THIRD EDITION

TUBEROUS SCLEROSIS COMPLEX

DEVELOPMENTAL PERSPECTIVES IN PSYCHIATRY

EDITED BY

MANUEL RODRÍGUEZ GÓMEZ

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Foreword

The title for this book, *Tuberous Sclerosis Complex*, has been consciously chosen to underscore salient, unusual, and problematic characteristics of this grave disorder, which is uniquely challenging clinically and scientifically. The volume draws together the experience of more than a century of clinical and pathologic analysis that has provided a characterization of tuberous sclerosis and outlined its patterns of distribution and transmission in large populations. It carries the discourse forward into findings unfolding from contemporary molecular genetic analysis that begin to clarify the genetic basis for certain of the knotty ambiguities relating to phenotypic heterogeneity and to patterns of transmission. It looks forward to an experimental developmental neuroscience that will enlarge our grasp of the relationship between mutant gene transcription and the cell biologic realization of phenotype. This last-mentioned perspective is of central importance. There is reason to expect that the genes mutated in tuberous sclerosis act within a critical and versatile molecular signal transduction system. At risk in malfunction of this system is the normal modulation of cell internal functions by cell external influences. The specific cell functions at risk are potentially diverse and central both to individual cell welfare and to fundamental processes of development of the entire organism.

The disorder has unusual and informative clinical pathologic features. It is a multisystem disorder typically expressed in brain, integument, and multiple visceral organs. It is mosaic in its expression in each of these organs, inducing circumscribed islands of hamartomatous perversions of normal tissue architecture. The hallmark lesion is the neocortical tuber, which is populated by gigantic cells that may express both neuronal and glial differentiation antigens. These are distributed within zones of disrupted cerebral architecture that span cortex and subcortex. These lesions are severely epileptogenic and are typically associated with mental retardation or autistic behavior, reflecting, perhaps, indirect consequences of circuitry perversions secondary to uncontrolled epilepsy.

Tuberous sclerosis complex (TSC) is transmitted as an autosomal dominant trait. Its mosaic pattern of expression predicted the discovery that, at a cellular level, the mutant allele would become penetrant only after loss or mutation of the corresponding normal allele, as by somatic mutation. It appears that the occurrence of such a "second hit" is highly probable in large populations of developing cells. Thus searching pathologic study identifies, at least microscopically, tubers in brains of virtually all who carry the primary mutation. But here arises one of the more problematic issues in the biology of this disorder. Apparently the typical clinical phenotype is equally probable and indistinguishable in association with mutation of two quite separate loci, 9q34 and 16p13.3, designated *TSC1* and *TSC2*, respectively. *TSC2* was the first of the two genes to be cloned and characterized. Its encoded protein, *tuberin*, is thought to function as a GTPase activating protein modulating signal transduction in a host of cell biologic processes. These include cell proliferation, specification of cell class, specification of regional domains, and the great cascade of cell biologic processes that are essential to growth and differentiation of mature tissue properties. Successful cloning of *TSC1* has been announced only very recently. Its encoded protein, *hamartin*, is large and hydrophilic, with a single predicted transmembrane segment and of unknown function.

This book is a welcome, well-conceived summary of a complex field at a strategic moment in the history of the analysis of tuberous sclerosis. It offers an organizing perspective that identifies, from what is known, what the next steps are likely to be and should be. I will highlight two that are surely among the most compelling. Among the first steps will be to go forward into the mystery of how it happens that mutation of two genes that are remote from each other in the genome, *TSC1* and *TSC2*, are associated with an identical developmental disorder. It is reasonable to expect that future investigations will establish that the two genes are separate processing elements within the same molecular biologic processing stream. Thus impairment or loss of either would disable the same molecular biologic mechanisms and would be expected to produce a common phenotype. The *scrambler* and *reeler* mutations in mice are a model for such a phenomenon.

A second recommended direction of investigation will be to work toward a less ambiguous identification of those aspects of the tuberous sclerosis phenotype that are directly a consequence of the disruption of *TSC1* or *TSC2* function. Ambiguity arises at two levels. First, there is the ambiguity that certain aspects of phenotype may be only indirectly consequences of *TSC* gene function. For example, the biologic basis of the associated mental retardation has been suggested to be a secondary consequence of circuitry perversions resulting from uncontrolled epilepsy. Might there be instead an as yet undisclosed, more pervasive direct effect of loss of *TSC* function upon the assembly of neocortical circuitry? Second, there is the ambiguity that certain of the protean features associated with the tuberous sclerosis phenotype, though still direct consequences of loss of gene func-

tion, are due to loss of function of genes other than *TSC*. For example, there is reason to think that at least certain aspects of the renal pathology associated with *TSC2* are an expression of deletion of the polycystic kidney disease type 1 gene (*PKD1*), which is positioned close to the *TSC2* gene on the short arm of chromosome 16. Insights into the importance of these as well as a host of other fundamental challenges relating to the biology of tuberous sclerosis will be among the rewards for the readers of this timely and informative monograph.

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Preface to the Third Edition

The first edition of *Tuberous Sclerosis* appeared in the year 1979, the second in 1988. Nine years later, when preparing a third edition, we found an avalanche of medical and scientific papers referring to Tuberous sclerosis complex (TSC). During this latter period, the number of published articles has more than doubled in comparison to the earlier 9-year period. The authors are from a variety of specialties. Virtually all medical and basic biologic branches, armed with new technologies, have contributed relevant data on TSC. Clinical and molecular genetics, imaging, pathology, and histochemistry are the chief contributors. Traditional and modern medical and biologic journals harvested such an abundance of knowledge that it is again appropriate to gather those new contributions and wed them to prevalent concepts on TSC.

For lack of a better name, we favor "the tuberous sclerosis complex," as Moolten called this disease half a century ago.[1] Our purpose is to avoid a well-entrenched ambiguity in medical nomenclature. The name "tuberous sclerosis" (without the word "complex") should refer only to the cerebral pathology of a pervasive disease. The finding of "tuberous sclerosis" (of the cerebral convolutions) is present in almost all patients and nearly exclusively to patients with TSC. The name "tuberous sclerosis complex" identifies a heritable disorder manifested by multiple hamartomatous lesions in one or several organs. Among other lesions, it includes cortical tubers and visceral harmatomas.

Significant discoveries in genetics and related fields have helped to improve understanding of the pathogenesis of TSC lesions. Several groups of geneticists have located by linkage analysis two separate gene loci, one each in chromosome region 9q34 (*TSC1*) and 16p13.3 (*TSC2*). *TSC1*, the first gene to be mapped, was not cloned until very recently. It is now known to encode a novel—130 kDa protein called hamartin. The other gene, *TSC2*, encodes a 5.5-kb transcript that is expressed in a great variety of tissues. Its

product is a 180-kDa protein named *tuberin*, the cellular function of which is probably to act as a GTPase-activating protein.

The *TSC2* gene is contiguous with the gene for adult polycystic kidney disease type 1 (*PKD1*). The proximity of these two genes unveils the mechanism for the development of multiple renal cysts in a proportion of patients with TSC. Both genes may be deleted together.

It is now apparent that both *TSC* genes act as tumor suppressors. Allelic loss (loss of heterozygosity) has been found in renal angiomyolipomas and cardiac rhabdomyomas of TSC patients. In both renal and cardiac hamartomas allelic loss is more often found at the *TSC2* locus than at the *TSC1* locus.[2] It is not clear as yet why cardiac rhabdomyomas will grow during fetal life but cease to grow and shrink during the first years of extrauterine life.

At least one type of malignancy may occur in TSC patients, the renal cell carcinoma. A possible second malignancy is angiosarcoma of the spleen.[3] There is now an animal model for TSC and renal cancer in the Eker rat. This rodent is predisposed to renal carcinoma as an autosomal dominant trait. The causative mutation is a retrotransposon inserted into and disrupting of the *TSC2* gene, located on rat chromosome 10.[4] There may be another animal model: Cardiac rhabdomyomas have been observed to spontaneously develop in 6-month-old swine.[5]

One of the most serious and common expressions of TSC is refractory epileptic seizures. The discovery and introduction of newer and more effective antiepileptic drugs (AEDs) are welcome. Some of these new AEDs are particularly effective in children with TSC (e.g., vigabatrin for infantile spasms). In rare instances a surgical approach is indicated; resection of the cortical tuber may abolish the partial or secondarily generalized seizures. To locate the cortical tuber where seizures originate, modalities such as magnetic resonance imaging with fluid-attenuated inversion recovery, single-photon emission computed tomography, and positron emission tomography, in addition to electroencephalography, are available.

