au K-S et al, unpublished data).[65,66] Given that the GAP domain of tuberin has been shown to hydrolyze guanosine triphosphate from at least three different guanosine triphosphatases (RAP1A, RAB5, and Rheb) and possibly more affecting several downstream biologic functions, as described above, the tuberin-hamartin complex can play a role as a signal "switchboard" to relay different cell signals to their corresponding downstream responses. Such speculation is consistent with the reported roles of tuberin and hamartin in different biologic pathways, as well as presentation of a wide spectrum of clinical findings described among patients with tuberous sclerosis complex involving multiple organ systems and even neurobehavioral abnormalities.

MUTATIONS IN THE *TSC1* AND *TSC2* GENES

Many different mutations have been described in the *TSC1* and *TSC2* genes since their discovery. The best way to determine general trends in mutation type and frequency is to compare the results in large databases and studies while keeping in mind specific biases that the different repositories of information might harbor. Until 2002, there was a public database of tuberous sclerosis complex gene mutations maintained through Brigham and Women's Hospital/Harvard Medical School (<http://zk.bwh.harvard.edu/projects/tsc/>). The database contained all published tuberous sclerosis complex gene mutations through 2000. In 2001, Dabora et al published a comprehensive genotype-phenotype study of 224 families affected with tuberous sclerosis complex.[67] In our database at The University of Texas Medical School at Houston, as of September 2003, we have documented 219 independent *TSC1* and *TSC2* gene mutations. The results contained within each of these resources are discussed to provide insight regarding the type and frequency of tuberous sclerosis complex gene mutation.

Nearly 500 different mutations have been reported in the Tuberous Sclerosis Complex Variation Database previously maintained through Brigham and Women's Hospital/Harvard Medical School (Table 1). All mutations reported in the medical literature until 2000 are included. There is potential for bias in the Brigham and Women's database for an excess of large gene deletions and rearrangements in the *TSC2* gene. This particular type of mutation was reported in the literature more commonly in the first few years after the *TSC2* gene was discovered. The discovery of the *TSC2* gene was facilitated by studies of tumor samples from patients with tuberous sclerosis complex that revealed loss of heterozygosity for portions of chromosome 16p13.[68] Additionally, soon after discovery of the *TSC2* gene, a contiguous gene syndrome was described involving the *TSC2* and *APKD1* genes.[69] A number of investigations following up on

Table 2. University of Texas Medical School at Houston Tuberous Sclerosis Complex Database as of September 2003

Type of Mutation	TSC1 *Gene (%)*	TSC2 *Gene (%)*
Frameshift and protein truncation	34/57 (59.6)	51/162 (31/5)
Nonsense	19/57 (33.3)	36/162 (22.2)
Splicing	4/57 (7.0)	20/162 (12/3)
In-frame	0/57 (0)	7/162 (4.3)
Deletion/insertion/duplication	0/57 (0)	44/162 (27.2)
Missense	0/57 (0)	2/162 (1.2)
Large deletion/rearrangement	0/57 (0)	1/162 (0.6)

the loss of heterozygosity studies and describing the contiguous gene deletion syndrome were immediately published, thus enriching the literature for examples of this mutation type.[24,67,70–72] On the other hand, the common use of sequencing to detect tuberous sclerosis complex gene mutations was not used until the late 1990s, therefore creating a bias against reporting of more subtle mutations in the literature. As of 2000, the Brigham and Women's database listed 268 small *TSC2* mutations, 159 small *TSC1* mutations, and 55 large *TSC2* deletions and rearrangements.

The University of Texas database information as of September 2003 is shown in Table 2. There might be a minor amount of overlap between the two databases because some of our data were published prior to the time that the Brigham and Women's database stopped cataloguing new findings of tuberous sclerosis complex mutations. In contrast to the Brigham and Women's database, the University of Texas database is biased against reporting of large gene deletions and rearrangements because only a subset of our patient samples has been tested for this type of mutation. On the other hand, all of the University of Texas patient samples have been tested by direct sequencing of the *TSC1* and *TSC2* genes, thus providing only a limited opportunity for missing subtle mutations. Our database currently includes 160 small *TSC2* mutations, 57 small *TSC1* mutations, and two large gene deletions/rearrangements.

The final dataset included here for comparison is the study published by Dabora et al in 2001.[67] Their study is the largest, most comprehensive study to date that includes both mutational and phenotypic information. Dabora et al reported findings on 224 families, with initial molecular testing performed by denaturing high performance liquid chromatography (DHPLC) and follow-up of all variants by direct sequencing. For the families with no mutation detected by these methods, additional testing was performed using long-range quantitative polymerase chain reaction (PCR) searching for large deletions and rearrangements. Mutations were identifiable in 186 (83%) individuals with 138 small *TSC2* mutations, 20 large *TSC2* mutations, and 28 small *TSC1* mutations. There is some overlap between the results reported in the Dabora et al study and the Brigham and Women's database because some of the patients included in the Dabora et al study had been reported in the literature prior to 2000. It is not possible to determine the extent of the overlap.

Several differences in mutation type between the *TSC1* and the *TSC2* genes are noted in both databases and the Dabora et al study. Although missense mutations represent a significant percentage of detectable mutations in the *TSC2* gene in all three studies, with percentages varying from 23.5 to 32%, there were only two cases with missense mutations reported in the *TSC1* gene with normal parental *TSC1* gene sequences. Of the 81 reported different *TSC2* missense mutations, 27 are located between amino acids 1500 and 1750 (within the GAP and calcium binding protein calmodulin domains), and approximately 50 are spread out between amino acids 200 and 1200 (the hamartin binding region and the protein kinaseB/Akt target site serine 939), with very few located between amino acids 1200 and 1500 (see Figure 1). No missense mutations are within or immediately adjacent to the three known Akt target sites. There are no "hot spots" for mutation in either gene. The *TSC2* gene does harbor two "warm spots," each accounting for 4% of reported mutations: substitution of arginine 611 for tryptophan or glutamine and an in-frame deletion of six amino acids in exon 38.

The only two *TSC1* missense mutations reported were noted in the Brigham and Women's database. Mutation studies at the University of Texas have identified several potential *TSC1* gene missense mutations that are currently pending analysis on parental DNAs for confirmation. The *TSC1* gene has only a few large deletion mutations reported; however, large deletions and rearrangements are commonly reported for the *TSC2* gene.[72] The University of Texas database reports only two such cases, representing 1.2%; however, only a subset of our patients was tested by the method of Southern blotting, and none of our patients were tested by long-range PCR or quantitative PCR; thus, our study has an underrepresentation of this mutation type. The Brigham and Women's database reports 55 of 323 (17%) cases, but, as stated above, the sample is probably enriched for these cases owing to a skewing from reports in the literature. The Dabora et al study likely represents the best estimate among the three sources reviewed here, with 20 of 158 (12.7%) detectable *TSC2* mutations of the large deletion or duplication type.[67]

As for comparison of frequency of mutations in *TSC1* versus *TSC2* in familial and sporadic tuberous sclerosis complex cases, all studies to date conclude that *TSC2* mutations are much more common in patients with sporadic

Table 3. University of Texas Medical School at Houston Database Comparing Patients With Familial and Sporadic Tuberous Sclerosis Complex

Type of Mutation	*Familial*		*Sporadic*	
	TSC1 *(%)*	TSC2 *(%)*	TSC1 *(%)*	TSC2 *(%)*
Frameshiftand protein truncation	7	6	27	45
Nonsense	7	5	12	31
Splicing	1	4	3	16
In-frame	0	1	0	7
Deletion/insertion/duplication	0	8	0	36
Missense	0	1	0	1
Large deletion/rearrangement	0	0	0	1
Total (219)	15	25	42	137
	(37.5)	(62.5)	(24)	(76)
Ratio (*TSC1* to *TSC2*)	1:1.7		1:3.3	

tuberous sclerosis complex than *TSC1* mutations. A range of 3:1 to as high as 7:1 for *TSC2* to *TSC1* mutations among patients with sporadic tuberous sclerosis complex has been reported by different research protocols.[67,71,72] The issue of the underlying mutation in patients with multigenerational familial tuberous sclerosis complex has been more controversial. A number of studies have concluded that mutations in the *TSC1* and *TSC2* genes were roughly equal as causative in multigenerational families affected with tuberous sclerosis complex.[71,73–75] The University of Texas database would support these conclusions. We observed a ratio of 1.7:1 for *TSC2* to *TSC1* gene mutations in familial patients and a 3.3:1 ratio in patients with sporadic tuberous sclerosis complex (Table 3).

On the other hand, the Dabora et al study did not find equal instances of *TSC1* and *TSC2* mutations in the multigenerational families they tested. They found *TSC2* mutations to far outnumber *TSC1* mutations in their multigenerational families and their patients with sporadic tuberous sclerosis complex. Several factors could have biased their results regarding multigenerational families affected with tuberous sclerosis complex. First, their sample of patients was in large part recruited through neurology clinics, thus creating bias for patients with a more prominent neurologic phenotype. As is described below, many studies have found that the *TSC1* phenotype is less severe (particularly with respect to the neurologic phenotype) than the *TSC2* phenotype. Second, their study included a very limited number of families, and all of the families were relatively small in size. They included only 11 multigenerational families: 3 with 4 members affected, 3 with 3 members affected, and 5 with 2 affected individuals. All of the families with two affected individuals represent a parent-child pair. Although the ratio might not be 1:1 for *TSC1* to *TSC2* mutations in multigenerational families, most studies indicate that it is not as skewed as in new-mutation cases of sporadic tuberous sclerosis complex.

A general comparison of the University of Texas study and the Dabora et al study is summarized in Table 4. Overall, our findings are similar, with the exception that our study has not accounted for large gene deletions and rearrangements.

GENOTYPE-PHENOTYPE CORRELATION

Most affected individuals who have reached adulthood will have the majority of the findings noted in tuberous sclerosis complex listed in Table 5. Multiple investigations have attempted to determine genotype-phenotype correlations in tuberous sclerosis complex between *TSC1* and *TSC2* gene mutations. All of the studies to date have investigated groups of patients with sporadic tuberous sclerosis complex or a combination of patients with sporadic and familial tuberous sclerosis complex. Several authors have reported finding a more severe phenotype with *TSC2* mutations.[67,70,71,76] Jones et al[71] reported increased mental retardation with *TSC2* mutations, whereas Al-Saleem[76] reported an increased risk of renal malignancy. Dabora et al, in the largest study to date that combined patients with familial and sporadic tuberous sclerosis complex, reported an overall increased incidence of seizures, mental retardation, and central nervous system tumors; more severe renal findings, increased retinal hamartomas; and more severe facial angiofibromas in patients with *TSC2* mutations.[67] Importantly, Dabora et al paid careful attention to the age of the patients because the findings of tuberous sclerosis complex tend to increase

Table 4. Comparison of University of Texas Medical School at Houston and Dabora et al Data Sets

	UTMS-Houston (%)	*Dabora et al, 2001[67] (%)*
Total families	351	224
Familial	49 (14)	38 (17)
Sporadic	302 (86)	183 (82)
Unknown	0	3 (1)
Mutation		
Small	217 (62)	166 (74)
Large	2 (0.6)	20 (8.9)
Pending	19 (5.4)	—
Ongoing	31 (8.8)	—
NMI	82 (23.4)	38 (17)
Methods	Direct sequencing, 77%	DHPLC followed up with sequencing, long-range PCR, and quantitative PCR, 83%

DHPLC =denaturing high performance liquid chromatography ; NMI =no mutation identified ; PCR = polymerase chain reaction; UTMS = University of Texas Medical School.

Table 5. Frequency of Disease Phenotype Observed Among Patients With Tuberous Sclerosis Complex

Phenotype	*Frequency (%)*
Cortical tuber	~ 90
Facial angiofibroma	> 80
Renal angiomyolipoma	> 80
Subependymal nodule	~ 80
Cardiac rhabdomyoma	~ 50
Ungual/subungual fibroma	51 to ~ 88

over the lifetime of the affected individual. A few studies have demonstrated no differences between the tuberous sclerosis complex phenotype and causative mutation.[74,75] Specifically, van Slegtenhorst and colleagues reported that they found no evidence for a milder phenotype in *TSC1* mutations.[75] The University of Texas study has gathered clinical data and is in the process of collating the data for genotype-phenotype correlations.

MOSAICISM AND GENETIC HETEROGENEITY

Mosaicism, differing genotypes in different cells of an individual's body, has been shown for a number of genetic conditions. For example, mosaicism has been proven in individuals affected by osteogenesis imperfecta type II and Duchenne muscular dystrophy, as well as for disorders associated with benign and malignant tumors, such as retinoblastoma, von Hippel-Lindau disease, and neurofibromatosis 1.[77–81] Mosaicism for causative mutations in tuberous sclerosis has been documented. There have been families described with more severely affected children and a less severely affected parent who was discovered to be mosaic for the child's germline mutation.[82,83] Other families have been reported with two or more affected children and clinically unaffected parents.[84,85] Although the exact percentage of individuals who have tuberous sclerosis complex as a result of mosaicism for a mutation has not been determined, it remains important to educate families about mosaicism when providing genetic counseling for a new diagnosis of tuberous sclerosis complex in a family. In addition, mosaicism might be an unstudied source of clinical variability between affected parents and children, as mentioned above, and should be considered when performing clinical examinations of first-degree relatives of an index case. For genetic counseling purposes, it will be important to clarify to families that clinical and molecular testing of parents might not uncover mosaicism. Providing a recurrence risk of 1 to 2% for the parents of seemingly sporadic cases is the most prudent course of action to account for the possibility of undetected mosaicism in one of the parents.[84]

Given the implication of two genes (*TSC1* and *TSC2*) in the pathogenesis of tuberous sclerosis complex and the relatively high mutation rate suspected for the *TSC2* gene, the phenomenon of genetic heterogeneity might further play a role in clinical variability, genetic testing, and genetic counseling for larger families with tuberous sclerosis complex. Families previously thought to be demonstrating non-penetrance for a tuberous sclerosis complex mutation were subsequently found to harbor two different mutations as a result of a sporadic mutation event.[86,87] In cases with apparent nonpenetrance, extremely variable expressivity, or when targeted familial mutation analysis yields unexpectedly negative results, genetic heterogeneity should be suspected.