Finally, in a new chapter of this book, we emphasize the importance of keeping patients and parents or guardians well informed on the diagnosis, treatment, and prognosis of the TSC lesions.

Rochester, Minnesota M. R. G.
June 1998

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TUBEROUS SCLEROSIS COMPLEX

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History of Tuberous Sclerosis Complex

Désiré-Magloire Bourneville (1840–1909) deserves credit for discovering, describing, and giving the name still in use—*tuberous sclerosis of the cerebral convolutions*—to the cerebral pathology of a previously practically unknown disease. However, before Bourneville's report of two patients in 1880 and 1881, there were two small contributions to the knowledge of tuberous sclerosis (Table 1.1).

First, in the year 1835, Pierre François Olive Rayer published an atlas illustrating skin diseases to accompany his voluminous text "Theoretical and Practical Treatise on Skin Diseases."[1] One of the last colored plates in this atlas depicts a young man's face dotted with small, erythematous papules in the characteristic distribution and with similar appearance to what Balzer and Ménétrier, 50 years later, reported as *adénomes sébacés* (adenoma sebaceum).[2] The lesions illustrated in the atlas closely resemble the facial angiofibromas often seen in patients with the tuberous sclerosis complex (TSC).

Second, on March 25, 1862, Friedrich Daniel von Recklinghausen, a pupil of Virchow, presented to the Obstetrical Society of Berlin the pathologic findings in a newborn infant who had "died after taking a few breaths." The heart had several tumors protruding on the cardiac surface, while others bulged into the cardiac chambers and still others were embedded in the ventricular walls. A tumor of the left ventricular wall was "the size of a pigeon's egg." von Recklinghausen labeled these cardiac tumors "myomata"[3] and added briefly that the infant's brain contained a "great number of scleroses." von Recklinghausen's brief report contains the first description of the two pathologic lesions most often present in newborn infants with TSC, cardiac rhabdomyomas and cortical tubers.

Bourneville studied medicine in Paris and became an intern of the Hospitals of Paris: Bicêtre, Salpêtrière, St. Louis, and Pieté. A pupil of Charcot, he was also influenced by Pascal, Claude Bernard, and Delasiauve. In 1879

TABLE 1.1. Historical Milestones of the Tuberous Sclerosis Complex

19th Century Pathological Discoveries	
1835	PFO Rayer: illustration of facial angiofibroma in atlas
1862	F. von Recklinghausen finds cardiac "myomata" in newborn
1879	Désiré-Magloire Bourneville finds cortical "tuberosities"
1881	Hartdegen reports cortical pathology
1885	Balzer and Ménétrier, Hallopeau and Lerede report "adenoma sebaceum"
Clinicopathological Developments of the 20th Century	
1901	Pellizzi: dysplasia, heterotopia, myelination defect
1905	Perusini, Campbell: pathology of brain-kidney-heart-skin
1908	Vogt: diagnostic triad
1910	Kirpicznik: hereditary nature
1912	Nieuwenhuijse: long life span of patients
1913	Berg: hereditary nature
1914	Schuster: *forme fruste* with normal intelligence
1918	Lutenbacher: lung involvement
1920	van der Hoeve: retinal phakoma
1924	Marcus: radiographic findings
1932	Critchley & Earl review clinical aspects and discover white spots
1935	van der Hoeve introduces concept of phakoma Dalsgaard-Nielsen: radiographic findings
1967	Lagos and Gomez: 38% of patients have normal intelligence
Impact of the New Technologies	
1974	Computed tomography of the head is invented
1979	Gomez: new criteria for diagnosis; decline of Vogt's triad
1982	Renal ultrasound & echocardiography demonstrate pathology
1984	Magnetic resonance imaging (MRI) demonstrates cortical tubers
1987	Positional cloning: mapping of *TSC1* to chromosome 9q34.3
1992	Mapping of *TSC2* to chromosome 16p13.3
1993	Cloning of *TSC2*; its product is called *tuberin*
1995	Loss of heterozygosity found in renal and cardiac hamartomas
1996	Introduction of MRI with fluid-attenuated inversion recovery
1997	Cloning of *TSC1*: its product is called *hamartin*

he became physician to a pediatric service, a position that he held until he retired in 1905. Bourneville's fame came after an event of May 7, 1879: at 3 o'clock in the morning, L. Marie, a 15-year-old epileptic, mentally handicapped and an inmate of Salpêtrière, died in her bed. She had suffered seizures most of her life, at first partial and, after the age of 2 years, generalized. She was then interned in Salpêtrière, and, at the age of 3 years, she suffered frequent episodes of status epilepticus and developed a right spastic hemiplegia. She had skin tags (molluscum pendulum) of the neck and a "confluent vesiculo-papular eruption on her nose, cheeks and forehead." Pathologic examination of the brain disclosed raised, opaque, and sclerotic areas in some of the cerebral convolutions. On sectioning the brain sagittally, Bourneville found what appeared to be white nodular tumors em-

bedded in the corpus striatum and protruding into the lateral ventricles. Bourneville coined the term *tuberous sclerosis of the cerebral convolutions* for this unique cerebral pathology. The sclerotic areas in many convolutions had a potatolike consistency, hence the adjective *tuberous*.[4] Notably, Bourneville also found small yellowish white tumors in the kidneys, protruding 3 to 5 mm over the surface, which he thought were unrelated to the cerebral pathology. He concluded that Marie's partial seizures originated in an extensive sclerotic area, indeed a large tuber, that occupied the left ascending frontal and parietal convolutions.

A year later Bourneville and Brissaud[5] reported a second patient with tuberous sclerosis. This 4-year-old boy had predominantly right-sided seizures and died in status epilepticus. The boy's cerebral pathology was similar to that of L. Marie.

In the same year, Hartdegen[6] reported the postmortem findings of a 2-day-old infant who had died in status epilepticus; he had spina bifida, purulent meningitis, areas of sclerosis throughout the cerebral cortex, and several small tumors protruding into the lateral ventricles of the brain. The cerebral lesions contained hyperplastic glia and large cells that looked like neurons. Hartdegen called the cerebral lesion "glioma gangliocellulare cerebri congenitum" and offered a neoplastic etiologic hypothesis later supported by Vogt[7] and Bielschowsky.[8] During the remainder of the 19th century, dermatologists led by Balzer and Ménétrier[2] in France and by Pringle[9] in Great Britain recognized and named "adenoma sebaceum" a facial lesion found in some patients with seizures and mental handicap.

Vogt[7] proposed in 1908 a clinical triad of seizures, mental handicap, and "adenoma sebaceum" as indicative of cerebral tuberous sclerosis. Since that time it has been possible to make the clinical diagnosis of TSC by finding this triad in a patient. Vogt noted that cardiac and renal tumors are also part of this disease. In 1905, 3 years before Vogt[7] defined the well-known triad of tuberous sclerosis, Campbell[10] described its ocular pathology.

Histopathologic studies of the cerebral lesions began with Pellizzi,[11] who in 1901 emphasized the dysplastic nature of the cerebral lesions: disordered cortical architecture, embryonic appearance of the abnormal cells, neuronal heterotopia, and defective myelination. Perusini[12] published similar findings in 1905 and included a precise microscopic report on the cortical tubers properly illustrated with ink drawings of atypical neurons, subcortical areas of hypomyelination, and subependymal nodules. Perusini also observed the association of cerebral, renal, and cardiac lesions with facial angiofibromas ("cutaneous adenoma") in TSC patients.

Kirpicznik[13] and Berg[14] reported on the hereditary nature of tuberous sclerosis observed in families both these authors studied. Schuster[15] confirmed the hereditary nature of TSC and recognized the exceptional patient with only the "adenoma sebaceum" component of the Vogt triad, that is, without mental handicap. This phenotype received the label *forme fruste* (from the French *frustre*, or defaced), a term not clearly defined but used

for any "incomplete" phenotype or to indicate reduced expression of the *TSC* gene.

Van der Hoeve, in 1920,[16] called attention to the retinal astrocytic hamartomas and other well-circumscribed lesions in organs of patients with TSC. Noting the similarity between TSC, neurofibromatosis and von Hippel–Lindau disease in the spotty distribution of these lesions and their tendency to grow as benign tumors, he introduced the term *phakoma* and the concept of *phakomatosis*. All diseases in this class have autosomal dominant inheritance. Phakomas are indeed hamartomas. However, the concept of phakomatosis lost its original meaning when van der Hoeve included Sturge-Weber disease under this class of diseases as a new phakomatosis. Sturge-Weber disease is a nonhereditary, congenital vascular anomaly of the skin, choroid, and leptomeninges, not associated with phakomas or hamartomas—indeed, not a phakomatosis or even a hamartosis.

In the first decades of the 20th century, abundant reports of isolated or small groups of patients with TSC indicated that TSC was not as rare a disease as had been earlier believed. The majority of reported patients were inmates of hospitals or asylums for the "feebleminded" or epileptic. At that time the diagnosis of TSC was based on the triad of seizures, mental retardation and facial angiofibroma; therefore, all identified patients had seizures and were mentally handicapped, many of them severely so. By counting the TSC patients among inmates of asylums and similar homes for mentally handicapped patients and interpolating the proportion from the total number of all mentally subnormal individuals in an entire population of a geographic region, the prevalence of TSC was then estimated at 1:100,000.

In 1924 Marcus,[17] and in 1935 Dalsgaard-Nielsen,[18] described roentgenographic intracranial calcifications as a sign of TSC, thus increasing the number of patients diagnosed. With the introduction of pneumoencephalography, the intraventricular subependymal nodules on the walls of the lateral ventricles could be demonstrated in a living patient for the first time.[19] The image was called "candle guttering" for its resemblance to the drippings of a burning candle.

Critchley and Earl, in 1932,[20] published a very complete description of their observations in 29 patients with TSC, almost all residents of an asylum. Their paper provides very detailed clinical description of TSC, for many years the best source of data on this disease. The authors were the first to find and emphasize the diagnostic value of white spots (hypomelanotic skin macules) in patients with TSC.

Moolten, in 1942, recognizing the complexity and "hamartial nature" of tuberous sclerosis,[21] renamed it "the tuberous sclerosis complex." This is now the preferred name, although in many in European countries, where eponymic names of diseases are often used, it is called Bourneville disease.

The old and erroneous idea that patients had to be mentally subnormal to have this disease suffered a severe blow in 1967 when, in a series of 71 patients with TSC from the Mayo Clinic, 38% were found to have average intelligence.[22] Even more striking was the finding that all TSC patients who

were mentally retarded had had seizures but, among those with average mental ability, some had had seizures and some had not. Critchley and Earl, in their classic 1932 paper,[20] reported psychiatric disorders and described autistic behavior 11 years before Kanner published his "classic" paper describing the paradoxical and bewildering disturbance of behavior that he called "early infantile autism."[23] Only in recent years has autism associated with infantile spasms often been identified and reported in patients with tuberous sclerosis[24–26] (see Chapter 5).

Only occasionally were asymptomatic relatives of the severely affected patients recognized as having TSC prior to the improvement of imaging methods, which began in the mid-1970s. The introduction of computed tomography of the head in 1974, followed by echocardiography and renal ultrasound and, in 1982, magnetic resonance imaging, provided reliable noninvasive methods of diagnosis that aided in establishing new and more extensive criteria for diagnosis of TSC.[27] With the expansion of the diagnostic criteria, the number of affected individuals with TSC rapidly increased. In 1972, Donegani et al.[28] estimated the prevalence of TSC at 1:10,000 based on autopsy records. Among the newly recognized affected subjects were some with few or no clinical features of TSC. Some were patients' relatives who had average intelligence but abnormal and characteristic neuroimaging findings. In one reported series,[18] 45% had normal intelligence, only 29% had the complete Vogt's triad, and 6% had none of the triad's features.

Fryer and coworkers, in 1987, reported the localization of the mutated *TSC* gene at chromosome 9q34 by genetic linkage studies.[29] Not all TSC families tested by several groups of investigators had the mutated gene in that chromosomal location. Indeed, TSC is a genetically heterogeneous disease. The first gene locus discovered at chromosome 9q34 was called *TSC1* and a second at chromosome region 16p13.3 called *TSC2*.[30] Clinical and imaging examinations have failed to demonstrate any sign corresponding with the chromosomal location of the mutation that consistently differentiates the associated phenotypes.

The European Chromosome 16 Consortium in 1993 reported that the *TSC2* gene had been identified and characterized.[31] The coding sequence of *TSC2* spans 5439 base pairs. The predicted product, a protein with 1830 amino acids and a calculated molecular mass of approximately 200 kDa is called *tuberin*. A region of similarity between *tuberin* and the human GTPase-activating protein GAP3 (rap1 GAP) and murine GAP, Spa 1, is maximal over a region of some 58 amino acids.[31] Several deletion mutations affecting different parts of *TSC2* gene have been identified in unrelated TSC patients.

Recent molecular genetic findings of loss of heterozygosity in a variety of hamartomas from patients with TSC support the idea that the product of each *TSC* gene is a tumor suppressor protein, the lack of which predisposes the individual to develop tumors in different organs. The mechanism proposed in the Knudson hypothesis seems to be operative.

Recently, the *TSC1* gene has been identified from a 900-kilobase region containing at least 30 genes. The 8.6 kilobase transcript encodes a protein of 130 kDa named *hamartin*.[32]

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Definition and Criteria for Diagnosis

Definition of Tuberous Sclerosis Complex

"Tuberous sclerosis of the cerebral convolutions" is the descriptive term Bourneville coined for the unique and distinctive cerebral pathology he found in a patient with seizures and mental subnormality.[1] The cerebral lesion, called a *cortical tuber*, is the hallmark of a protean autosomal dominant disease with variable expression in one or more organs or tissues. The various lesions are encompassed under the umbrella term proposed by the pathologist Moolten,[2] *tuberous sclerosis complex* (TSC). "Tuberous sclerosis of Bourneville" and "Bourneville disease" are historically correct eponymic terms that refer only to the cortical tuber, but this is a single pathologic feature in a single organ, the brain. "Pringle disease" pertains only to one type of skin lesion and is historically incorrect. "Bourneville-Pringle disease" combines two eponyms, one correct and one incorrect. "Epiloia" is a cacophonic, obsolete combination of "epilepsy" and "anoia," the latter, in classical Greek, meaning "mindlessness." The term *tuberous sclerosis complex* embraces not only the brain pathology but also the lesions in the multiple organs involved by this disease. Approximately 96% of patients with TSC have one or more types of skin lesions, 90% have symptoms or signs of cerebral pathology, 84% have or have had seizures, an estimated 60% have renal pathology, and nearly 50% have retinal hamartomas.

TSC is a hamartiosis and hamartomatosis[2] that may affect any human organ with well-circumscribed, benign, noninvasive lesions. Table 2.1 contains a list of the characteristic lesions of TSC arranged by organ and type of pathologic lesion: hamartia or hamartoma. Except for the limited lesions, the remaining parenchyma of the affected organs is normal. The skin, brain, retina, heart, kidney, lung, and liver are the organs most often involved, usually with tumors called *hamartomas*. Other tissues that may be affected include bone, dental enamel, gums, nasal mucosa, pituitary gland, thyroid,

TABLE 2.1. The Characteristic Lesions of TSC

Organ	Hamartias	Hamartomas
Brain	**Cortical tuber**	**Subependymal nodes or SEGAs**
Retina	Retinal depigmented spots	**Astrocytic retinal hamartomas**
Skin	Hypomelanotic macules	**Facial angiofibromas**
		Fibrous forehead plaques
		Ungual fibromas
		Shagreen plaque(s)
Kidney	Cysts	*Angiomyolipomas*
Lung		*Lymphangioleiomyomas*
Liver		Angiomyolipoma(s)
Pancreas		Islet cell adenoma
Colon-rectum junction		Hamartomatous polyp
Adrenal		Angiomyolipoma
Thyroid		Fetal or papilliform thyroid adenoma
Gonads		Testicular angiomyolipoma
Teeth		Enamel pits in primary or deciduous teeth
Bones		Phalangeal cysts; skull thickenings
Arteries		Wall defects resulting in aneurysm of aorta or subclavian, cranial, or renal arteries

Note: The lesions in bold type are pathognomonic when two or more are present in an organ or more than one organ is affected. The association of the two lesions in italics is not always diagnostic of TSC.

adrenals, thymus, gonads, uterus, vagina, pancreas, spleen, lymph nodes, lymphatics, synovia, aorta, and other large-caliber arteries. The spinal cord is rarely involved.[3] Neither the skeketal muscles nor the peripheral nerves have been reported to be affected in patients with TSC.