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Special Article

Aberrant Cellular Differentiation and Migration in Renal and Pulmonary Tuberous Sclerosis Complex

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ABSTRACT

This review is focused on pathways and mechanisms that might provide molecular links between the pathogenesis of renal and pulmonary disease in tuberous sclerosis complex and the pathogenesis of the neurologic manifestations of tuberous sclerosis complex. Tuberous sclerosis complex is an autosomal dominant disorder in which the manifestations can include seizures; mental retardation; autism; benign tumors of the brain, retina, skin, and kidneys; and pulmonary lymphangiomyomatosis. Lymphangiomyomatosis is a life-threatening lung disease affecting almost exclusively young women. Genetic data have demonstrated that the cells giving rise to renal angiomyolipomas, the most frequent tumor type in patients with tuberous sclerosis complex, exhibit differentiation plasticity. Genetic studies have also shown that the benign smooth muscle cells of angiomyolipomas and pulmonary lymphangiomyomatosis have the ability to migrate or metastasize to other organs. These findings indicate that hamartin and tuberin play functional roles in the regulation of cell migration and differentiation. The biochemical pathways responsible for these effects are not yet fully understood but might involve dysregulation of the small guanosine triphosphatase Rho. Similar pathways might contribute to aberrant neuronal differentiation and migration in tuberous sclerosis complex. (*J Child Neurol* 2004;19:710–715).

Tuberous sclerosis complex is a tumor suppressor gene syndrome in which manifestations can include seizures, mental retardation, autism, and benign tumors of the brain, retina, kidney, heart, and skin.[1] The most frequent tumors include subependymal giant cell astrocytomas, facial angiofibromas, cardiac rhabdomyomas, and renal angiomyolipomas. Mutations in two genes, *TSC1* on chromosome 9q34[2] and *TSC2* on chromosome 16p13,[3] cause tuberous sclerosis complex. The clinical features of *TSC1*- and *TSC2*-linked disease are very similar,[4] although, generally, *TSC1*-linked disease tends to have milder clinical manifestations. Loss of heterozygosity in the *TSC1* or *TSC2* region occurs in most angiomyolipomas, rhabdomyomas, and astrocytomas from patients with tuberous sclerosis complex,[5] as expected, given their roles as tumor suppressor genes. Consistent with the clinical similarities between *TSC1*- and *TSC2*-linked disease, the protein products of the *TSC1* and *TSC2* genes, hamartin and tuberin, respectively, are known to physically interact[6,7] and appear to function as a complex.

Lymphangiomyomatosis, which occurs sporadically and in women with tuberous sclerosis complex, is characterized by widespread pulmonary proliferation of abnormal smooth muscle cells and cystic changes within the lung parenchyma.[8] The incidence of radiographic evidence of lymphangiomyomatosis among women with tuberous sclerosis complex is 26 to 39%,[9–11] although many women with radiographic evidence of lymphangiomyomatosis have only mild clinical symptoms. In a Mayo Clinic series, lymphangiomyomatosis was the third most frequent cause of tuberous sclerosis complex–related death, after renal disease and brain tumors.[12] Germline mutations in both *TSC1* and *TSC2* are associated with lymphangiomyomatosis, including missense mutations in the final exon of *TSC2* (exon 41).[10,13]

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RENAL ANGIOMYOLIPOMAS EXHIBIT DIFFERENTIATION PLASTICITY

Renal angiomyolipomas occur in most patients with tuberous sclerosis complex[4] and in approximately 50% of patients with sporadic lymphangiomyomatosis.[14] Angiomyolipomas are distinctive tumors with three components: dysplastic vessels, smooth muscle cells, and fat. Genetic analyses indicate that all three components are derived from a common progenitor cell.[15,16] Angiomyolipomas from patients with sporadic lymphangiomyomatosis contain vessels with at least five different morphologic patterns: cellular, hemangiopericytic, glomeruloid, aneurysmal, and collagenous.[16] These different vessel types have clear differences in diameter, wall thickness, lumen size, aneurysmal wall dilatations, collagen deposition, and glycogen deposition. To determine whether each vessel type is composed of neoplastic cells, we microdissected cells from the walls of each vessel type and analyzed the DNA for loss of heterozygosity. Four of the five vessel types (cellular, hemangiopericytic, glomeruloid, and aneurysmal) had loss of heterozygosity, and in each case, the loss of heterozygosity pattern was identical to the loss of heterozygosity pattern in the smooth muscle and fat cells.[16] This confirms that the cells in the walls of these vessels are neoplastic and supports a model in which angiomyolipomas are derived from a mesenchymal cell that retains the ability to differentiate into multiple different lineages. In contrast, collagenous vessels did not have loss of heterozygosity, consistent with an earlier study.[17] Loss of heterozygosity was also not detected in endothelial cells from cellular, aneurysmal, or collagenous vessels. The lack of a loss of heterozygosity in the endothelial cells might be related to the previously demonstrated expression of vascular endothelial growth factor by angiomyolipomas,[18] which could recruit genetically normal endothelial cells. Taken together, these results demonstrate that multiple genetic mechanisms contribute to angiomyolipoma blood vessel formation, including both neoplastic and non-neoplastic vessel wall formation and the recruitment of non-neoplastic endothelial cells.

To our knowledge, angiomyolipomas represent the only benign vascular tumor for which the vascular component has been shown to be neoplastic.[19] Studies of von Hippel-Lindau disease–associated hemangioblastomas and retinal angiomas have consistently demonstrated that the stromal cell component, not the vascular component, contains the second-hit mutation.[20–22] Tuberous sclerosis complex, therefore, appears to reflect a novel genetic mechanism of blood vessel formation.

"BENIGN METASTASIS" MODEL FOR PULMONARY LYMPHANGIOMYOMATOSIS

The occurrence of angiomyolipomas in both sporadic lymphangiomyomatosis and tuberous sclerosis complex led to the hypothesis that lymphangiomyomatosis and tuberous sclerosis complex have a common genetic basis. In 1998, loss of heterozygosity in the *TSC2* region of chromosome 16p13 was found in angiomyolipomas from patients with sporadic lymphangiomyomatosis,[23] consistent with this hypothesis. However, analysis of DNA from 21 women with sporadic lymphangiomyomatosis did not reveal germline *TSC2* mutations.[24] This led us to ask whether somatic, rather than germline, *TSC2* mutations were present in lymphangiomyomatosis cells. To address this possibility, DNA from seven sporadic lymphangiomyomatosis angiomyolipomas was analyzed. Inactivating *TSC2* mutations were detected in five of the seven angiomyolipomas, including four in which *TSC2* loss of heterozygosity had been previously detected, thereby validating Knudson's two-hit model in these sporadic lymphangiomyomatosis angiomyolipomas.[25] The *TSC2* mutations found in the angiomyolipomas were not present in morphologically normal kidney tissue or in the peripheral blood, indicating that they arose somatically.

Laser capture microdissection was used to isolate lymphangiomyomatosis cells and normal lung cells from the patients whose angiomyolipomas contained *TSC2* mutations. The same *TSC2* mutations present in the angiomyolipomas were found in the pulmonary lymphangiomyomatosis cells but not in the normal lung.[25] To further address whether pulmonary lymphangiomyomatosis cells and angiomyolipoma cells had a common origin, the region of loss of heterozygosity was compared between angiomyolipoma and lymphangiomyomatosis in two patients using a panel of 12 markers spanning chromosome 16. At each heterozygous marker, the pattern was the same (either retention of heterozygosity or loss of heterozygosity) between angiomyolipoma and pulmonary lymphangiomyomatosis cells.[26] Previous work has demonstrated that different tuberous sclerosis complex tumors have different somatic mutations and that these differences can often be detected using loss of heterozygosity analyses.[5,27,28] These data, therefore, are consistent with a model in which lymphangiomyomatosis cells and angiomyolipoma cells have a common genetic origin. These findings led to the hypothesis that pulmonary lymphangiomyomatosis results from the metastatic spread of histologically benign angiomyolipoma smooth muscle cells.

Sato and colleagues both confirmed and extended these observations. They studied 22 women with sporadic lymphangiomyomatosis. Germline *TSC2* mutations were not found in any of the patients with sporadic lymphangiomyomatosis, but one patient with sporadic lymphangiomyomatosis had a germline *TSC1* mutation.[29] This is of interest because germline *TSC1* mutations are known to cause a less severe clinical phenotype than germline *TSC2* mutations.[4] In addition, Sato and colleagues found *TSC1* or *TSC2* loss of heterozygosity in four of seven microdissected pulmonary lymphangiomyomatosis specimens. These results confirm that lymphangiomyomatosis cells from patients with sporadic lymphangiomyomatosis contain somatic *TSC1* or *TSC2* mutations. In a ninth patient, they tested a lymph node containing lymphangiomyomatosis and found different somatic point mutations in the two alleles of *TSC2* (in contrast to other patients,

in whom a point mutation was found on one allele; the remaining wild-type allele was "lost"). In this ninth patient, identical point mutations were found in five other lymph nodes containing lymphangiomyomatosis, strongly supporting the metastatic model.

RECURRENT LYMPHANGIOMYOMATOSIS AFTER LUNG TRANSPLANT: ADDITIONAL SUPPORT FOR THE METASTATIC MODEL

Additional data supporting a metastatic model of lymphangiomyomatosis pathogenesis were recently obtained from a patient with sporadic lymphangiomyomatosis who underwent lung transplant. We examined the genetic basis of recurrent lymphangiomyomatosis using laser capture microdissection and two genetic approaches: microsatellite marker fingerprinting and *TSC2* mutational analysis. The microsatellite patterns revealed that foci of recurrent lymphangiomyomatosis contained both patient-derived and donor-derived cells. To determine definitively the genetic origin of recurrent lymphangiomyomatosis cells after lung transplant, DNA from the native pulmonary lymphangiomyomatosis (before the lung transplant) was screened for mutations in all 41 exons of the *TSC2* gene. A one base-pair frame-shifting deletion in exon 18 was identified. Morphologically, normal bronchial epithelial cells from this patient did not contain the mutation, indicating that the exon 18 mutation in the lymphangiomyomatosis cells arose somatically.[30] This is consistent with previous studies in which patients with sporadic lymphangiomyomatosis were found to have somatic *TSC2* mutations.[25,29] This patient also had lymphangiomyomatosis in four separate mediastinal lymph nodes, which were removed at the time of diagnostic lung biopsy. All four lymph nodes also showed the exon 18 mutation and had a loss of heterozygosity in the *TSC2* gene region.[30] Microdissected recurrent lymphangiomyomatosis cells after the lung transplant also contained the exon 18 *TSC2* mutation.

The presence of the same *TSC2* mutation in pulmonary lymphangiomyomatosis, recurrent lymphangiomyomatosis, and lymph node lymphangiomyomatosis strongly supports the hypothesis that lymphangiomyomatosis cells migrate or metastasize in vivo, despite the fact that they are histologically benign. This challenges the conventional definition of malignant tumors as tumors with malignant potential and blurs the distinction between benign and malignant tumors. There are at least two other histologically benign diseases in which cells appear to metastasize: benign metastasizing leiomyoma and disseminated peritoneal leiomyomatosis.[31] Approximately 40% of patients with sporadic lymphangiomyomatosis do not have angiomyolipomas,[14] including the patient with recurrent lymphangiomyomatosis described above. To our knowledge, this patient represents the first time that a *TSC1* or *TSC2* mutation has been identified in a patient with lymphangiomyomatosis in whom the absence of angiomyolipomas has been documented. The inactivation of *TSC2* in the lymphangiomyomatosis cells from this patient challenges a model in which lymphangiomyomatosis cells migrate from the angiomyolipoma to the lung and raises the possibility that lymphangiomyomatosis cells can arise in other sites. The occurrence of lymphangiomyomatosis cells primarily in axial lymph nodes, lung, and kidney, rather than other sites, indicates a specific affinity for the cells of these organs.

In summary, the genetic data suggesting that lymphangiomyomatosis cells metastasize include (1) identical *TSC2* mutations and loss of heterozygosity patterns between lymphangiomyomatosis cells and angiomyolipoma cells and (2) identical *TSC2* mutations in native lymphangiomyomatosis cells and recurrent lymphangiomyomatosis cells after lung transplant. In addition, numerous studies have demonstrated that the abnormal smooth muscle cells of sporadic pulmonary lymphangiomyomatosis, tuberous sclerosis complex–associated pulmonary lymphangiomyomatosis, and renal angiomyolipomas are indistinguishable at the histologic, immunohistochemical, and ultrastructural levels.[12,32]

TSC1 AND *TSC2* GENE PRODUCTS, HAMARTIN AND TUBERIN, ACTIVATE Rho

If lymphangiomyomatosis cells containing *TSC2* gene mutations have the potential to migrate in vivo, this might indicate that the tuberous sclerosis complex proteins normally function as inhibitors of cellular migration or metastasis. *TSC2* encodes tuberin, a 200 kDa protein that functions in a complex with hamartin, the product of the *TSC1* gene. Hamartin and tuberin function in multiple cellular pathways in mammalian cells, including vesicular trafficking,[33] regulation of the G1 phase of the cell cycle,[34–38] corticosteroid hormone regulation,[39] and Ras-related homolog (Rho) activation.[40,41] Tuberin has a highly conserved domain with homology to Ras-related protein (rap)1 guanosine triphosphatase activating protein (GAP), and it has been shown to possess GAP activity for Rap1A,[42] rabaptin (Rab)[55,33] and Ras homolog enriched in brain (Rheb).[43–46] Hyperphosphorylation of p70S6 kinase and/or its substrate, ribosomal protein S6, occurs in cells lacking hamartin from a murine model of *TSC1*,[47] in cells lacking tuberin from the Eker rat model of *TSC2*,[48,49] and in tumor cells containing *TSC2* mutations,[16] demonstrating that the hamartin-tuberin complex negatively regulates p70S6 kinase. Studies in *Drosophila*[37,38,50] and mammalian cells suggest that regulation of p70S6 kinase by the hamartin-tuberin complex is mediated by the mammalian target of rapamycin (mTOR),[51,52] although controversy exists because one group found that it is mTOR independent.[53]

Hamartin interacts with the ezrin-radixin-moesin (ERM) family of cytoskeletal proteins and activates the guanosine triphosphatase Rho.[40] The Rho family of guanosine triphosphatases (cdc42, Rac1, and Rho) regulates the cytokine-induced reorganization of the actin cytoskeleton and is critical to cell migration, invasion, and metastasis.[54–57] We found that expression of tuberin, the product of the *TSC2*

tumor suppressor gene, is also associated with activation of Rho.[41] ELT3 cells, which lack endogenous tuberin, are derived from the Eker rat model of *TSC2*. ELT3 cells in which tuberin was stably re-expressed had increased Rho activation, increased cell attachment, and decreased cell migration. Increased cell attachment and decreased cell migration are consistent with activation of pathways downstream of Rho. We hypothesize that abnormal Rho regulation in cells lacking tuberin results in aberrant cell migration and is central to the pathogenesis of lymphangiomyomatosis. It is also possible that Rho participates in the aberrant cellular differentiation seen in renal angiomyolipomas and the pathogenesis of the central nervous system manifestations of tuberous sclerosis complex.