Hamartoma is the term coined by Albrecht in 1904 from the Greek word *hamartenein*, meaning "to err." Moolten proposed[2] that the lesions of TSC belong to one of three types:

1. *Hamartias*, or well-circumscribed, misaligned or misarranged groups of dysplastic cells that nevertheless are appropriate for the organ or tissue involved. The undifferentiated cells do not multiply or grow more rapidly than the normal cells of the affected organ (e.g., cortical tubers).
2. *Hamartomas*, or well-circumscribed groups of dysplastic cells that, in addition, have a propensity to multiply excessively, thus growing as benign tumors that may or may not cause symptoms (e.g., cardiac rhabdomyoma and renal angiomyolipoma).
3. *Hamartoblastomas*, or rare malignant tumors derived from hamartomas.

In Moolten's own words, "the basic lesion is hamartial, becoming in turn tumor-like but benign (hamartoma) or truly neoplastic (hamartoblastoma)."[2]

Five essential attributes of TSC are (1) autosomal dominant inheritance; (2) age-dependent phenotypic expression; (3) characteristic lesions visible directly or indirectly with the aid of scopes or with imaging equipment; (4) lesions that are often multiple in the affected organ(s); and (5) well-circumscribed lesions in the affected organ(s) surrounded by normal parenchyma, except where a hamartoma causes mechanical displacement or compression.

Analogous to other hamartomatoses, in TSC there is mutation involving a *TSC* gene that is either inherited from a parent (in approximately 40% of cases) or arises de novo during spermatogenesis or oogenenesis, or in the first divisions of the fertilized egg. TSC is the only hamartomatosis exhibiting lower heterogeneity. Two genes have been found in separate parts of the genome: about half of the affected families have the defective gene in chromosomal region 9q34 (*TSC1*); in the remainder the locus is in region 16p13 (*TSC2*). Patients with TSC have a defective *TSC1* or *TSC2* gene. At the present time in most cases there is no clinical, imaging, or histopathologic feature in the phenotypes that distinguishes which of the two genes is defective.

Analysis of genomic DNA of some TSC hamartomas has shown that, in addition to the germline defective gene *TSC1* or *TSC2*, the other allele is also defective or absent; that is, there is loss of heterozygosity (LOH), a finding suggesting that both *TSC1* and *TSC2* function as tumor suppressor genes.[4,5] This concept conforms with Knudson's two-hit model for explaining the inheritance of retinoblastoma.[6] However, the formation of hamartias is not explained by this hypothesis. Tubers are not tumors, and LOH does not seem to occur in cortical tubers, as occurs with hamartomas.[7] It is only speculative that, in addition to functioning as a tumor suppressor, the product of these two genes also plays an important role in neuroblast migration and differentiation during embryogenesis.

The phenotype of patients with TSC varies according to the organ or organs involved, the number and size of the lesions, and sometimes the exact location of the lesions. The age of the patient is another factor to be considered; there are lesions that do not appear until certain age (i.e., angiomyolipomas), and other hamartomas that appear during fetal life may disappear in infancy (i.e., rhabdomyomas). A great variety of clinical signs results from the random distribution, number, location, and size of the lesions in the affected organ(s), which are more often the brain, heart, skin, and kidneys. The phenotypic heterogeneity of TSC is both extra- and intrafamilial.

Hamartomas histologically identical to those found in TSC patients are also found in patients who do not appear to have TSC because they lack other diagnostic features of TSC. These isolated lesions apparently result from random somatic mutations. The isolated hamartomas that develop in individuals without TSC present at a later age than do those in patients with

TSC. Consequently, when an unusually young subject harbors an isolated hamartoma of the type frequently found in patients with TSC, such as lymphangioleiomyoma, renal angiomyolipoma (AML), or renal clear cell carcinoma, TSC should be suspected.

There is reduced penetrance and possibly even nonexpression of TSC genotypes. Nevertheless, the search for signs of TSC in a proband and his or her first-degree family members is essential when attempting to establish the diagnosis of TSC.

Criteria for the Diagnosis of TSC

The criteria for the diagnosis of TSC presented here are based on published clinical and pathologic experiences[8] and on computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, and echocardiographic images performed in the last two decades.[9,10] The criteria are conventional; up to this time the cloning and characterization of TSS1 and *TSC2* have not significantly changed the diagnostic criteria, still considered to be provisional. Table 2.2 lists the major and minor features of TSC and the diagnostic criteria adopted at a recent National Tuberous Sclerosis Association consensus conference.[11] Essentially, the diagnosis is based on the type of lesion(s) found by direct inspection, imaging, or pathologic examination of an affected organ(s).

Generally, a definitive clinical diagnosis of TSC is made when a propositus meets at least one of the following conditions:

1. Two or more hamartias (cortical tubers) in the brain
2. Two or more hamartomas characteristic of TSC in any organ
3. One or more hamartomas characteristic of TSC in at least two different organs
4. One or more hamartomas characteristic of TSC in any organ plus at least one cortical tuber
5. One hamartoma characteristic of TSC or one cortical tuber plus a first-degree relative with TSC who meets one of the criteria of TSC above outlined

Table 2.3 is a modified Table 2.2 to include *3 or more migration lines in cerebral white matter* as a definitive or major diagnostic feature of TSC and to change *hypomelanotic macules* and *shagreen patches* to the category of presumptive or minor diagnostic features.

Skin

Among the pathognomonic and well-recognized skin lesions are facial angiofibromas (FAFs), fibrous forehead plaques (FFPs), and ungual (periungual or subungual) fibromas. When there is more than one FAF or FFP, or a combination of both FAF and FFP, the diagnosis of TSC is justified. The

TABLE 2.2. Features of TSC and Diagnostic Criteria

Major Features

Facial angiofibromas or forehead plaques
Nontraumatic ungual or periungual fibroma
Hypomelanotic macules (more than 3)
Shagreen patch (connective tissue nevus)
Multiple retinal nodular hamartomas
Cortical tuber
Subependymal nodule
Subependymal giant cell astrocytoma
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 radial migration lines
Gingival fibromas
Nonrenal hamartomas
Retinal achromic patch
"Confetti" skin lesions
Multiple renal cysts

Diagnostic Criteria[a]
Definitive TSC: either 2 major features or 1 major feature plus 2 minor features
Probable TSC: 1 major plus 1 minor feature
Possible TSC: either 1 major feature or 2 or more minor features

[a]These diagnostic criteria were adopted at the Consensus Conference on TSC held in Annapolis, Maryland, on July 10, 1998, under the auspices of the National Tuberous Sclerosis Association.[11]

FFP, although histologically identical to the FAF and the shagreen patch, favors the diagnosis of TSC if present in a newborn with seizures.

With few exceptions ungual fibromas (periungual or subungual) are diagnostic of TSC. However, an injury to the toenail or fingernail disrupting the matrix, followed by granuloma formation, may be mistaken for a periungual fibroma. Also patients with incontinentia pigmenti may develop subungual fibromas similar to those seen in TSC. A painful "omega-nail" may be formed by the growth of a subungual fibroma in TSC patients.

White spots, or hypomelanotic macules, are not pathognomonic for TSC. If four or more white spots typical of TSC are found in an infant with partial or generalized seizures (infantile spasms), an obstructive cardiac tumor, the Wolff-Parkinson-White (WPW) syndrome, or multiple renal cysts, the diagnosis of TSC should be presumptive, but if there is an affected first-degree relative, the diagnosis is definitive. White spots alone, even when multiple, not sufficient for the definitive diagnosis of TSC (see also Chapter 11); their presence should lead to further investigation to seek confirmation.

TABLE 2.3. Diagnosis of TSC by Clinical Features (1999)

Definitive Clinical Features
Multiple cortical tubers on MRI (>2)[a]
Radial migrating lines on head MRI (>3)
Subepenymal nodules or giant cell astrocytoma (>2)
Astrocytic retinal hamartomas (>2)
Facial angiofibromas or fibrous forehead plaques (>2)
Ungual fibroma (>2)
Cardiac rhabdomyomas (fetus, infant, or child)
Multiple renal cysts and AML or renal cell carcinoma
Presumptive Clinical Features
Hypomelanotic macules (>4)
Shagreen patches (>3)
Gingival fibromas (>2)
Dental enamel pits (>3)
Pulmonary lymphangioleiomyoma/spontaneous pneumo- or chylothorax
Thyroid adenoma
Parathyroid adenoma
Hamartomatous colon–rectum polyps
Infantile spasms, partial or complex partial seizures
One or more first-degree relatives with established TSC diagnosis

[a]Number needed for diagnosis is given in parentheses.

The absence of additional skin signs, abnormal eye findings, or abnormal head CT scan and MRI, echocardiogram, and renal ultrasound or CT scan does not completely rule out the diagnosis but makes it extremely unlikely.

The shagreen patch is a *connective tissue nevus* that, by itself, should not be accepted as diagnostic of TSC; approximately 50% of patients with such a lesion do not have any other sign of TSC. If unaccompanied by any other characteristic TSC lesion in the propositus or in a first-degree relative, the shagreen patch is only suspicious of the diagnosis of TSC.

Retina

The astrocytic retinal hamartoma, also called retinal phakoma, has long been recognized as a diagnostic sign of TSC. Nevertheless, when single its significance is controversial, particularly when it is not typical or it is peripapillary; in the latter case, drüsen could be mistaken for hamartoma. When more than one is found in one eye, or it is present in both eyes, it is an unquestionable diagnostic sign of TSC (see also Chapter 10).

Central Nervous System

Cortical tubers, subependymal nodules, or giant cell astrocytomas, and radial migration lines of high-intensity signal in cerebral white matter displayed

by MRI, are dependable signs to establish the diagnosis of TSC. Calcified or uncalcified and contrast-enhancing subependymal nodules, or a mass found near the foramina of Monro visualized by head CT scan, have great diagnostic value. Head MRI may clearly reveal subcortical hypomyelinated areas or radial lines of increased signal in more than one region of the white matter, corresponding, respectively, to cortical tubers or to nests of heterotopic and undifferentiated neurons or glia.

Kidney

Imaged by ultrasound, CT scans, or MRI, the kidney is the third most common organ, after the brain and the skin, found to be affected in adults with TSC. The kidneys should be imaged in all adults at risk of TSC. The presence of multiple congenital renal cysts in a child or an adult with TSC constitutes a contiguous gene syndrome caused by deletion of *TSC2* and the autosomal dominant adult polycystic kidney disease (*PKD1*) genes. Multiple renal AMLs in a young female should be attributed to TSC, and the finding of renal cysts or a clear cell carcinoma would confirm this diagnosis.

Teeth

Dental enamel pits are of varying size, from microscopic to visible with the naked eye, especially if they are previously stained with a food dye. A recent study by Russell and colleagues found dental enamel pits in all 87 shed deciduous teeth from 20 patients with TSC, but in none of the 253 deciduous teeth from 142 patients with cerebral palsy, phenylketonuria, and Down syndrome and healthy individuals used as controls.[12] The results of other studies have not been as unconditional.[13]

Heart

Cardiac rhabdomyoma is the most common cardiac tumor in infancy. Approximately 50% of infants with multiple cardiac rhabdomyomas demonstrable by echocardiography have TSC[10] (see "Antenatal Diagnosis of TSC" later in this chapter). Among the rare patients with multiple rhadomyomas not having TSC are those with the rare autosomal recessive syndrome known as "cardiac rhabdomyomas–megacystis-microcolon-hypoperistalsis syndrome."[14] Patients with this syndrome have, in addition to multiple cardiac rhabdomyomas, a nonobstructive megabladder and intestinal pseudoobstruction; they die in the first 3 years of life.

The cardiac tumors of TSC patients may not cause any symptoms[15] or may produce cardiac failure as a result of obstruction of blood flow through cardiac chambers or outflow tracts. Cardiac arrhythmia, particularly the WPW syndrome, may be detected prenatally or neonatally and be the first

sign of TSC.[16] Fetal echocardiography can sometimes detect rhabdomyomas after the 25th week of gestation. It is the most useful test for antenatal diagnosis of TSC and should be considered for any fetus at risk of this disease. Although an uncommon presentation of TSC, cardiac rhabdomyomas or arrhythmias in recent years have been recognized with increasing frequency in the fetus and neonate.

Lungs

Pulmonary lymphangioleiomyomatosis (LAM) may be found in adult females at risk of TSC, and rarely in males. Lung CT scan is preferable to chest radiography and MRI for early detection of LAM. Finding LAM in a woman whose child presents with seizures of unknown cause should make the diagnosis of TSC presumptive in both mother and child. Recurrent pneumothorax or chylothorax are rare but important features of TSC to inquire about when obtaining the history and to search for when investigating individuals at risk of TSC.

Any one of the features listed in Table 2.3 under "Definitive Clinical Features" is sufficient to suspect the diagnosis of TSC. If two or more of the features listed under "Presumptive Clinical Features" are present, a presumptive diagnosis of TSC is justified.

Patients' Age and Phenotype

The relative patient age at which different types of TSC lesions and signs are likely to be manifest clinically or by imaging methods is shown in Table 2.4. Cardiac rhabdomyomas grow during fetal life, reaching their maximum in size at birth, and may remain static or, more often, regress in the first postnatal years.[15] With rare exceptions they do not develop or grow in the adult years. FAFs usually appear after the age of 3 years, may grow rapidly during puberty, and sometimes may continue to grow or may cease growing in the adult years. FFPs present at birth appear to be pathognomonic of TSC because histologically they are identical to FAFs

A subependymal giant cell astrocytoma (SEGA) may be found in a newborn with partial seizures and a calcified mass in a cerebral hemisphere. More often symptoms begin after the child is a few years old as the hamartoma begins a period of rapid growth from a small subependymal nodule. The median age for this event is 9 years and the range is between birth and 18 years. The lesion's growth peaks at puberty, and it may slow down and possibly stop growing as late as the end of the third decade of life. About one third of SEGAs are bilateral, but they do not always grow together and obstruct the two foramina of Monro at the same time.

Renal AMLs and pulmonary lymphangioleimyomas as a rule are very small or inapparent during the first decade of life, begin to increase in size

TABLE 2.4. Ages When Certain Lesions or Signs of TSC May First Appear

Age	Lesion/Sign
Between 20th week of gestation and birth	Cardiac rhabdomyoma
Perinatal period	Subependymal nodule/tumor (by CT/MRI)
	Multiple renal cysts (ultrasound)
	Hydrops fetalis
	Seizures in utero
	WPW syndrome
Newborn period	FFPs
	Partial seizures with or without generalization
	Abdominal distention/uremia
Infancy (<1 year of age)	Infantile spasms/West syndrome
	Cutaneous hypomelanotic macules
	Retinal hamartomas
	Regression of social-adaptive behavior
	Delayed motor development
Early childhood (<5 years)	Seizures/Lennox-Gastaut syndrome
	Autism/atypical autism/mental handicap
	FAFs
	Aberrant behavior/learning disability
Late childhood (6 to 12 years)	Mental retardation/autism/intractable seizures
	Subependymal nodules/giant cell astrocytoma
	Status epilepticus
Adulthood	No mental handicap if no seizures
	Late-onset seizures; status epilepticus
	FAF
	Renal AML causing hematuria or sudden bleed
	Pulmonary LAM
	Pneumothorax/chylothorax/respiratory failure/ hypoxemia/hypercarbia
	Ungual fibromas

at the end of the second decade, and may grow faster in the fourth decade of life. Large renal cysts may be present at birth and continue to grow by accumulating fluid, compromising renal function. This may be part of a contiguous gene syndrome.[17]

Varieties of Expression of TSC Genes

Whereas some TSC phenotypes have been called severe, others mild, and still others a *forme fruste*, these terms have not been clearly defined. Most often they refer to the prognosis in terms of morbidity or mortality. It is reasonable to denominate as severe or lethal those phenotypes leading to early loss of life (in a few months or less). Examples of patients with the lethal variety include:

1. The infant with cardiac rhabdomyomas obstructing an atrioventricular foramen or a ventricular outflow tract, or disturbing the

cardiac conduction system to cause a persistent arrhythmia and cardiac failure
2. The patient with large renal cysts obliterating enough renal parenchyma to cause renal failure
3. The patient, usually in the second decade of life with frequent and intractable generalized seizures often culminating in status epilepticus, with all its possible complications
4. The youngster with a growing SEGA that blocks the cerebrospinal fluid circulation in one or both foramina of Monro, and needs an immediate ventricular decompression
5. The patient of any age with a SEGA that suddenly and spontaneously bleeds internally, causing acute intracranial hypertension and possibly sudden death
6. The adult patient with extensive renal parenchymal loss from multiple and large renal AMLs
7. The adult patient with abrupt bleeding within a renal AML from ruptured aneurysmatic renal arterioles, causing a retroperitoneal hemorrhage and hypovolemic shock
8. The child with ruptured aneurysm of the thoracic or abdominal aorta, or of any other major artery
9. The rare adult patient who develops renal cell carcinoma that may be metastatic
10. The woman with severe respiratory failure from pulmonary LAM, hypoxemia, and hypercarbia.