The individual roles of hamartin and tuberin in activating Rho are not yet understood. Hamartin stabilizes tuberin by protecting tuberin from ubiquitin-mediated degradation, and tuberin can also stabilize hamartin, but to a substantially lesser extent.[58] If tuberin is the key effector in Rho activation, the previously observed Rho activation by hamartin could have resulted from stabilization of tuberin by hamartin. Consistent with this model, it has been reported that hamartin activates Rho via amino acids 145 to 510,[40] a region that overlaps with hamartin's tuberin-interaction domain (amino acids 302–430).[59,60]

WHAT CAN RENAL AND PULMONARY TUBEROUS SCLEROSIS COMPLEX TELL US ABOUT THE PATHOGENESIS OF NEUROLOGIC DISEASE IN TUBEROUS SCLEROSIS COMPLEX?

Renal and pulmonary tuberous sclerosis complex appear to involve aberrant cellular migration and differentiation, potentially related to dysregulation of Rho signaling, as detailed in the sections above. Aberrant neuronal migration and differentiation are also hallmarks of neurologic disease in tuberous sclerosis complex. Cerebral cortical tubers have been pathologically categorized with neuronal migration disorders[61] and/or cortical dysplasia,[62] and conditional astrocyte knockout of *TSC1* leads to abnormal neuronal organization.[63] Cells from tuberous sclerosis complex lesions exhibit heterogeneous expression patterns,[64] including the inappropriate expression of embryonic markers,[65] suggesting defective terminal differentiation. Whether Rho dysregulation contributes to the neurologic manifestations of tuberous sclerosis complex is unknown. Intriguingly, Rho and Rho family members are believed to play pivotal roles in neuronal morphogenesis and migration,[66–68] and mutations in p21-activated kinase 3 (PAK3), a Rho effector, are a cause of X-linked mental retardation.[69] In conclusion, the underlying pathogenesis of renal and pulmonary tuberous sclerosis complex might be closely linked to the pathogenesis of the neurologic manifestations of tuberous sclerosis complex.

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Special Article

Molecular Pathogenesis of Tuber Formation in Tuberous Sclerosis Complex

Peter B. Crino, MD, PhD

ABSTRACT

Tuberous sclerosis complex results from mutations in the *TSC1* (hamartin) and *TSC2* (tuberin) genes. Tubers are cortical developmental malformations in patients with tuberous sclerosis complex that are associated with intractable epilepsy and are composed of histologically distinct cell types, including giant cells and dysplastic neurons. We recently showed that tubers can be dynamic lesions characterized by populations of cells undergoing proliferation, migration, and death. We demonstrate that there is cell-specific activation of the mammalian target of rapamycin (mTOR)/p70S6 kinase/ribosomal S6 cascade in tubers and that giant cells express activated (phosphorylated) p70S6 kinase and ribosomal S6 protein. These findings support impaired hamartin- and tuberin-mediated mTOR pathway regulation. Tubers likely form by constitutive activation of the mTOR cascade during brain development as a consequence of impaired hamartin or tuberin function. (*J Child Neurol* 2004;19:716–725).

Tuberous sclerosis complex is an autosomal disorder resulting from mutations in the *TSC1* or *TSC2* genes.[1,2] Although tuberous sclerosis complex affects multiple organ systems, the neurologic and psychiatric symptoms of tuberous sclerosis complex clearly cause the most significant patient morbidity.[3] Many individuals with tuberous sclerosis complex exhibit mental retardation and autism,[4] and over 75% of patients with tuberous sclerosis complex develop seizures.[5] Examination of the brain of patients with tuberous sclerosis complex demonstrates cortical tubers, focal developmental malformations of the cerebral cortex characterized by disorganized lamination, and the presence of a unique cell type, giant cells, which exhibit cytomegaly.[6] Adjacent to giant cells are smaller dysplastic neurons, which are characterized by aberrant dendritic arborizations and a dysmorphic cell body. Tubers can be multiple or solitary lesions; however, the cerebral cortex surrounding tubers exhibits intact cytoarchitecture.

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TUBER FORMATION AND CELLULAR PHENOTYPE

Tubers are cortical developmental lesions that likely form during the midgestational portions of corticogenesis (Figure 1). A single histopathologic study has shown that a tuber can be detected as early as 19 weeks' gestation,[7] and more recent advances in fetal magnetic resonance imaging (MRI) have demonstrated tubers by gestational weeks 20 to 26. Given that tubers typically affect only a restricted region of the cerebral cortex, it has been proposed that tuber formation likely occurs in later rather than earlier epochs of cortical development. The reason is that an early event affecting cortical progenitor cells would have more time to undergo mitosis and thus generate a larger lesion. In the rare cases of hemispheric tubers in tuberous sclerosis complex, it is conceivable that these lesions form earlier in cortical development.

Several studies have attempted to define the phenotype of cells in tubers. There is obvious cellular heterogeneity in tubers, with giant cells, dysmorphic neurons, and astrocytes noted. Giant cells are the hallmark histologic cell type within tubers that are unique to tuberous sclerosis complex. They are large (80–150 μm in diameter) polygonal or ovoid cells that exhibit glassy eosinophilic cytoplasm and that extend short, thickened processes of unclear identity

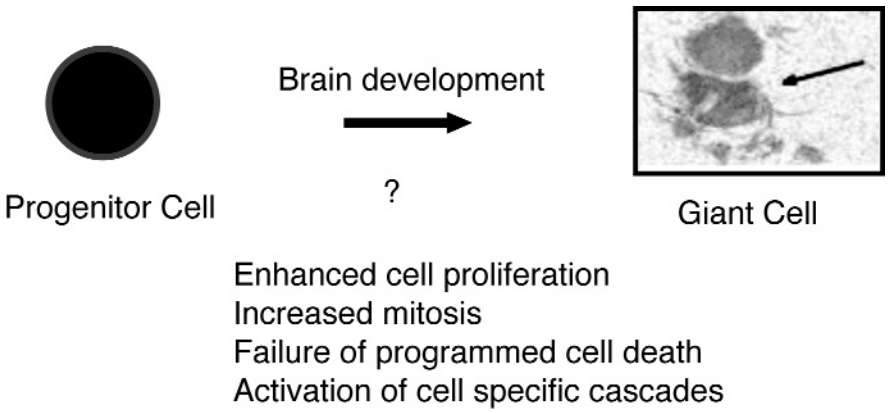

Figure 1. Possible cell mechanisms leading to giant cell formation from progenitor cells during brain development, including enhanced cell proliferation and increased mitosis, failure of programmed cell death, and activation of cell-specific cascades leading to cytomegaly.

(ie, axons or dendrites). Giant cells are distributed from the pial surface to the subcortical white matter without clear radial or laminar orientation. They do not exhibit preference for superficial or deeper parts of cortex.

A pivotal issue in tuberous sclerosis complex research has been defining the cells of origin for giant cells and dysmorphic neurons. Whereas dysmorphic neurons more clearly exhibit phenotypic features consistent with neurons, giant cells are more complex. Dysmorphic neurons express a variety of neuronal markers, for example, cytoskeletal elements, neurotransmitter receptor subunits that suggest that they are indeed of the neuronal phenotype. Early evidence argued independently for neuronal or glial lineage of giant cells based on identification of neuronal or glial structural features using electron microscopy.[8–10] For example, the presence of rough endoplasmic reticulum, intermediate filaments extending into processes of ambiguous morphology, prominent paranuclear Golgi zones, and dense-core granules (suggestive of secretory vesicles) in giant cells are suggestive of neuronal features. In contrast, giant cells have been shown by immunohistochemistry to express both glial and astrocytic markers. Recent immunohistochemical and molecular analyses have identified neuronal messenger ribonucleic acids (RNAs) and proteins such as neuron-specific enolase, tubulin, microtubule-associated proteins (MAPs), and intermediate filaments in giant cells within tubers.[11–13] Coversely, a subpopulation of giant cells in tubers is immunoreactive for glial marker proteins, such as glial fibrillary acidic protein (GFAP), vimentin, or S-100, which suggests an astrocytic phenotype. CD44, a cell adhesion molecule, mediates cell-cell and cell-matrix interactions and is expressed near giant cells in tubers.[14] CD44 is expressed in astrocytic processes, predominantly in white-matter and subpial regions, suggesting its involvement in the maintenance of a stable central nervous system cytoarchitecture. Giant cells in tubers also express messenger RNA and proteins that are typically found in immature neurons and neuroepithelial precursor cells, such as nestin,[13] MAP2C,[9] and the *N*-methyl-D-aspartate (NMDA) 2D receptor subunit.[15] One possible explanation is that these markers indicate that giant cells might have failed to terminally differentiate prior to migration into cortex or that they have retained an immature cellular phenotype. Tuber sections probed with antibodies recognizing the neural marker NeuN, a DNA binding protein present in mature neurons, exhibit a heterogeneous staining pattern.[16] Some giant cells and most dysmorphic neurons exhibit NeuN immunolabeling, but a small proportion are unlabeled, suggesting either a mixed neuroglial phenotype or that these cell types are phenotypically immature.

ROLE OF GROWTH FACTORS IN TUBER FORMATION

Neurotrophins and their cognate receptors, including brain-derived neurotrophic factor, nerve growth factor, neurotrophin 3, and neurotrophin 4, and trks A to C, comprise a family of proteins that modulate cell proliferation, differentiation, migration, and process outgrowth during cortical development and, thus, are ideal candidate genes to investigate in tubers. We hypothesized that expression of select neurotrophins would be altered in tubers resected from patients with tuberous sclerosis complex with medically intractable epilepsy and that these changes can be defined in select cell types.[17] We demonstrated that observed alterations in neurotrophin messenger RNA expression predicted changes in protein expression in tubers using Western blotting and immunohistochemistry. The relative abundance of neurotrophin 3 and trkB messenger RNAs was reduced, and the relative abundance of neurotrophin 4 and trkC messenger RNAs was increased in whole tuber sections compared with control cortex. Changes in the abundance of neurotrophin messenger RNAs identified in whole tuber sections predicted altered levels of neurotrophin 4 and trkC by Western analysis in frozen tuber homogenates. The expression of neurotrophin messenger RNAs was distinct in microdissected dysmorphic neurons and giant cells when compared with control neurons and highlighted the cellular specificity of the changes in messenger RNA expression determined in whole sections. Neurotrophin 4 messenger RNA expression was increased and trkB messenger RNA expression was reduced in dysmorphic neurons compared with giant cells and control neurons. Neurotrophin 3 messenger RNA levels were reduced in giant cells when

compared with dysmorphic neurons and control neurons. In contrast, trkC messenger RNA abundance was increased in both dysmorphic neurons and giant cells when compared with control neurons. These results identify differential expression of neurotrophin genes and proteins that might be central to the pathogenesis of tuber formation and that might directly reflect loss of hamartin-tuberin function.

EPILEPTOGENESIS AND TUBERS

The number of cortical tubers in patients with tuberous sclerosis complex is highly correlated with an increased risk of epilepsy and increased seizure severity.[5,18] Although a putative imbalance between excitatory and inhibitory synaptic transmission can be linked with seizure initiation in tubers, the mechanistic relationship between seizures and tubers has not been characterized. Until recently, the pharmacologic features of dysmorphic neurons and giant cells in tubers had not been addressed. Moreover, the electrophysiologic properties of dysmorphic neurons and giant cells (eg, excitatory versus inhibitory) have not been investigated, and, to date, there has been no direct electrophysiologic analysis or characterization of dysmorphic neurons or giant cells in acute slice preparations from tubers obtained intraoperatively. One early study showed variable GAD65 and NR 1 receptor immunoreactivity in "tuberous sclerosis complex–like lesions."[19] Another important question is to what extent giant cells and dysmorphic neurons participate in synaptic connectivity or electrophysiologic activity in tubers. Synaptophysin immunoreactivity has been reported along the cell membrane of giant cells.[20] One compelling question is whether giant cells are capable of synaptic transmission and, if so, whether giant cells potentiate excitatory or inhibitory activity. Electron microscopic analyses have also identified desmosomal or gap junction–like connections between giant cells and surrounding neurons,[10] and giant cells express the gap junction messenger RNA connexin 26.[13]

We were the first laboratory to define the expression of messenger RNAs encoding the glutamate (NMDA receptor and glutamate receptors [GluRs]) and γ-aminobutyric acid $(GABA)_A$ receptor subunit messenger RNAs, as well as the neuronal glutamate (EAAC1) and the vesicular GABA transporter in tubers (Figure 2).[15] We hypothesized that genes encoding glutamate and $GABA_A$ receptor subunits and uptake sites were differentially expressed in tubers. We found increased expression of EAAC1, GluR3 and GluR6, and NMDA 2B and 2D receptor subunit messenger RNAs and diminished levels of GAD65, vesicular GABA, GluR1, and $GABA_A R\ \alpha_1$, and α_2, in tubers ($P < .05$) compared with control neocortex. Ligand binding experiments in tuber homogenates with the NMDA 2B selective ligand ifenprodil revealed increased NMDA 2B sites in tubers and thus corroborated increased NMDA 2B messenger RNA expression. EAAC1 and GAD65 messenger RNA expression was corroborated by immunohistochemistry in tuber sections. These findings demonstrate that there is altered expression of EAAC1, GluR, NR, and $GABA_A R$ messenger RNAs in tubers and support the hypothesis that seizures can be generated in tubers as a consequence of enhanced excitability.

MOLECULAR NEUROBIOLOGY OF TUBEROUS SCLEROSIS COMPLEX

The identification of the *TSC1* and *TSC2* genes has aided in understanding the molecular events that lead to tuber formation. Hamartin messenger RNA and protein are widely expressed in normal tissues, including brain, liver, adrenal cortex, cardiac muscle, skin, and kidney. Hamartin is highly expressed in G(0)-arrested cells and throughout the ongoing cell cycle. Hamartin likely interacts directly with tuberin and can be localized to cytoplasmic vesicles.[21] Recent studies suggest that hamartin interacts with the ezrin-radixin-moesin (ERM) family of actin binding proteins and thus might play an important role in mediating cell-cell interactions, cell adhesion, and, potentially, cell migration.[22] Interaction of endogenous hamartin with ERM family proteins is required for activation of the intracellular signaling protein Ras-related homolog (Rho). Inhibition of hamartin function in vitro disrupts the formation and maintenance of focal adhesions and results in loss of attachment to the cell substrate. In contrast, hamartin overexpression in vitro in cells lacking focal adhesions results in activation of the small guanosine triphosphate binding protein Rho, assembly of actin stress fibers, and formation of focal adhesions.