By contrast, the moderate and mild forms of TSC are represented by those patients whose lives are not threatened by a hamartoma or hamartoblastoma and do not have or rarely have a seizure. These patients may have only skin, retinal, or small renal hamartomas, few or no cortical tubers, and no intraventricular SEGA, and have attained normal intellectual development. The term *forme fruste* was originally used to refer to affected individuals with skin lesions or renal tumors but with normal mentation and no seizures. Members of a family with TSC may reveal only FAF, FFP, or periungual fibroma, or imaging studies may demonstrate typical features of TSC, such as calcified subependymal nodules or renal AMLs.

At the other extreme is the form of TSC that is more difficult for parents to accept and handle, and for anyone to care for. These infants and children live with severe and intractable seizures, and fail to attain normal mental development or learn the basic rules of social behavior at home and school. Some have autistic or aggressive behavior and do not become independent (see Chapters 4 and 5).

Antenatal Diagnosis of TSC

Cardiac tumors have been identified in fetal or neonatal life by echocardiography,[18] and can be the only finding of TSC at this early age. After infancy,

such tumors would not be the sole indication of TSC. In a report of five infants with these tumors, there were no other signs of TSC until weeks or months later, when hypomelanotic macules or neuroimaging abnormalities appeared.[19]

Antenatal seizures caused by TSC have not been well documented, although the prenatal diagnosis of this disease is now easily made by imaging when cardiac or cerebral tumors, or rarely polycystic kidneys are present. Two of our patients had antenatal onset of seizures. The mother of one of these infants spontaneously related that, by placing her hand on her abdomen, she had felt violent paroxysmal and rhythmic movements of brief duration in the last 3 weeks of gestation. After birth, the infant had partial motor seizures that apparently had their onset before birth. The infant's electroencephalogram demonstrated a right frontal epileptic focus, and CT revealed a large amorphous calcification in the right frontal lobe. Histologic examination of the biopsied lesion revealed a SEGA. Skin examination with the Wood lamp did not reveal white spots until the patient was 2 months old. In the event of neonatal or antenatal presentation of partial motor seizures, CT or MRI of the head will demonstrate a structural lesion, probably calcified and located in the frontal lobe on the side opposite the partial motor seizures.

Causes of Death Among TSC Patients

Mortality data were obtained from 40 TSC patients seen at least once at the Mayo Clinic who died from causes related to TSC. Eleven patients 10 years

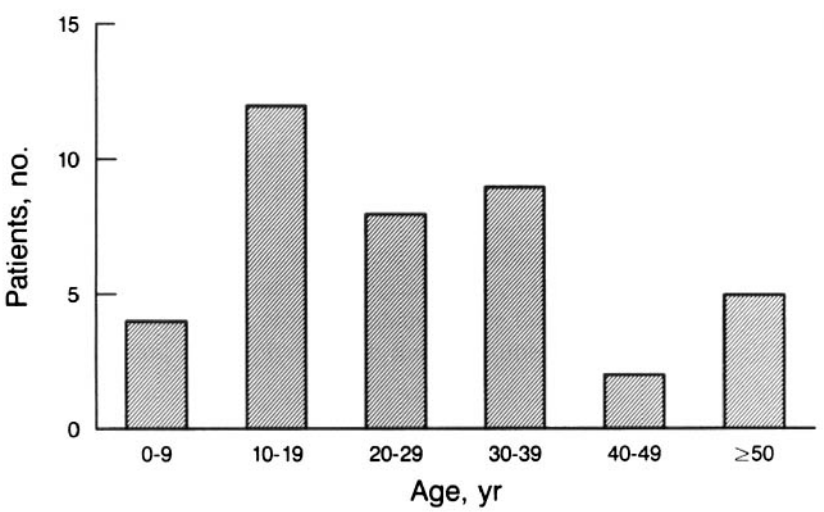

FIGURE 2.1. Distribution of ages of 40 patients (22 female and 18 male; mean age, 26 years) who died of TSC-related causes. (Reproduced from Shepherd et al.[20] by courtesy of the publisher.)

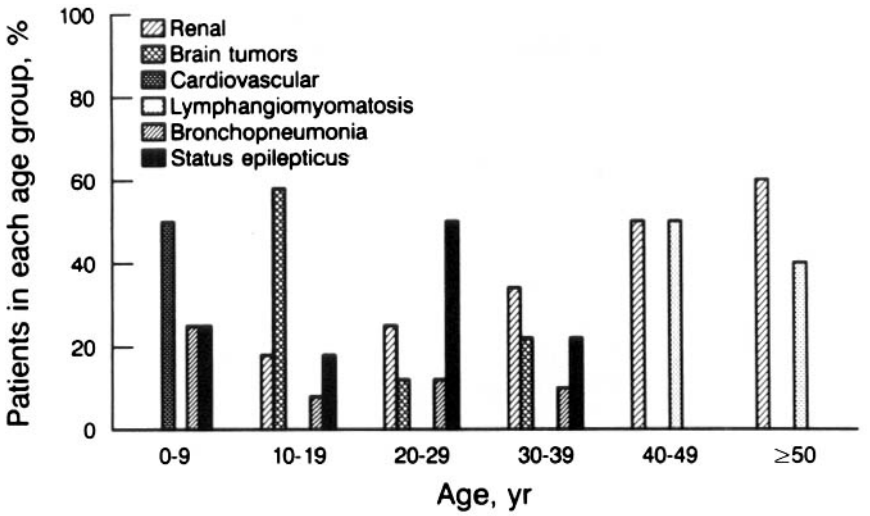

FIGURE 2.2. Distribution of causes of death and ages of patients at time of death from TSC-related causes. (Reproduced from Shepherd et al.[20] by courtesy of the publisher.)

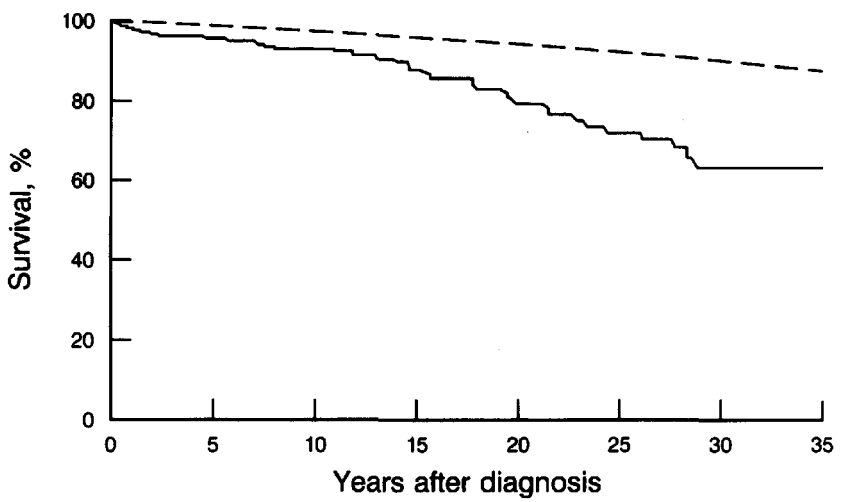

FIGURE 2.3. Kaplan-Meier survival curves for patients with TSC *(solid line)* and for the white population of the United States in 1970 (*broken line*). (Reproduced from Shepherd et al.[20] by courtesy of the publisher.)

of age or older died of renal disease, seven of them in renal failure, two from a bleeding AML, and another two from a renal clear cell carcinoma. Ten patients died with a brain tumor, either as a direct result of the tumor in six instances or as a result of the surgical attempt to remove the tumor. All the tumors were SEGAs. Four patients died of LAM. An infant died of heart failure caused by cardiac rhabdomyomas. Thirteen patients who had seizures and severe mental handicap died, nine of them in status epilepticus and four with bronchopneumonia.[20] Figure 2.1 shows the age distribution of these 40 patients with TSC. The distribution of the causes of death and ages of the patients at the time of death are shown in Figure 2.2. The survival curve for all Mayo Clinic patients with cause of death related to TSC is plotted in Figure 2.3. For comparison, the survival curve for the white population of the United States is also shown.[20]

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Charles W. Shepherd, M.D.

The Epidemiology of the Tuberous Sclerosis Complex

Introduction

Early studies into the prevalence and incidence of tuberous sclerosis complex (TSC) were inaccurate because TSC was perceived to occur only in people who had seizures, were mentally handicapped, and had facial angiofibroma. Early studies also tended to focus on hospital populations rather than look at total populations. Recent studies to ascertain the prevalence of TSC in defined populations have been more accurate because investigators now appreciate the different phenotypes of TSC.[1] These studies have looked for patients with renal, lung, and skin disease suggestive of TSC, even if there is no evidence of mental handicap or seizures. A knowledge of all the different phenotypes of TSC is essential for an accurate population study.

Hospital-Based Studies

In 1926, Brushfield and Wyatt[2] found that 1:150 cases in an institution for the mentally handicapped had TSC. Table 3.1 lists the studies that have documented the incidence of TSC in institutions for the mentally handicapped.[2–5] These studies are of general interest only and are of no real value in the study of the epidemiology of TSC.

Table 3.2 lists those studies that, by finding the numbers of patients with TSC in one or more institutions for the mentally handicapped, attempted to estimate the prevalence of TSC in the general population. The first study was by Gunther and Penrose[6] in 1935. They examined all inmates in five hospitals for the mentally retarded and found that 1:300 of the cases had TSC. The diagnosis was made mainly by the presence of facial angiofibroma. They concluded that, since 1% of the population had mental handicap, then 1:30,000 patients in the general population had TSC. Although

TABLE 3.1. Estimates of TSC in a Single Institution

Year	Authors	Incidence
1926	Brushfield and Wyatt[2]	1:150
1936	Ferraro and Doolittle[3]	1:300
1938	Penrose[4]	1:150
1965	Gastaut et al.[5]	1:190

they looked only at one phenotype of TSC, their results were compatible with other later population studies. Similar studies were done by both Ross and Dickerson[7] in 1943 and Paulson and Lyle[8] in 1966. Dawson's study, published in 1954,[9] was mainly focused on looking for patients with TSC who had lung disease. He surveyed all the hospitals for the mentally handicapped between 1930 and 1951 and found 1:300 admissions had TSC. At that time records indicate 1:1000 of the population were admitted to such hospitals. Dawson[9] also found several cases of patients with TSC who were not handicapped in the outpatient records of other London hospitals. By assuming that the mild form of TSC was as common as the severe form, he estimated a prevalence of 1:150,000 of cases of TSC in the general population. Although this figure was later shown to be inaccurate, he was one of the first people to include other phenotypes of the condition. In 1968, Zaremba[10] found 26 cases of TSC in Polish institutions and 14 cases among their relatives. From these figures he estimated the prevalence to be 1:23,000. A study in 1960 by Crome[11] documented cerebral pathology diagnostic of TSC in 2.5% of 282 mentally handicapped patients. Another study in Switzerland[12] of 49,000 routine autopsies found cerebral changes consistent with TSC in 6 cases. The authors estimated a population incidence of 1:10,000.

TABLE 3.2. Estimates of Prevalence of TSC From Studies in Institutions

Year	Authors	Incidence	Estimated Prevalence in Population
1935	Gunther and Penrose[6]	1:300	1:30,000
1943	Ross and Dickerson[7]	1:175	1:50,000
1954	Dawson[9]	1:190	1:150,000
1966	Paulson and Lyle[8]	—	1:20,000–1:40,000
1968	Zaremba[10]	1:100	1:23,000

Population-Based Studies

The most accurate method of determining the true prevalence of TSC is population-based studies. Table 3.3 lists the different population studies that have been undertaken. All the surveys were based on reported clinical cases and, in some studies, the detection of TSC in asymptomatic relatives. Mass diagnostic testing is at present impractical. The earlier studies of Stevenson and Fischer,[13] Nevin and Pearce,[14] and Singer[15] reported very low figures. These were done prior to the introduction of computed tomography in 1974, which allowed detection of subependymal nodules, and ultrasound scanning in 1982, which identified renal tumors and cysts. Like earlier hospital-based studies, these surveys did not take account of all the different phenotypes. The publication of primary and secondary diagnostic criteria by Gomez[16] in 1979 enabled a more accurate diagnosis of TSC to be made in patients and allowed investigators to take account of the different phenotypes. The Oxford study of Nevin and Pearce[14] was updated in 1984 by Hunt and Lindenbaum.[17] Using computed tomography and ultrasound scanning to aid diagnosis, they detected 68 cases in a population of 2,328,100. Total prevalence was estimated at 1:34,200; for people under 45 years of age it was 1:24,600, for people under 15 it was 1:17,300, and for people under 5 it was 1:15,400. They suggested that the birth prevalence may be higher. Another total population study was undertaken in the west of Scotland.[18] In this study 101 cases were identified, giving a total prevalence of 1:27,000 and a prevalence of 1:10,000 for children under 10 years of age. Similar results were obtained by Webb et al.,[19] who found 131 cases of TSC among 3.4 million people in the south of England. The prevalence in the population was 1:26,500.

TABLE 3.3. Population Studies of TSC

Year	Authors	Prevalence	Population Sampled
1956	Stevenson and Fischer[13]	1:150,000	Population of Northern Ireland
1968	Nevin and Pearse[14]	1:100,000	Population of Oxford Regional Health Authority
1971	Singer[15]	1:70,000	Chinese population of Hong Kong
1984	Hunt and Lindenbaum[17]	1:34,200	Population of Oxford Regional Health Authority
1985	Wiederholt et al.[20]	1:9704	Population of Rochester, Minnesota
1989	Sampson et al.[18]	1:27,000	Population of the west of Scotland
1991	Shepherd et al.[21]	1:14,492	Population of Olmsted County, Minnesota (includes Rochester)
1996	Webb et al.[19]	1:26,500	Population of part of the south of England

Two studies were done as part of the Olmsted County Epidemiological Project. In this project, access to medical records of nearly all the population of Rochester, Minnesota, allowed for identification of all cases of TSC. In the first study, from 1950 through 1982, Wiederholt and colleagues[20] found a prevalence of 1:9434 and an incidence of 0.56 per 100,000 person-years. This study was updated by Shepherd et al.,[21] who located all cases of TSC in Olmsted County (of which Rochester is the largest town) using the medical records available to the Olmsted County Epidemiological Project. The incidence of TSC was 0.28 per 100,000 person-years between 1950 and 1989. The incidence between 1950 and 1974 was 0.13 per 100,000 person-years, and that between 1974 and 1989 was 0.46 per 100,000 person-years. The increased incidence of TSC after 1974 is believed to be due to the introduction of computed tomography in 1974, which, by detecting subependymal nodules, allowed for a more accurate and earlier diagnosis. The prevalence was 1:14,492 of the population. The lower prevalence compared to the previous study is due to two cases being excluded because one had left the county and the other was a false diagnosis.

In population studies of genetic diseases, there is a possibility that large family clusters could give a higher figure for the prevalence of the condition, especially if the population is small. This was not the case in the Olmsted County study: The total population studied was 100,000. Although total acertainment was almost certainly not obtained, it is believed that this study is the most accurate because of the comprehensive medical record system used.

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4

Natural History of Cerebral Tuberous Sclerosis

The tuberous sclerosis complex (TSC) is a hamartomatosis with protean phenotypes because it may affect in diverse ways almost any human organ. Patients with TSC most often present with symptoms caused by cerebral pathology. However, the diagnosis is frequently suspected and very often made with signs found on inspection of the skin.

Histopathologically, cerebral TSC is a disorder of cellular migration, proliferation, and differentiation. The central nervous system (CNS) lesions of TSC patients, and in particular the cortical tubers, are very characteristic. Cells found in the cerebral lesions of TSC patients differ from those of normal cell lineage, and it is difficult to know from what cell type they derive.[1] Patients with TSC usually have other lesions, generally hamartomas in one or more organs. The descriptive term originally used for the cerebral lesions, *tuberous sclerosis*, is also used for other manifestations of this inheritable disorder.

This chapter deals specifically with symptomatology and treatment of the cerebral component of TSC. It focuses on the chief four neurologic features of TSC: seizures, mental handicap, motor signs, and intracranial hypertension (for definitions and cerebral pathology, see, respectively, Chapters 2 and 8). Chapter 5 deals with the behavioral complications of TSC, including childhood autism, hyperactivity, attention deficit, aggressive behavior, and sleep disturbances, thus completing the long list of clinical features manifested by cerebral lesions of TSC.