Tuberin messenger RNA and protein are widely expressed in normal tissues, including brain, liver, adrenal cortex, cardiac muscle, skin, and kidney.[23] Tuberin messenger RNA and protein have been detected throughout the developing and adult brain (eg, cortical and hippocampal pyramidal neurons, cerebellar Purkinje cells, brainstem motor nuclei, choroid plexus epithelium, and spinal cord).[23,24] In the mouse, tuberin expression is greatest during embryogenesis and in non-neuronal tissues (eg, lymphocytes and epithelia, which undergo high mitotic turnover, suggesting a role in cell division). In patients with tuberous sclerosis complex, alterations in tuberin messenger RNA or protein expression have been reported in tubers.[25,26] Tuberin immunoreactivity was moderate to strong in neurons and reactive astrocytes of control brains but was reduced in brains with tuberous sclerosis. A surprising finding in some patients with tuberous sclerosis complex is the detection of intense tuberin immunoreactivity in giant cells within tubers.

Tuberin contains a hydrophobic N-terminal domain and a conserved 163–amino acid carboxy-terminal region that exhibits sequence homology to the catalytic domain of a guanosine triphosphatase activating protein (GAP) for Ras-related protein 1 (Rap1). As a member of the superfamily of Ras-related protein, Rap1 likely functions in regulation of DNA synthesis and cell-cycle transition. Tuberin displays GAP activity for Rap1 but not Rap2, Ha-Ras, Rac, or Rho and colocalizes with Rap1 in the Golgi apparatus in several cell lines.[27,28] The GAP activity of functional tuberin can modu-

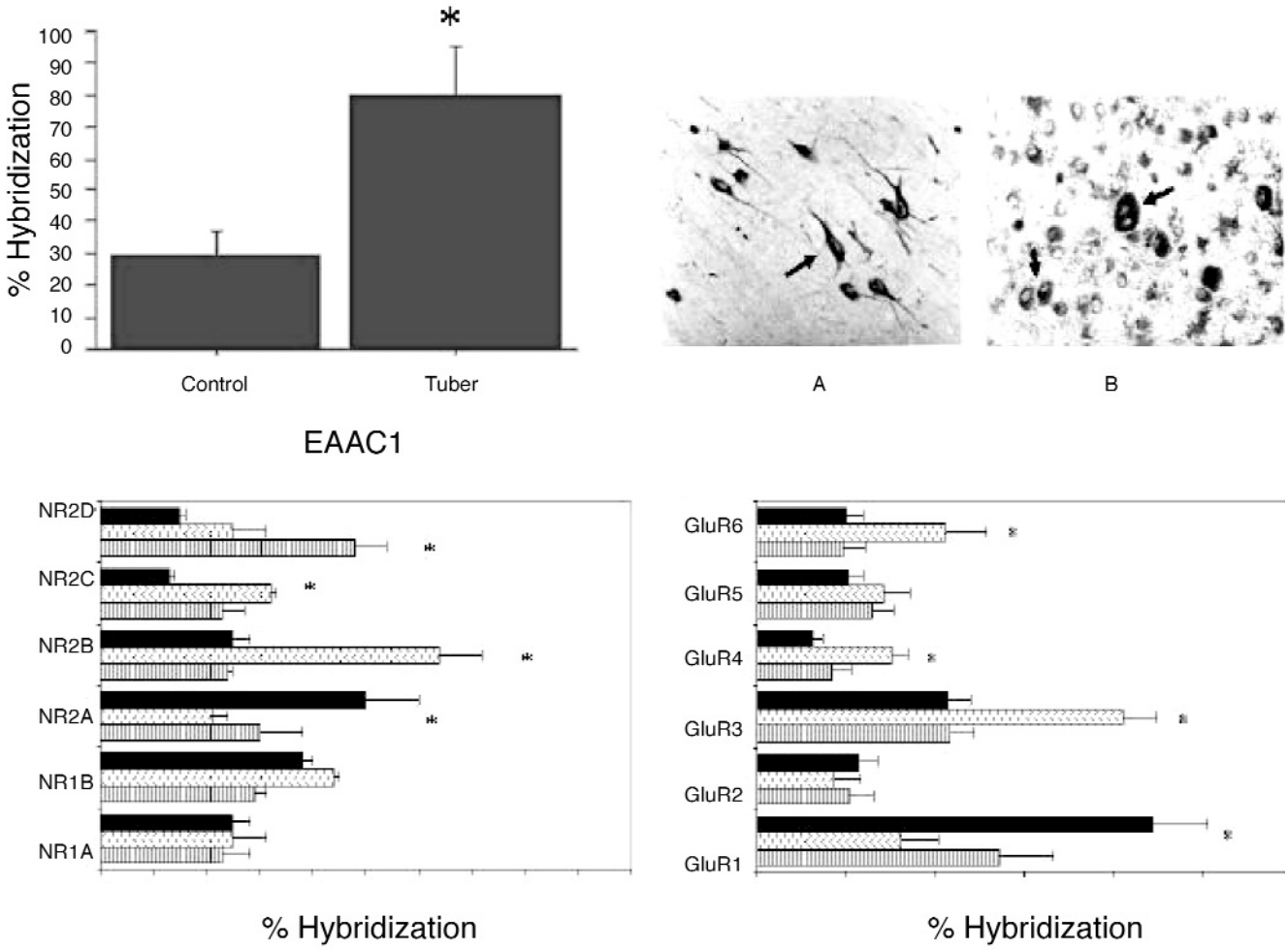

Figure 2. *Top left,* increased expression of EAAC1 messenger ribonucleic acid (RNA) in tuber versus control cortex sections. *Top right,* increased EAAC1 immunoreactivity (*arrows*) was demonstrated in dysmorphic neurons (*A*) and giant cells (*B*). *Bottom left,* differential expression (mean percentage of hybridization intensity) of *N*-methyl-D-aspartate (NR), and *bottom right,* glutamate receptor (GluR) subunit messenger RNAs in single microdissected control neurons (*black*), dysmorphic neurons, (*vertical-hatch*), and giant cells (*horizontal hatch;* $*P < .05$).

late the effects of Rap1 on G- to S-phase transition during cell division. Thus, *TSC2* mutations might result in constitutive activation of Rap1. For example, antisense inhibition of tuberin expression in cultured fibroblasts induced quiescent G0-arrested cells to re-enter the cell cycle and shortened the time in G1 of actively dividing cells.[29] In neuroblastoma cell lines, antisense inhibition of tuberin expression inhibited neuronal differentiation.[30] Loss of hamartin or tuberin function following *TSC1* or *TSC2* mutations can result in enhanced proliferation of neural and astrocytic precursor cells and increased cell size characteristic of dysplastic neurons and giant cells. Enhanced cell size can compromise neuronal migration and account for the loss of lamination within tubers. Alternatively, loss of hamartin or tuberin function can independently compromise neural migration via an interaction with ERM or actin binding proteins. Recently, it has been shown that hamartin and tuberin interact with the G2/M cyclin-dependent kinase 1 and its regulatory cyclins, A and B,[31] and, thus, might alter the kinetics of cell division.

ACTIVATION OF mTOR/p70S6 KINASE/RIBOSOMAL S6 CASCADE IN TUBEROUS SCLEROSIS COMPLEX

Mutations in *TSC1* or *TSC2* likely have a significant impact on neuronal proliferation, differentiation, and migration.[32] Recent studies in *Drosophila* suggest that hamartin and tuberin form a functional heteromeric complex that is an important component of a pathway that modulates insulin receptor– or insulin-like growth factor–mediated signaling.[33,34] This pathway functions downstream of the cell signaling molecule Akt to regulate cell growth and potentially cell size (Figure 3). Hamartin and tuberin interact via a coiled-coil domain encoded in the carboxy region of hamartin that permits a direct protein-protein interaction with tuberin.[35] The hamartin and tuberin heteromeric complex constitutively inhibits the mammalian target of rapamycin (mTOR)/p70S6 kinase/ribosomal S6 cascade that contributes to ribosomal assembly and protein translation.[36,37] The mTOR/p70S6 kinase/ribosomal S6 pathway is downstream of the insulin- and insulin-like growth factor receptors and likely serves as

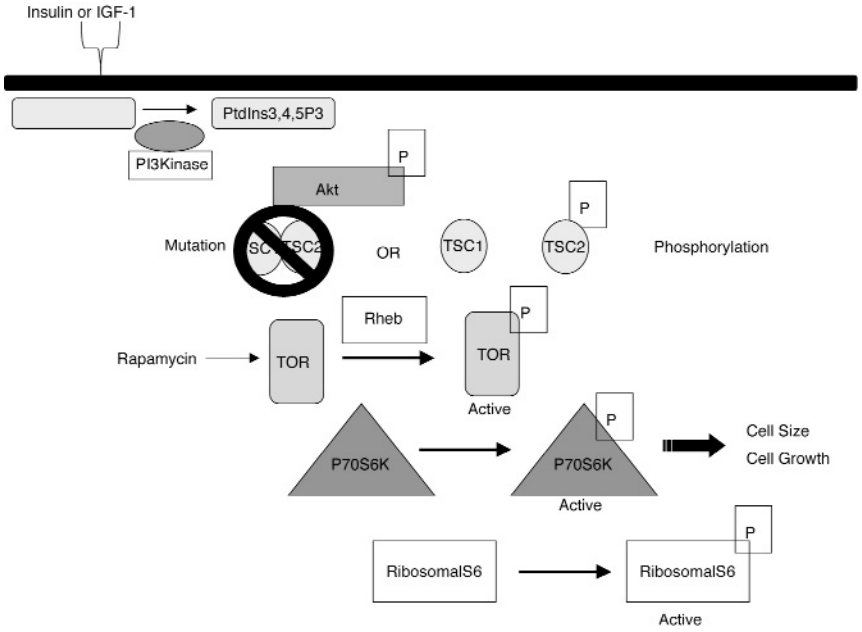

Figure 3. Schematic diagram depicting activation of the mammalian target of rapamycin (mTOR)/p70S6 kinase/ribosomal S6 pathway in tuberous sclerosis complex. Activation of the mTOR pathway proceeds under normal conditions from growth factors such as insulin-like growth factor (IGF) via phosphatidylinositol-3 (PI3) kinase activation of phosphotadylinositol. The hamartin-tuberin complex acts to constitutively inhibit further activation of the cascade. In the setting of loss of function mutations or phosphorylation of tuberin by Akt, the complex dissociates, and via Rheb (Ras homolog expressed in brain), p70S6 kinase is activated by phosphorylation. Activation of p70S6 kinase leads to activation (by phosphorylation) of ribosomal S6 protein, a component of the 40S ribosomal subunit. The net result of this path is enhanced protein translation and increased cell size.

a regulator of cell proliferation, cell size, and organogenesis.[38,39] Negative modulation of this cascade by hamartin and tuberin results in growth suppression and restricted cell size. However, phosphorylation (activation) of mTOR, p70S6 kinase, and ribosomal S6 is observed following tuberin inactivation by Akt-mediated phosphorylation in vitro or in cells lacking tuberin or hamartin in vitro.[40,41] Phosphorylation of ribosomal S6 protein is increased in cytomegalic neuroepithelial cells derived from *Tsc2* knockout mice (but not in neuroepithelial cells from heterozygote animals)[42] and in renal angiomyolipoma cells from patients with tuberous sclerosis complex in vitro.[43]

MOLECULAR PATHOGENESIS OF TUBER FORMATION

An intensive debate surrounds the molecular mechanisms governing tuber formation during brain development. A pivotal question is whether tubers derive from a single neuroglial progenitor cell or whether tubers form from a population of cell types. That is, are all cells in tubers clonally derived, or are they a nonclonal mixture of cell types? Loss of heterozygosity at either *TSC* gene locus demonstrated in hamartomas in other organ systems from patients with tuberous sclerosis complex supports a two-hit mutational mechanism that results in biallelic *TSC* gene inactivation.[44–46] In contrast, loss of heterozygosity in tubers has not been conclusively demonstrated,[44,47] although reports of variably reduced hamartin or tuberin expression in tubers suggest that loss of hamartin or tuberin likely underlies tuber formation.[48–50] Thus, although a two-hit mechanism might be responsible for formation of other tuberous sclerosis complex lesions and in other tumor phenotypes, it is not known whether tubers also require biallelic inactivation. A recent study has demonstrated that enhanced phosphorylation of tuberin can provide another mechanism for tuberin inactivation.[51] A key technical limitation in defining whether tubers form as a consequence of biallelic inactivation is that loss of heterozygosity is not a sensitive assay to detect single base-pair mutations that occur commonly in *TSC1* and *TSC2*.[52] Another complicating issue is that only a subpopulation of cells in tubers (eg, giant cells) can form as a consequence of a somatic *TSC* gene mutation, and, thus, the admixture of giant cells and dysmorphic neurons in tubers further confounds attempts to identify second-hit *TSC* gene mutations when using tuber tissue homogenates.

We used immunohistochemistry to define activation of the mTOR/p70S6 kinase/ribosomal S6 cascade because a marker of diminished hamartin-tuberin function has not been studied in tubers. Tubers were obtained from 15 patients with clinically diagnosed tuberous sclerosis complex (mean age 8.25 years; 9 boys and 6 girls) undergoing surgery for the treatment of intractable epilepsy.[53] Histologic examination in all specimens revealed abnormal cortical lamination and the presence of giant cells. Control frontal lobe neocortex was obtained postmortem from four patients who died of non-neurologic causes without a history of tuberous sclerosis complex or epilepsy (mean age 20.7 years; 2 men and 2 women; average postmortem interval to autopsy 14 hours). Human tissue studies were approved by the University of Pennsylvania Institutional Review Board and Committee on Human Research.

Paraffin-embedded, 7 μm sections from human tissue specimens were immunolabeled with phospho-S6 (serine 235 and 236, 1:500 dilution) and p70S6 kinase (threonine 389, 1:200 dilution; Cell Signaling, New England Biolabs, Beverly, MA) antibodies overnight at 4°C and were visualized using avidin-biotin conjugation (Vectastain ABC Elite, Vector Labs, Burlingame, CA) with 3,3´-diaminobenzidine.

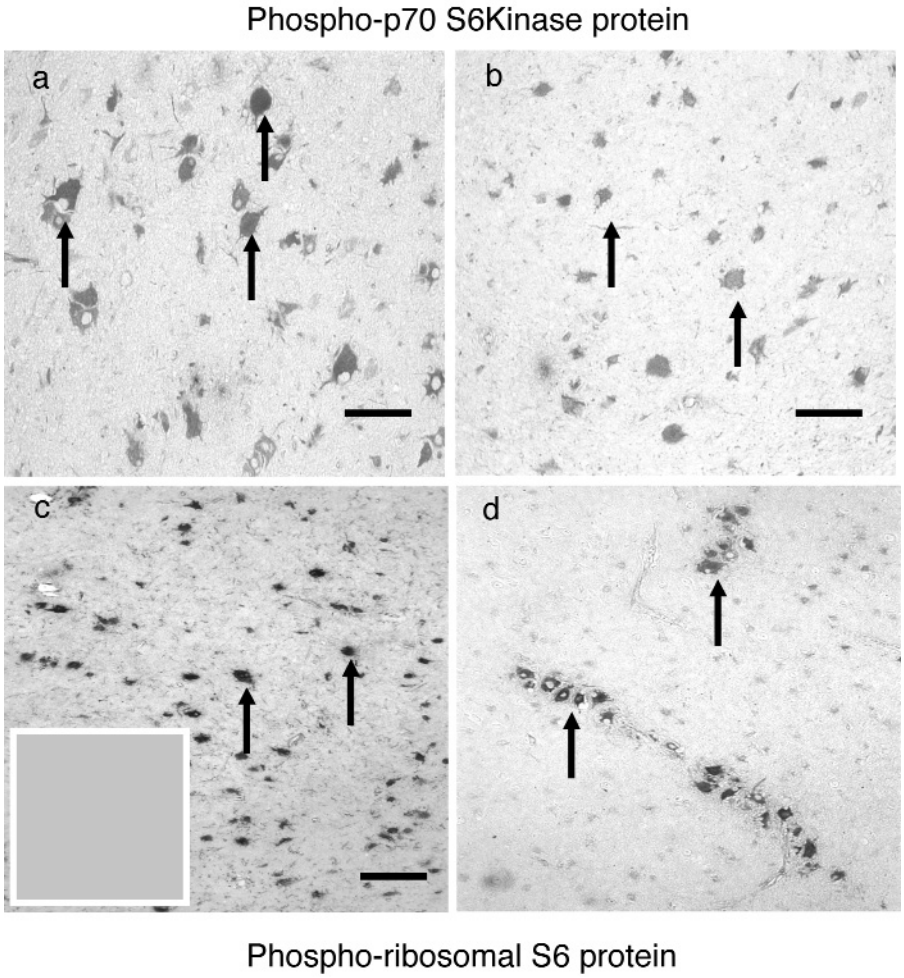

Figure 4. Selective activation of the mammalian target in rapamycin (mTOR) pathway in giant cells in human tubers. *A* and *B,* Phospho-p70S6 kinase immunoreactivity in giant cells in human tubers (*arrows*). Scale bars = 200 μm. *C* and *D,* Phospho-S6 immunoreactivity in giant cells in two tuber specimens (*arrows*). *Inset,* minimal phospho-S6 labeling in control cortex. Scale bars = 350 μm.

There was robust expression of phospho-S6 in giant cells, but not in adjacent dysmorphic neurons, in 15 human tuber specimens (Figure 4). Virtually all giant cells within the 15 tuber samples exhibited phospho-S6 immunoreactivity. There was a virtual absence of phospho-S6 immunoreactivity in the control cortex. In further support of cell-specific mTOR pathway activation, we found selective expression of phosphorylated p70S6 kinase in giant cells but not in adjacent dysmorphic neurons or in control tissue samples (see Figure 4). These results demonstrate that giant cells represent a cell subpopulation in human tubers in which there is selective activation of the mTOR pathway as a consequence of altered hamartin-tuberin function. Selective expression of phosphorylated p70S6 kinase and ribosomal S6 in giant cells across 15 tuber specimens from 15 patients with tuberous sclerosis complex provides a compelling anatomic image of a selected cell type in tubers that lacks normal hamartin-tuberin function.

Our results provide a new view of how tubers can form during brain development. Although all cells in each patient with tuberous sclerosis complex express a common germline mutation, focal lesions in the kidney, heart, and eye in tuberous sclerosis complex form in the setting of somatic, inactivating *TSC* mutations.[45,46] We propose a model in which hamartin or tuberin inactivation of neuroepithelial progenitor cells in the embryonic brain results in constitutive activation of mTOR signaling and leads to cytomegaly (giant cells) in the progeny derived from this progenitor cell, as has been modeled in the *Drosophila dTsc2* mutant *gigas*.[33] In contrast, the mTOR pathway is not activated in adjacent neuroepithelial cells, and these progeny migrate into the cortical plate and likely become dysmorphic neurons. Given that giant cells are found throughout the full thickness of tubers, we can assume that these cells migrate, albeit abnormally, into the cortical plate. Further studies will be necessary to define second-hit *TSC* gene mutations to determine whether cell-specific bialleleic gene inactivation occurs. An alternative hypothesis is that additional mutations in other genes that modulate the mTOR pathway might also contribute to tuber formation.

ARE TUBERS DYNAMIC LESIONS IN THE BRAIN?

Several studies have shown that giant cells in tubers, subependymal nodules, and subependymal giant cell astrocytomas express proteins that are characteristic of neuroglial progenitor cells, such as nestin and vimentin.[9,13] In an attempt to further define the developmental phenotype

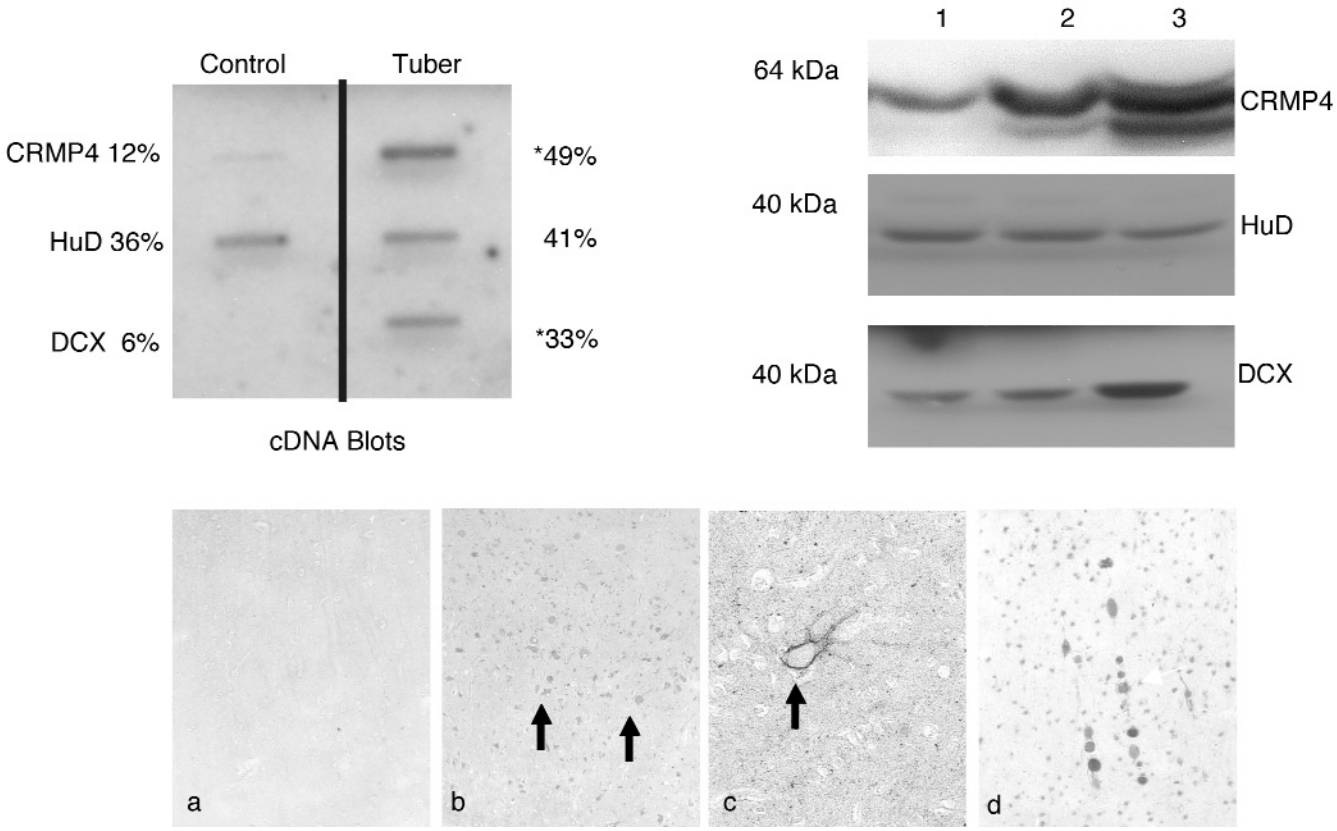

Figure 5. Expression of collapsin response mediator protein 4 (CRMP4), HuD, and doublecortin (DCX) in tubers. *Top left,* increased expression of CRMP4 and doublecortin messenger ribonucleic acid (RNA) in whole tuber homogenates (*$P < .05$). There was no change in expression of HuD messenger RNA. *Top right,* enhanced expression of CRMP4 and doublecortin protein in whole tuber homogenates but no change in HuD. *Bottom, A,* CRMP4 expression in control cortex; *B,* CRMP4 in a giant cell in a tuber; *C,* CRMP4 expression in a single giant cell; *D,* doublecortin expression in giant cells in subcortical white matter. cDNA = complementary deoxyribonucleic acid.

of cells in tubers, we recently evaluated the expression of several markers, incuding p34cdc2, collapsin response mediator protein 4 (CRMP4), doublecortin, LIS-1, HuD, and NeuN in tuber and subependymal giant cell astrocytoma specimens (Figure 5).[16] These markers were selected because they are expressed at distinct neurodevelopmental epochs, such as neuroglial cell mitosis (p34cdc2, CRMP4), neural migration (doublecortin, LIS-1), and neural differentiation (HuD, NeuN). The majority of dysmorphic neurons exhibited HuD and NeuN immunolabeling consistent with a differentiated neural phenotype. In contrast, giant cells exhibited CRMP4, doublecortin, and LIS-1 immunoreactivity. Increased expression of CRMP4 and doublecortin proteins was demonstrated in tubers by Western analysis. Interestingly, CRMP4 and doublecortin expression was also observed in subependymal nodules and subependymal giant cell astrocytomas from patients with tuberous sclerosis complex, suggesting that these lesions share a common cellular origin during development and can be derived from the subventricular zone. CRMP4, doublecortin, and LIS-1 expression can identify newly generated cells that actively migrate from the subventricular zone or from subependymal nodules and subependymal giant cell astrocytomas into tubers, where they then differentiate and express NeuN or HuD. Thus, tubers can be dynamic lesions that contain a subpopulation of newly generated cells. Recurrent seizures in the rodent are associated with ectopic migratory pathways of doublecortin-labeled neurons into the forebrain.[54] White-matter abnormalities adjacent to tubers[55] can highlight the pathway of migrating neurons, and, in fact, many of the doublecortin or CRMP4 immunoreactive cells were in the subcortical white matter, possibly en route to tubers. Tuber and subependymal nodule number are positively correlated,[56] and although there is no current evidence to suggest that tubers can enlarge, the variable MRI characteristics of tubers, in concert with the finding of multiple proliferative markers, suggest that these lesions can be dynamic over time. There might be an interplay between progenitor cells in the subventricular zone that gives rise to cells in both subependymal nodules and subependymal giant cell astrocytomas and tubers. Indeed, the detection of similar marker proteins in subependymal nodules and subependymal giant cell astrocytomas and tubers might indicate that cells in subependymal lesions migrate into tubers.

In a separate set of experiments, we found that there is evidence of ongoing cell death in tubers.[57] Using gene

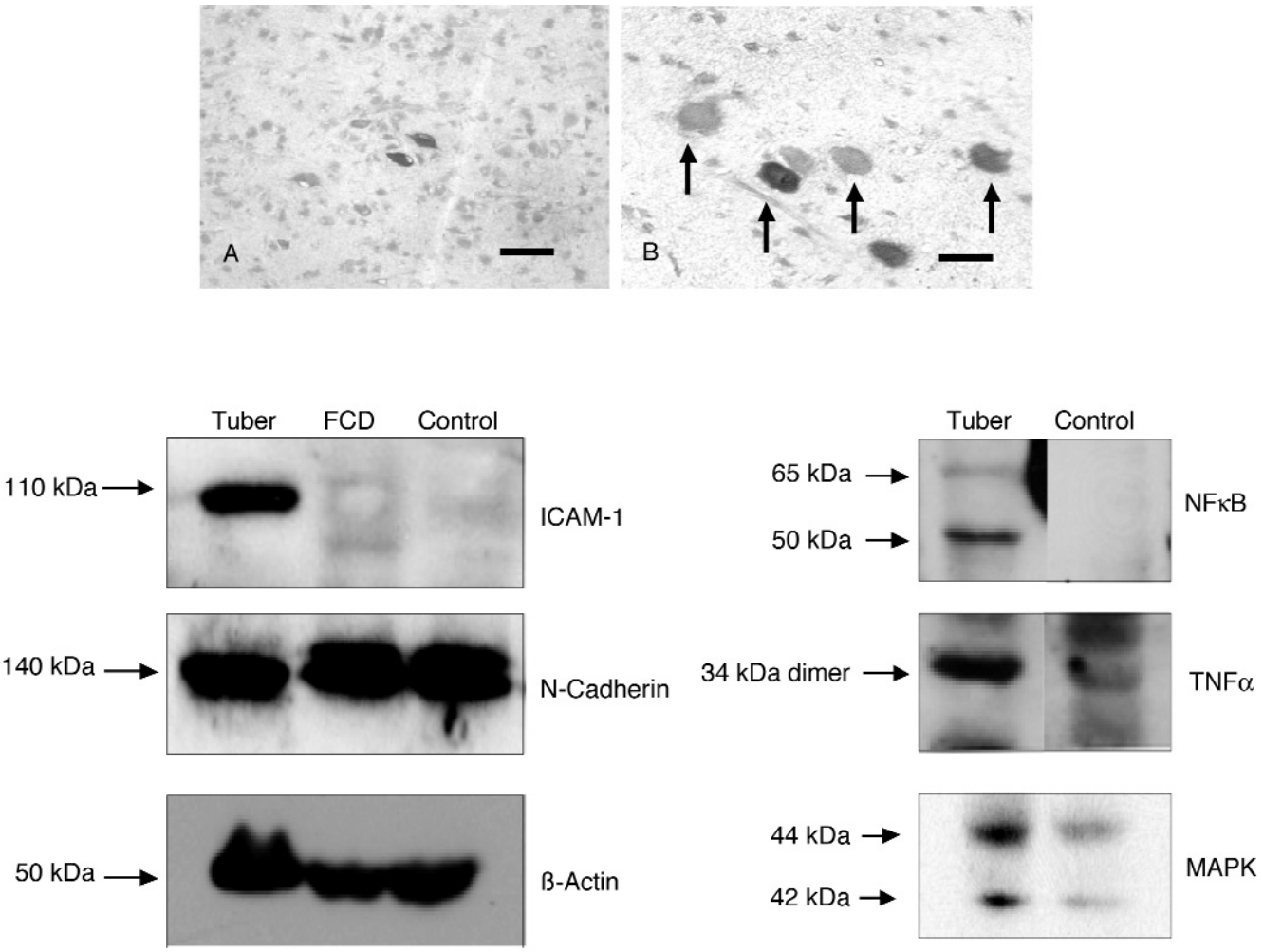

Figure 6. Selective expression of intercellular adhesion molecule 1 (ICAM-1) in giant cells in tubers. *Top,* note enhanced intercellular adhesion molecule 1 immunoreactivity in single giant cells (*A* and *B, arrows*). Scale bar: *A,* 300 μm; *B,* 80 μm. *Bottom,* increased levels of intercellular adhesion molecule 1 in tuber homogenate compared with non–tuberous sclerosis complex focal cortical dysplasia (FCD) and control cortex (N-cadherin and β-actin serve as protein loading controls). Note increased levels of nuclear factor κB (NFκB), tumor necrosis factor α (TNFα), and activated mitogen-activated protein kinase (MAPK) by Western assay in tubers.

expression profiling analysis, we found that expression of intercellular adhesion molecule 1, a cell-surface glycoprotein that functions in cytokine signaling, is enhanced in tubers (Figure 6). Western and immunohistochemical analysis revealed that intercellular adhesion molecule 1 protein was selectively expressed in tubers but was only minimally expressed in control cortex, adjacent nontuberal cortex, or non–tuberous sclerosis complex focal cortical dysplasia. Expression of molecules involved in intercellular adhesion molecule 1 activation and signaling was increased in tubers, including tumor necrosis factor α, mitogen-activated protein (MAP) kinase, and nuclear factor κB. We demonstrated similar increased expression of these cytokine-related proteins in mice in which the *Tsc1* gene was conditionally inactivated in astrocytes. The specific alterations in intercellular adhesion molecule 1, tumor necrosis factor α, nuclear factor κB1, and MAP kinase expression, coupled with the presence of numerous CD68 immunoreactive macrophages clustered around giant cells, suggest that tuber formation might involve activation of proinflammatory cytokine signaling pathways. Tuber specimens obtained intraoperatively were then stained with TUNEL, a marker for ongoing cell death and antibodies that recognize caspase 8, a pivotal catalyst in the extrinsic cell death pathway (Figure 7). Numerous cells exhibited TUNEL and caspase 8 labeling consistent with the notion that a population of cells might undergo cell death in tubers. Thus, the detection of cell proliferation and cell death markers in tubers might suggest that tubers are, indeed, dynamic lesions that are subject to progressive changes in cell populations.

CONCLUSIONS

Critical future studies in tuberous sclerosis complex will need to focus on how changes in mTOR cascade expression during brain development can lead to cytomegaly. Another

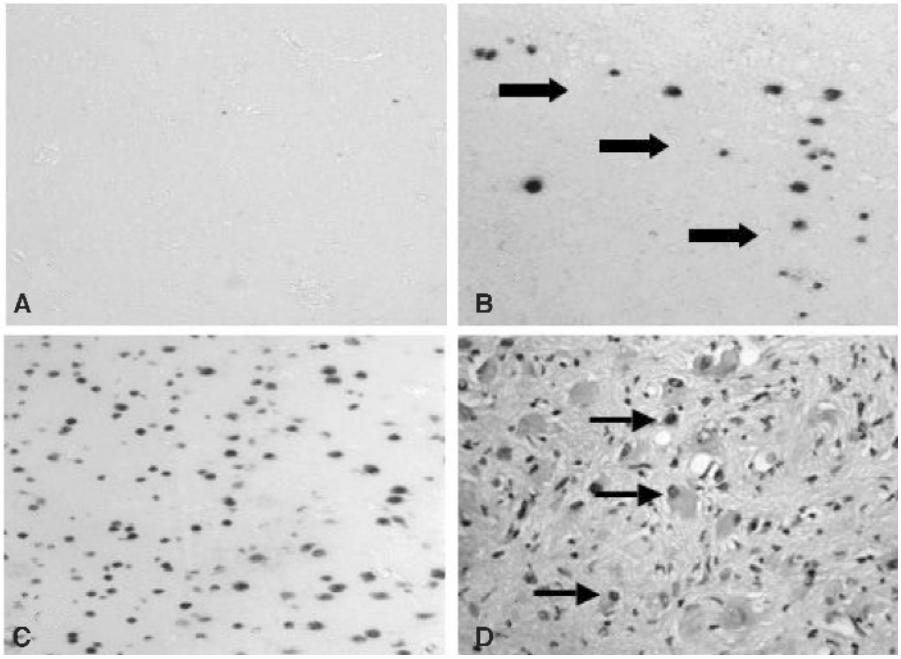

Figure 7. *A,* Minimal caspase 8 expression in control cortex. *B,* Border zone (*arrows*) between perituberal cortex (*left of arrows*) and tuber (*right of arrows*). Note the increase in activated caspase 8 immunolabeling in the tuber (*right portion of figure*). *C,* Numerous activated caspase 8–labeled cells in tubers. *D,* TUNEL-positive cells in tubers. Some giant cells and dysmorphic neurons exhibit TUNEL reactivity, whereas others do not. Rare giant cells exhibit morphologic features of apoptosis (arrows). Numerous smaller cell types (astrocytes and macrophages) express TUNEL as well.

important area of research will be to define the developmental epoch in which tuber formation is initiated because this timepoint can shed light on the particular cell types that can be affected in tuberous sclerosis complex. In older patients, further studies to prove that tubers are dynamic lesions and that cells in subependymal nodules can migrate to tubers seem warranted. Finally, a deeper understanding of the role that astrocytes play in tuber formation and in epileptogenesis also seems pivotal. A realistic goal in the future will be to define the mechanisms of tuber formation so that there can be strategies to prevent tuber formation.

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Special Article

Mouse Models of Tuberous Sclerosis Complex

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ABSTRACT

The most devastating complications of tuberous sclerosis complex affect the central nervous system and include epilepsy, mental retardation, autism, and glial tumors. Mutations in one of two genes, *TSC1* and *TSC2*, result in a similar disease phenotype by disrupting the normal interaction of their protein products, hamartin and tuberin, which form a functional signaling complex. Disruption of these genes in the brain results in abnormal cellular differentiation, migration, and proliferation, giving rise to characteristic brain lesions called cortical tubers. Relevant animal models, including conventional and conditional knockout mice, are valuable tools for studying the normal functions of tuberin and hamartin and how disruption of their expression gives rise to the variety of clinical features that characterize tuberous sclerosis complex. In the future, these animals will be invaluable preclinical models for the development of highly specific and efficacious treatments for children affected with tuberous sclerosis complex. (*J Child Neurol* 2004;19:726–733).

Tuberous sclerosis complex is an autosomal dominant tumor predisposition syndrome that affects approximately 1 in 7500 individuals worldwide.[1] Tuberous sclerosis complex is characterized by benign, hamartomatous growths in multiple organs, including the kidney, skin, retina, lung, and brain. Rarely, malignant tumors, including renal cell carcinoma, can develop. Although it has been nearly a decade since linkage analyses first revealed the two genetic loci associated with tuberous sclerosis complex, the mechanisms by which disruption of these genes results in the variety of tuberous sclerosis complex–associated abnormalities remain poorly understood. Current efforts aimed at developing preclinical animal models of tuberous sclerosis complex have provided important insights into the pathogenesis of tuberous sclerosis complex and will serve as useful tools for studying potential therapies for this disorder.

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NERVOUS SYSTEM ABNORMALITIES IN TUBEROUS SCLEROSIS COMPLEX

Clinically, the central nervous system complications of tuberous sclerosis complex are the most disabling. Cortical tubers are the pathognomonic lesions that characterize tuberous sclerosis complex. Tubers are detected as high-intensity signals on T_2-weighted magnetic resonance images (MRIs) that are often located at the junctions between the gray and white matter. The size and location of cortical tubers have been suggested to correlate with the major central nervous system manifestations of tuberous sclerosis complex: seizures, mental retardation, and autism.[2–5] The number and location of cortical tubers and the age at seizure onset are highly correlated with neurologic outcome. Individuals who experience seizures at an earlier age have poorer cognitive outcomes.[2,6]

Epilepsy occurs in approximately 80% of affected individuals. Infants affected with tuberous sclerosis complex commonly present with partial motor seizures or infantile spasms within the first year of life.[7] An increase in seizure frequency and severity is common during early childhood. Tuberous sclerosis complex–associated infantile spasms frequently evolve into other seizure types, including partial motor, complex partial, and secondarily generalized seizures, with age.[8] Epileptogenic foci identified by electroencephalography frequently correlate with cortical tubers visualized by MRI.[9,10] In addition, the progression from infantile spasms to other seizure types might reflect the formation of tubers at different times during cortical development.

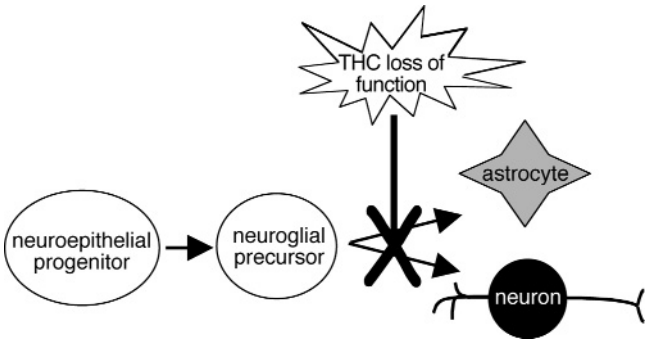

Figure 1. Inactivation of the tuberin-hamartin complex (THC) alters cellular differentiation in the central nervous system. Neurons and astrocytes arise from neuroglial precursors during development. Central nervous system lesions associated with tuberous sclerosis complex contain giant cells that express a variety of cellular markers, including those characteristic of immature nervous system cells. This suggests that disruption of the tuberin-hamartin complex by tuberin or hamartin loss interferes with the normal maturation of precursor cells and impairs their ability to differentiate into more developmentally mature neurons or astrocytes.

Thus, tubers in areas of the brain that become functionally mature earlier (eg, temporal or occipital regions) can become epileptogenic before tubers located in brain regions that mature more slowly, including the frontal lobes.[8] Because clinicians cannot accurately visualize each of these lesions, predicting the progression of epilepsy in patients with tuberous sclerosis complex is difficult.

Tuberous sclerosis complex–associated seizures are often refractory to conventional pharmacologic therapies. Improved seizure control has been achieved using the γ-aminobutyric acid (GABA) agonist vigabatrin in some patients with tuberous sclerosis complex.[11] Surgical treatment might be helpful for selected patients, especially those who have a single epileptogenic tuber.[12]

Pathologic evidence suggests that cortical tubers represent areas of abnormal neuronal differentiation and migration. Because surrounding areas of cortex are not disrupted, it has been hypothesized that tubers result from the defective differentiation, migration, and proliferation of a subpopulation of precursor cells (Figure 1). Tubers are formed early in development and have been identified as early as 20 weeks of gestation.[13] Because tubers often disrupt normal cortical lamination, it has been suggested that tubers reflect a defect in cell migration occurring early in brain development.

Tubers contain dysmorphic neurons, increased numbers of astrocytes, and characteristic "giant cells". The cellular origin of these giant cells is not known, but they can arise from a neuroglial precursor cell because giant cells often express both mature neuronal and astrocyte proteins. Many giant cells also express proteins found in immature central nervous system cells, including nestin and vimentin.[14–16] Similarly, the morphology of giant cells is variable.[17] Recent evidence suggests that cells of cortical tubers can undergo active proliferation and that, despite their early formation, these lesions can be more dynamic than previously assumed.[16]

In addition to cortical tubers, individuals affected with tuberous sclerosis complex develop low-grade astrocytic neoplasms. Subependymal nodules are benign, proliferative lesions that line the surface of the lateral ventricles. Unlike the cortical tubers, these lesions are often asymptomatic but can develop into subependymal giant cell astrocytomas. Although not frankly malignant, subependymal giant cell astrocytomas can cause ventricular obstruction and hydrocephalus, which require neurosurgical intervention.[18] In addition to glial cells, subependymal nodules and subependymal giant cell astrocytomas can also contain giant cells. These lesions, like cortical tubers, are thought to develop early in life and have been identified as early as 27 weeks of gestation.[19] Like cortical tubers, these lesions are thought to arise from abnormal development of precursor cells during brain development.

TSC GENES

Linkage studies have identified two distinct loci that undergo mutational inactivation in individuals with tuberous sclerosis complex.[20] The clinical tuberous sclerosis complex phenotype occurs when inactivating mutations occur in one of the two genes, *TSC1* or *TSC2*. The *TSC1* gene is located on chromosome 9q34. The gene contains 23 exons and encodes the 130 kDa hamartin protein.[21] Hamartin has little sequence homology to other known proteins. The *TSC2* gene on chromosome 16p13 encodes the 180 to 200 kDa tuberin protein.[22] Tuberin contains a small region with sequence similarity to proteins with guanosine triphosphatase activating protein function. Guanosine triphosphatase activating proteins are molecules that negatively regulate small guanosine triphosphatase proteins related to the Ras oncogene. These Ras-like molecules are active when bound to guanosine triphosphate and are inactivated by the conversion of guanosine triphosphate to guanosine diphosphate by guanosine triphosphatase. Guanosine triphosphatase proteins accelerate the conversion of the guanosine triphosphate–bound active form to the inactive guanosine diphosphate–bound form by activating this intrinsic guanosine triphosphatase activity of Ras and related proteins.

Both hamartin and tuberin contain predicted coiled-coil domains that mediate their interaction to form the tuberin-hamartin protein complex.[23,24] The formation of this complex is critical for tuberin and hamartin functions, and tuberous sclerosis complex–associated mutations frequently disrupt this interaction and render the tuberin-hamartin complex functionally inactive. Identified *TSC* gene mutations include missense and nonsense mutations, in-frame deletions, and large deletions.[25,26] Whereas familial cases of tuberous sclerosis complex have an approximately equal distribution of families with *TSC1* and *TSC2* mutations, *TSC2* mutations are approximately four times more common in sporadic cases.[27] Because two thirds of all patients with tuberous sclerosis complex have sporadic mutations, the high frequency of *TSC2* mutations might reflect a higher intrinsic mutation rate for the *TSC2* gene.

The observation that individuals who are born with a mutated copy of the *TSC1* or *TSC2* gene in all cells of their bodies are predisposed to develop tumors in several different organs, including the central nervous system, suggested that these genes act as tumor suppressors. Tumors develop in these individuals when one or more somatic cells undergo a "second hit" and the remaining, wild-type *TSC* gene is inactivated. This loss of *TSC1* or *TSC2* expression results in abnormal cell growth and proliferation. Indeed, hamartomas from patients with both familial and sporadic forms of the disease have shown loss of the normal copy of *TSC1* or *TSC2*, termed loss of heterozygosity, indicating that these genes act as classic tumor suppressors.[21,28,29] In vitro studies have also supported the role of these genes as tumor suppressors. When either tuberin or hamartin is overexpressed in cultured cells, the cells undergo growth arrest.[30–32] Similarly, down-regulation of *TSC2* expression in cultured fibroblasts by antisense inhibition resulted in increased cell proliferation.[33]

Tuberin and hamartin are highly expressed in the central nervous system of humans and mice in both neurons and glial cells.[34,35] Tuberin and hamartin appear to be required for central nervous system development, and tuberin might be required for neuronal differentiation.[36] Similar to other organs, these proteins likely act as tumor suppressors in the central nervous system because tuberin expression is reduced or absent in 30% of sporadic astrocytomas.[37] There have been conflicting reports as to whether somatic mutations of the wild-type *TSC1* or *TSC2* allele in a precursor cell are required for cortical tuber formation in patients who have only one normal copy of one of the two *TSC* genes.[28,29,38] Because these lesions are composed of many different cell types, it can be difficult to identify the subset of cells that have undergone a second mutational event. In support of the hypothesis that two genetic "hits" are required for tuberous sclerosis complex–associated tuber formation, recent evidence suggests that, in mice, neuroepithelial cell progenitors that lack *TSC2* expression have many features of the giant cells found in cortical tubers.[39]

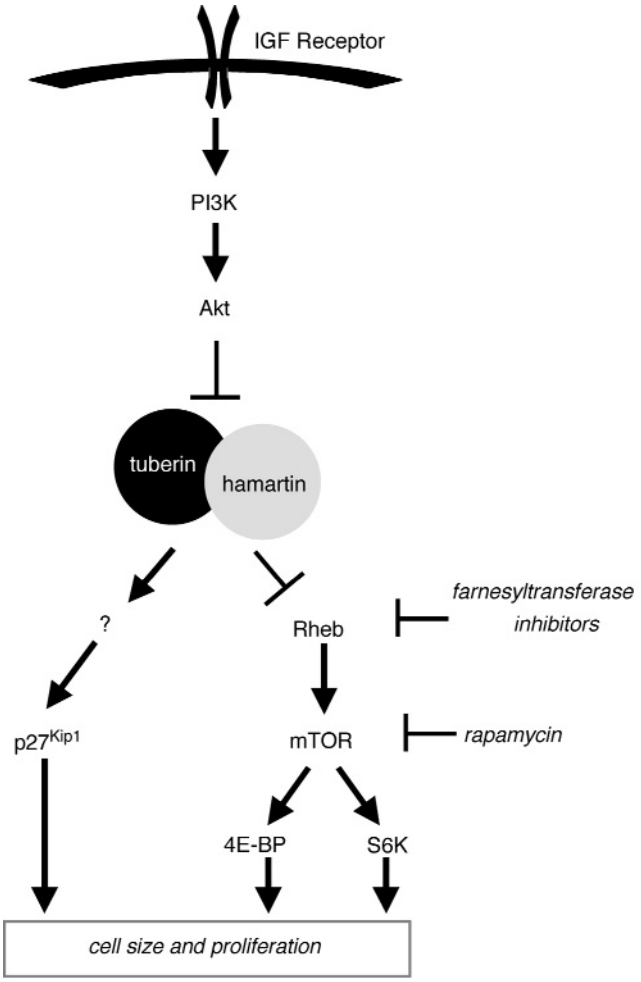

Figure 2. Functions of the tuberin-hamartin complex. The tuberin-hamartin complex negatively regulates Rheb, a small guanosine triphosphatase that activates the mammalian target in rapamycin (mTOR)/S6 kinase (S6K)/factor 4E binding protein (4E-BP) 1 signaling cascade important for the regulation of cell size and proliferation. In addition, the tuberin-hamartin complex may regulate the expression of the cyclin D kinase inhibitor $p27^{Kip1}$ involved in both cell growth and size control. To relieve the tuberin-hamartin complex–mediated inhibition of cell size and proliferation, phosphatidylinositol-3 kinase (PI3K) and Akt are activated following stimulation of the insulin-like growth factor (IGF) receptor. Akt phosphorylates tuberin, inactivating the complex and resulting in increased cell size and proliferation.

FUNCTION OF THE TUBERIN-HAMARTIN COMPLEX

The two abnormal cellular phenotypes in tuberous sclerosis complex involve an increase in both cell proliferation and cell size. Insights into how the tuberin-hamartin complex might regulate these processes first came from studies in *Drosophila*. When the homologs of *TSC1* or *TSC2* were mutated in flies, organogenesis proceeded normally, but dramatic increases in organ size were observed.[40,41] These size defects were attributed to both an increase in cell proliferation, determined by measuring the number of mitotic cells, and an increase in individual cell size when *TSC1* was mutated.[41] Overexpression of both *TSC1* and *TSC2* in these flies rescued these defects. Because the insulin signaling pathway had been linked to the regulation of cell growth and proliferation,[42] the possibility that the tuberin-hamartin complex might regulate this signaling pathway seemed plausible. In support of this hypothesis, overexpression of *TSC1* or *TSC2* rescued lethality in *Drosophila* mutants in which the insulin receptor was nonfunctional and reversed the cell proliferation defects of flies that overexpressed the insulin receptor.[41,43,44]

Additional studies, first in *Drosophila* and then in mammalian cells, indicated that the tuberin-hamartin complex functions in the insulin-like growth factor receptor pathway downstream of phosphatidylinositol-3 kinase and Akt (Figure 2). This pathway is known to regulate both cell proliferation and cell size. When the insulin receptor is activated, phosphatidylinositol-3 kinase is recruited to the membrane and triggers the production and release of the second

messenger phosphatidylinositol 3,4,5-triphosphate. Akt, a serine/threonine kinase, is activated by phosphatidylinositol 3,4,5-triphosphate. Akt has been shown to phosphorylate tuberin, resulting in its dissociation from hamartin and inactivation of the tuberin-hamartin complex.[45–48] The functional tuberin-hamartin complex normally inhibits the activity of the mammalian target of rapamycin (mTOR).[39,46,47,49] However, when the tuberin-hamartin complex is inactivated by Akt-mediated phosphorylation of tuberin, mTOR inhibition is relieved. Activation of mTOR has been shown to trigger the phosphorylation of ribosomal S6 kinase and factor 4E-binding protein 1.[50,51] Activation of these proteins results in an increase in protein synthesis and, ultimately, cell growth. In cells in which the tuberin-hamartin complex is permanently inactivated owing to genetic mutation, increased levels of phosphorylated S6 kinase and factor 4E binding protein 1 are constitutively present, resulting in unregulated cell growth. Several research groups have demonstrated that *TSC1* and *TSC2* mutant cells have elevated levels of phosphorylated S6 kinase and factor 4E binding protein 1, whereas overexpression of *TSC1* or *TSC2* inhibits S6 kinase pathway hyperactivation.[39,46,47,52] S6 kinase phosphorylation is also increased in cells that express a *TSC2* gene containing a human *TSC* gene mutation.[53]

How the tuberin-hamartin complex regulates mTOR, however, remained unclear until Rheb (Ras homolog enriched in brain), a Ras-like guanosine triphosphatase (GTPase), was identified as a target of tuberin guanosine triphosphatase activating protein activity in *Drosophila*.[53–58] Rheb is required for cell-cycle progression and cell growth in *Drosophila*.[59] In the presence of the tuberin-hamartin complex, the guanosine triphosphate bound to Rheb is hydrolyzed to guanosine diphosphate, resulting in Rheb inactivation. When the tuberin-hamartin complex is inactivated, either by Akt-mediated tuberin phosphorylation or genetic mutation, Rheb is constitutively active, resulting in up-regulation of mTOR-dependent pathways, resulting in increased cell size. In this regard, increased S6 kinase phosphorylation in Rheb-overexpressing cells can be blocked by treatment with rapamycin, a drug that inhibits mTOR.[54]

Although all of the functions of Rheb in mammalian cells are not understood, post-translational farnesylation of Rheb is required for Rheb activity and cell-cycle progression.[60] In this fashion, farnesyltransferase inhibitors that block the post-translational activation of Rheb have been shown to inhibit mTOR-dependent S6 kinase activity.[54] The mechanism by which Rheb regulates mTOR is not understood and is presently an area of active investigation.

In addition to the effects on cell size, Rheb overexpression resulted in an increase in cell proliferation in *Drosophila*.[61,62] It is not known if this effect of Rheb is mTOR dependent. In mammalian cells, cyclin-dependent kinases are required for the transition from a quiescent state to one of active proliferation. Inhibitory molecules, such as $p27^{Kip1}$, regulate these kinases. The expression of the cyclin-dependent kinase inhibitor $p27^{Kip1}$ is reduced in cells in which *TSC1* or *TSC2* expression is decreased.[36,63] Tuberin can regulate nuclear localization of $p27^{Kip1}$ because the molecule is mislocalized to the cytoplasm in tuberin-deficient fibroblasts.[36] Moreover, inactivation of $p27^{Kip1}$ in mice is associated with increases in both cell size and proliferation.[64] It is not clear whether Rheb or mTOR is required for regulation of $p27^{Kip1}$ expression.

ANIMAL MODELS OF TUBEROUS SCLEROSIS COMPLEX

Despite advances in understanding the signaling activities of these proteins using *Drosophila* genetic approaches and in vitro systems, a more desirable approach is to analyze the signaling and developmental roles of tuberin and hamartin in vivo, both individually and as a protein complex, using animal models. Initial attempts at studying these proteins in an animal model were performed in Eker rats. These animals carry a spontaneous germline mutation in the rat homolog of the human *TSC2* gene and have been used as a model of hereditary renal cell carcinoma.[65] These animals develop bilateral renal tumors and subependymal nodules.[66] Unfortunately, genetic manipulations of rats have been hampered by a lack of suitable methods for generating targeted mutations.[67] In contrast, methods for generating mice with disease-associated genetic changes are well established and have been widely used to study tumor suppressor gene function.[68] Not only do transgenic mice allow for analysis of disruption or overexpression of single genes, but these mice can be interbred to assess the effects of multiple genetic changes. In this way, these genetically engineered mice can serve as useful preclinical models for the study of disease pathophysiology and potential therapies.

Initial attempts to recapitulate the human tuberous sclerosis complex phenotype in mice were performed using conventional knockout mice in which germline expression of *Tsc1* or *Tsc2* was inactivated. Mice homozygous for loss of *Tsc1* or *Tsc2* die in midembryogenesis of apparent cardiac malformations and liver hypoplasia.[52,69,70] Heterozygous animals are viable but develop renal and liver tumors. $Tsc2^{+/-}$ mice developed tumors at younger ages than $Tsc1^{+/-}$ animals. This finding is interesting in light of the observation that patients who harbor *TSC2* mutations often develop more severe disease than those with *TSC1* mutations.[71] In the central nervous system of $Tsc2^{+/-}$ and $Tsc1^{+/-}$ mice, the numbers of astrocytes were increased, suggesting that hamartin and tuberin are important astrocyte growth regulators.[63] However, when grown in vitro, $Tsc2^{+/-}$ astrocytes did not demonstrate a cell autonomous growth advantage. Expression of the cell cycle–associated protein $p27^{Kip1}$ was reduced in $Tsc2^{+/-}$ astrocytes compared with wild-type astrocytes, suggesting that tuberin might regulate cell growth via regulation of $p27^{Kip1}$ expression.[63] In addition, compound heterozygotes that lack one copy of both *Tsc1* and *Tsc2* had further increases in the number of astrocytes. Together, these observations support the role of tuberin and hamartin as regulators of cell growth and proliferation in the central nervous system.

GENERATION OF A MOUSE MODEL OF TUBEROUS SCLEROSIS COMPLEX NEUROLOGIC DISEASE

The observations that reduced expression of tuberin or hamartin resulted in abnormal astrocyte proliferation, that individuals affected with tuberous sclerosis complex frequently develop astrocytic tumors (subependymal giant cell astrocytomas), and that *TSC2* expression is reduced or absent in 30% of high-grade sporadic astrocytomas[37] suggested that *TSC* gene expression in astrocytes might be of particular importance to the central nervous system defects that characterize this disease. Because conventional knockout mice for either of these two genes die before brain development is complete, it is not possible to study astrocyte defects in these animals. To circumvent this, our laboratory has used a conditional knockout approach to inactivate *Tsc1* in astrocytes (Figure 3). In this approach, two recombinase recognition sequences (LoxP sites) are inserted into the noncoding region of the *Tsc1* gene (termed "floxed" *Tsc1* allele) and therefore do not affect hamartin production. When mice expressing this allele are crossed with mice that express the Cre recombinase protein under the control of a cell type–specific promoter (eg, astrocytes), the LoxP sites are cleaved, and the *Tsc1* gene is inactivated only in those cells expressing Cre recombinase.

To study the role of hamartin in astrocyte growth regulation and function, mice that express a "floxed" *Tsc1* gene were crossed with mice that express Cre recombinase under the control of the human glial fibrillary acidic protein (Gfap) promoter, which is expressed predominantly in astrocytes. The resulting *Tsc1*; *Gfap*-Cre conditional knockout mice exhibit abnormal astrocyte cell size, proliferation, differentiation, and neuronal excitability.

Although the *Tsc1* conditional knockout mice do not have frank cortical tubers, these animals do have defects in astrocyte proliferation and cell size regulation.[72] Brains from these animals have an increased number of GFAP-immunoreactive astrocytes by 3 weeks of age and four times as many actively proliferating astrocytes at 6 weeks of age. The hippocampus exhibited the most pronounced enlargement in these animals and demonstrated significant architectural disorganization. Astrocytes from *Tsc1* conditional knockout mice grown in vitro exhibit a growth advantage at confluence, suggesting that the normal inhibitory cues that trigger cells to stop dividing when they become too densely packed are defective in hamartin-deficient astrocytes. Similar to studies of $Tsc1^{+/-}$ and $Tsc2^{+/-}$ cells, this increased cell proliferation was associated with decreased expression of the cyclin-dependent kinase inhibitor $p27^{Kip1}$. In addition to cell proliferation defects, astrocytes from *Tsc1* conditional knockout mice also have abnormal cell size regulation when grown in culture.[73]

Tsc1 conditional knockout mice also exhibited defects in cellular differentiation in the brain. Nestin is an intermediate filament protein expressed in central nervous system precursor cells. Astrocyte cultures derived from the brains of *Tsc1* conditional knockout animals have an increased number of nestin-immunoreactive cells compared with control cultures, suggesting that the cells were developmentally immature. Analysis of brains from these mice shows increased expression of the glial precursor markers vimentin and brain fatty acid binding protein.[74] These changes parallel expression changes in cortical tubers from patients with tuberous sclerosis complex and support previous findings that giant cells express markers of precursor cells and might be proliferative.[14–16] The finding that hamartin deficiency in astroglial precursor cells recapitulates many of the molecular changes observed in the human disease suggests that tuberous sclerosis complex–associated brain lesions might be related to abnormal differentiation of a neuroglial precursor cell. The mechanism(s) underlying tuberin and hamartin regulation of neuroglial cell differentiation is not completely understood.

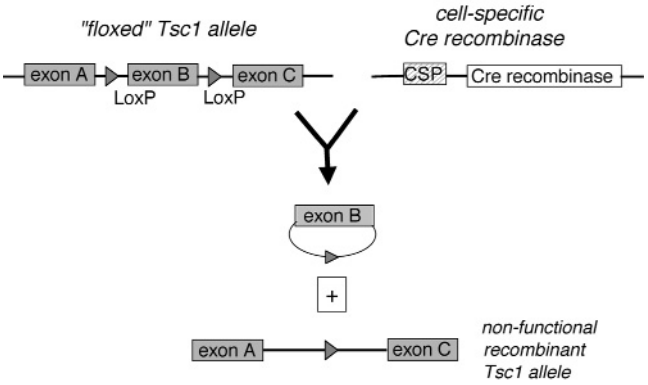

Figure 3. Generation of a *TSC1* conditional knockout mouse. Two LoxP sites were inserted within introns of the mouse *Tsc1* locus (H. Onda and D. J. Kwiatkowski, Harvard Medical School). Mice expressing the flanking LoxP ("floxed") alleles can produce hamartin and have no disease phenotype. Mice that are homozygous for the "floxed" *Tsc1* allele are crossed with mice that express Cre recombinase under the control of a cell-specific promoter (CSP). This limits the expression of the Cre recombinase to specific cells, such as astrocytes. In those cells that express both Cre recombinase and the "floxed" *Tsc1* genes, the DNA between the LoxP sites is removed, resulting in *Tsc1* gene inactivation. In this fashion, the effects of *Tsc1* gene loss in a particular cell type can be studied in vivo.

Despite a grossly normal cortical organization and a normal phenotype at birth, *Tsc1* conditional knockout mice develop severe, electroencephalographically confirmed seizures by 2 months of age.[72] These seizures increase in frequency until 4 months of age, when these mice die. One potential explanation for seizures developing in these mice relates to specific astrocyte defects that contribute to increased neuronal excitability. Astrocytes function to remove glutamate from the synaptic cleft using two transporters, glutamate transporter-1 (GLT-1) and glutamate-aspartate transporter (GLAST). *Tsc1*-null astrocytes express significantly lower levels of GLT-1 and GLAST.[75] Currents through these transporters are also significantly diminished in *Tsc1*-deficient astrocytes, even before the onset of clinically evident seizures.

Researchers are just beginnning to explore the utility of conventional and cell type–specific conditional *Tsc*

knockout mouse models. There are a number of different ways in which these animals could be used to better understand the pathogenesis of this disorder and design targeted therapies. First, the ability to generate a variety of genetic changes in mice, singly or in combination, will be useful for analyzing specific tuberous sclerosis complex phenotypes. Second, animal models in which tuberin or hamartin is deficient will allow for assessment of tuberin and hamartin functions that might be independent of the tuberin-hamartin complex. It has been presumed that the important functions of tuberin and hamartin are mediated by the tuberin-hamartin complex, but these proteins can have other important, tuberin-hamartin complex–independent functions. Third, to better understand how *Tsc1* or *Tsc2* heterozygosity contributes to the abnormal growth of cells that lack *Tsc1* or *Tsc2* expression, as seen in patients with tuberous sclerosis complex, it will be of interest to study the complete inactivation of *Tsc1* or *Tsc2* in one cell population, for example, astrocytes, in mice heterozygous for a germline *Tsc1* or *Tsc2* mutation. Recent studies in our laboratory have shown that astrocytoma formation in mice lacking expression of the neurofibromatosis 1 tumor suppressor gene in astrocytes requires an $Nf1^{+/-}$ cellular milieu.[76] Finally, animal models have recapitulated some important aspects of the central nervous system abnormalities observed in tuberous sclerosis complex. It will now be of interest to use these animals to better understand how *TSC1* and *TSC2* regulate neuroglial precursor differentiation relevant to normal cortical development.

SUMMARY

In the last several years, much has been learned about the cellular functions of the *TSC1* and *TSC2* genes and the proteins they encode. Improved understanding of the molecular pathogenesis of tuberous sclerosis complex is critical to the development of effective, highly specific therapies. Rapamycin, a specific inhibitor of mTOR, is currently in clinical cancer trials and might prove to be useful in some tuberous sclerosis complex–related tumors, including those that affect the central nervous system. Similarly, farnesyltransferase inhibitors are used clinically as antineoplastic agents.[77] These drugs might prove useful in disrupting the constitutive activation of Rheb that occurs in cells lacking tuberin-hamartin complex function. The development of mouse models that accurately recapitulate features of the human disease will facilitate our understanding of the pathogenesis of developmental defects and tumor formation associated with tuberous sclerosis complex and will likely identify new pharmacologic targets that can be exploited for the development of specific and efficacious therapies for patients affected with tuberous sclerosis complex.

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Special Article

Advances in Tuberous Sclerosis Complex Research: The October 1, 2003, Child Neurology Society Workshop

Robert Finkelstein, PhD

Although tuberous sclerosis complex (TSC) affects as many as 1 in 6000 live births (more than either amyotrophic lateral sclerosis or Huntington's disease), public awareness of this often devastating disorder remains minimal. The discovery of the two genes that cause tuberous sclerosis complex is providing an important opportunity for understanding and ultimately treating this disease. Researchers are elucidating the pathways in which the tuberous sclerosis complex gene products act, developing sophisticated animal models that will permit testing of candidate therapeutics, and initiating clinical trials based on their preliminary molecular understanding of tuberous sclerosis complex. To capitalize on this opportunity, the National Institute of Neurological Disorders and Stroke (NINDS) and the Tuberous Sclerosis Alliance jointly sponsored a workshop on tuberous sclerosis complex, held in conjunction with the 32nd annual meeting of the Child Neurology Society in Miami Beach, Florida. This was the third in a series of five conferences organized by Dr Bernard Maria (Medical University of South Carolina) intended to expose pediatric neurologists to cutting-edge research on selected neurologic disorders. These workshops not only provide a forum for researchers to update clinicians about their recent discoveries but also give clinicians a chance to discuss specific problems that arise in treating their patients. In the following paragraphs, I briefly highlight some of the issues raised by the speakers and suggest possible directions for future research. Some of these issues are discussed in greater detail in other articles in this special issue of the *Journal of Child Neurology*.

One of the major challenges in treating tuberous sclerosis complex is that it is a disorder with many manifestations. Dr Steve Roach (Wake Forest University Baptist Medical Center) summarized current diagnostic criteria and gave an overview of the effects of tuberous sclerosis complex on brain, skin, lungs, kidneys, heart, and other organs. He pointed out that there are no consistent phenotypic differences between individuals with *TSC1* and *TSC2* mutations, aside from the observation that patients with *TSC2* appear to be more prone to neurologic problems. At the present time, the clinical manifestations of tuberous sclerosis complex cannot be reliably predicted by genotype, but further genotype-phenotype studies will be important for counseling patients about what symptoms to expect and making decisions about when and how to intervene therapeutically.

Although genotype does not reliably predict symptom severity, analysis of brain lesions can be more informative. Francis DiMario (University of Connecticut School of Medicine) and Ed Bullmore (University of Cambridge) each spoke about how morphometric measures derived from magnetic resonance imaging correlate with various clinical symptoms, including cognitive impairment, autism, seizure frequency, and behavioral problems. Although the overall amounts of gray and white matter remain approximately unchanged in patients with tuberous sclerosis complex, abnormalities in specific regions predispose the patient to specific cognitive problems. For example, memory deficits in tuberous sclerosis complex are correlated with localized subcortical gray-matter deficits. Studies such as these will ultimately make it possible to anticipate potential problems during development and intervene early to ameliorate them. Penny Prather (Harvard Medical School) discussed neurodevelopmental risk factors for a range of tuberous sclerosis complex–associated symptoms. She stressed the importance of early intervention (including counseling and medication) to optimize social development and the acquisition of language, attention, and executive control.

The most common presenting symptom in individuals with tuberous sclerosis complex is epilepsy. Epilepsy occurs in 80 to 90% of patients with tuberous sclerosis complex, with the majority exhibiting seizures (infantile spasms) before 1 year of age. Elizabeth Thiele (Massachusetts General Hospital, Boston) described how epilepsy is managed in this population. Treatment modalities include anticonvulsant

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medications, the ketogenic diet, vagus nerve stimulation, and surgery. She summarized the body of evidence that strongly suggests that vigabatrin should be the first-line intervention for tuberous sclerosis complex–associated epilepsy. Howard Weiner (New York University) discussed the surgical approaches currently applied to these patients. Several studies have shown that surgery is safe and effective in children with tuberous sclerosis complex, particularly in older children with single tubers. However, many children are rejected as candidates for surgery because they have multiple epileptogenic tubers that are difficult to localize. To overcome this problem, Dr Weiner's group is using a novel invasive monitoring approach that permits more precise tuber localization and tailoring of surgery to each child. Their experience suggests that many children would benefit from this more aggressive approach because functional outcome is improved if seizures can be prevented at an early age.

Several speakers presented advances in our understanding of the molecular genetics of tuberous sclerosis complex. Hope Northrup (University of Texas Medical School at Houston) described the mapping and isolation of the *TSC1* and *TSC2* genes, which encode the proteins hamartin and tuberin, respectively. She also summarized conflicting results relating to genotype-phenotype correlations. In addition to evidence suggesting that patients with *TSC2* mutations have more severe neurologic problems (see above), several investigators have found that a variety of tuberous sclerosis complex symptoms occur at higher frequencies in these patients. However, several other studies have demonstrated no differences between patients with *TSC1* and those with *TSC2*. More comprehensive investigations will be critical to resolve these inconsistencies. Elizabeth Henske (Fox Chase Cancer Center, Philadelphia) proposed a new model for lymphangioleiomyomatosis, a rare pulmonary disease that occurs in a subset of women with tuberous sclerosis complex and as an isolated disorder. She hypothesized that kidney cells lacking the *TSC2* gene acquire abnormal migratory properties that enable them to metastasize to the lungs, where they form abnormal growths and cause cystic destruction of lung tissue. She also proposed that *TSC2* mutant cells are developmentally plastic, which causes the variegated phenotypes of tuberous sclerosis complex–associated growths in the central nervous system and other tissues.

Peter Crino (University of Pennsylvania School of Medicine) focused on the effects of tuberous sclerosis mutations in the brain. He described the histopathologic characteristics of tubers, which consist of a mixture of cell types expressing neuronal and glial markers, some of which are normally restricted to embryonic stages of development. He proposed a "two-hit" molecular model of tuber formation and described his laboratory's analysis of proteins expressed in tuber-forming cells. David Gutmann (Washington University School of Medicine) summarized attempts to develop mouse models that effectively mimic the symptoms of tuberous sclerosis complex. In principle, tissue-specific inactivation of either the *TSC1* or the *TSC2* gene should permit the recapitulation of particular tuberous sclerosis complex manifestations. For example, Dr Gutmann's laboratory used the Cre-LoxP system to generate a mouse strain in which the *TSC1* gene is specifically inactivated in astrocytes. These mice exhibit defects in astrocyte cell size and proliferation and abnormalities in neuronal organization. They also undergo seizures after 1 month of development and begin to die by 3 to 4 months. Producing additional strains in which *TSC* gene expression is similarly spatially or temporally regulated will be crucial not only for understanding this disease but for testing candidate therapeutics.

In addition to the presentations mentioned above, there were excellent talks by other speakers focusing on additional manifestations of tuberous sclerosis complex. A more complete discussion of research priorities related to specific aspects of tuberous sclerosis complex can be found in the National Institutes of Health Research Plan (<http://www.ninds.nih.gov/about_ninds/tscler_research_plan.htm>). It is worth noting that a general conclusion that emerged from many of the talks is the need for comprehensive natural history studies. By determining which parameters (including genotype) predict which clinical outcomes, we will be increasingly able to devise rational, prophylactic treatment plans for patients suffering from specific symptoms of tuberous sclerosis complex.

The meeting revealed several reasons why this is an exciting time for tuberous sclerosis complex research. First, the causal genes are known, and we are beginning to understand the functions of the proteins they encode. Second, there are increasingly sophisticated animal models for tuberous sclerosis complex (both invertebrate and vertebrate) that permit further dissection of the signal transduction pathways in which these proteins act. Third, these investigations are beginning to suggest candidate drugs that modulate the activities of these pathways and can be tested in animal models and human clinical trials. Finally, tuberous sclerosis complex provides an opportunity for investigating how a single genetic mutation affects the development of multiple tissues. For all of these reasons, further studies not only will lead to better treatments for individuals with tuberous sclerosis complex but will also promote our understanding of other genetic disorders.