There is much variation in the neurologic symptomatology of patients with cerebral TSC. Phenotypic heterogeneity may be found even amid members of the same family, monozygous twins included.[2] Not all individuals who have inherited the TSC mutant gene will express CNS lesions, and not all subjects who harbor CNS lesions in the brain, as detected by neuroimaging or neuropathologic examination, will necessarily express neurologic symptoms or signs. The clinical features when there is cerebral involvement

of TSC depend on the number, size, histologic nature, and sometimes precise location of the lesions. These lesions are found first in limited areas of the cerebral cortex and subjacent white matter and second in the subependymal region of the lateral ventricles. About 15% of patients with cerebral lesions also have cerebellar lesions demonstrable by imaging or by pathologic examination, and yet the patient with TSC who exhibits cerebellar symptoms or signs is very rare. The patient with TSC who becomes ataxic most likely is receiving antiepileptic drugs (AEDs) and becoming intoxicated.

Spinal cord and brain stem lesions in TSC patients have seldom been reported in the medical literature.[3,4] Koprowski and Rorke[4] reported the clinical and postmortem findings of two patients. One was a 14-year-old boy with frequent seizures, spastic quadriplegia, and mental handicap who at autopsy, in addition to renal angiolipomas, cardiac rhabdomyomas, and "probable adenoma sebaceum," had multiple cortical tubers and subependymal nodules (SENs). The spinal cord abnormalities consisted of "loss of neurons at thoracic levels and astrocytic proliferation in the white matter." The other patient at 5 months had infantile spasms and white spots on the skin, at age 3 years his developmental quotient was 44, and at age 4-1/2 years was found dead at home. The necropsy revealed cortical tubers and SENs in the brain. The spinal cord "showed astrocytic proliferation at all levels," clusters of fiber-forming astrocytes, and "unusually large, bizarre glial forms."[4] It may be that, because the spinal cord is not always removed at the time of necropsy, the descriptions of spinal cord pathology are uncommon, or perhaps the clinical findings of cord involvement are not sought or recognized. As far back as the year 1905, Perusini described a 14-year-old patient with quadriplegia that he attributed to TSC (cited by Koprowski and Rorke[4]). One of the few reported cases of this pathology is not valid; the patient was purported to have a spinal cord lesion caused by TSC,[5] but at necropsy years later the findings were not of TSC but most probably of neurofibromatosis type 1.[6]

In one of the few necropsy reports of brain stem and cerebellar findings in TSC,[3] microscopic examination of both the brain stem and the cerebellum of one patient disclosed "giant heterotrophic neurons." In the same report another patient, an 8-week-old girl, had "focal cortical and subependymal glial abnormalities in many parts of the cerebrum and brain stem and disorganization of the mantle layer of the cerebellum."[3]. Unfortunately, the clinical data on these patients are exiguous; no clinical signs of brain stem involvement are mentioned.

The neurologic symptoms and signs of cerebral TSC were present in the first patient ever reported to have TSC.[7] She was a severely mentally handicapped 15-year-old female inmate of the Salpêtrière Hospital in Paris who had neither walked nor talked, had intractable right partial motor seizures with secondary generalization since infancy, and had a right spastic hemiplegia. She had suffered frequent bouts of status epilepticus and expired during one of them. Postmortem examination disclosed two types of lesions in the brain, cortical tubers and SENs.[7]

In his classic paper on TSC, the pathologist Moolten referred to the two types of lesions of this disease as *hamartias* and *hamartomas*.[8] The hamartias, the cortical tubers, were the cause of partial motor and secondarily generalized seizures; hemiparesis that initially was only transient and recognized only postictally, but later became a permanent hemiparesis; or hemiplegia usually corresponding to a large cortical hamartia where seizures originated. The hamartomas are benign periventricular tumors that may or may not grow. Generally, they remain stable and asymptomatic as SENs and usually calcify. However, a SEN may continue to grow as a subependymal giant cell astrocytoma (SEGA, also called subependymal giant cell tumor). Growth of these hamartomas may cause blockage of the cerebrospinal fluid (CSF) circulation, most likely at one or both foramina of Monro, resulting in intracranial hypertension. These tumors do not become malignant.

Epileptic Seizures

The principal symptoms and signs of cerebral TSC are a wide variety of epileptic seizures originating in the cortical tubers and, apparently, in normal or transitional cortex surrounding them. Less often the partial seizures originate in cerebral cortex adjacent to a large SEGA extending from a location deep in the white matter into subcortical white matter and impinging onto cerebral cortex. In this site the SEGA, being away from the ventricular system, does not obstruct the CSF circulation. In rare cases a large cortical tuber overlies a SEGA, with both occupying the major part of a cerebral lobe, usually the frontal.

Approximately 84% of all individuals with TSC who come to medical attention, including affected first-degree relatives, some with reduced phenotype expression, have suffered epileptic seizures at some time in their lives. Although seizures may occur at any age in TSC patients, they rarely are the initial symptom of TSC in adults. Seizures are the initial symptom in 92% of TSC patients of all ages, almost of all them children. Thus one may conclude that no less than 92% of all individuals with TSC have typical cerebral involvement. The exact proportion is unknown.

Most often children with TSC begin having seizures in the first months or the first year of life. The seizure types include all varieties listed in the classification of the International League Against Epilepsy except pure absences or classic petit mal. The most frequent seizure types are partial motor, complex partial, and partial secondarily generalized, including infantile spasms.

Antenatal seizures caused by TSC may occur, as mentioned in Chapter 2 (see "Antenatal Diagnosis of TSC"). In the event of neonatal or antenatal presentation of partial motor seizures, head computed tomography (CT) or magnetic resonance imaging (MRI) will often demonstrate signs of TSC and a well-defined structural lesion, usually a calcified mass located in the frontal lobe and opposite the side of the partial motor seizures. This was the case

in the two girls reported by Sugita et al.,[9] who began having partial motor seizures in the first and the fifth day of life, respectively. The head CT scan showed in the first patient a calcified area "from the midbrain to the left cerebellum and in the occipital lobe." The second patient had a calcified lesion and a "wedge-shaped dilated Sylvian fissure in the left fronto-temporal region." This second patient underwent a partial surgical lesionectomy at age 60 days, and the histologic examination demonstrated a SEGA. Therefore, because partial seizures starting antenatally or neonatally may be caused by a cortical tuber or a SEGA, a thorough examination looking for other signs of TSC is in order in these patients.

Infantile spasms or massive bilateral myoclonic seizures may begin abruptly, but these generalized seizures seldom occur or are recognized within the first 6 weeks of the patient's life. Their onset is commonly reported to occur after the second month of life. The exact age of onset peaks between the fourth and sixth months. This is the age when parents have already seen their infant smile socially and explore visually his or her immediate surroundings. As seizures begin, the parents, unaware of them, may only observe the infant's loss of interest in people and objects in the immediate environment. The social smile disappears and the infant's demeanor is one of indifference or irritability preceding or coinciding with the onset of complex partial or generalized seizures. The first sign of seizures one mother observed was that, while she was nursing her 2-month-old infant there were interruptions of suckling each lasting a few seconds and frequently repeated. Only weeks later, when seizures were obvious, did this mother realize that suckling interruptions occurred during subtle seizures. A slightly asymmetric facial smile with one corner of the mouth pulling back may be the first or only sign of a seizure, and may go unnoticed by parents for many weeks unless they are exceptional observers. The electroencephalogram (EEG) can be very useful to determine and localize focal epileptic abnormalities. The waking EEG may disclose multifocal independent spikes and disorganized high-amplitude slow waves. Some patients' EEGs may show typical hypsarrhythmia. All 63 patients with TSC in the Mayo Clinic series who showed this pattern had onset of infantile spasms between 1 and 18 months of life (see Chapter 6).

During the fourth through ninth months of life, TSC patients may present with either partial motor seizures or complex partial seizures. These may become secondarily generalized in the form of infantile spasms, or they may be generalized from onset as infantile spasms or tonic, myoclonic, tonic-clonic, or atypical absences. Patients who begin having generalized seizures at this age may later have partial or partial complex seizures with secondary generalization. The onset of generalized seizures in TSC patients may precede but most often follows the onset of partial seizures.

The seizure type at onset is in part determined by the patient's age at that time. Thus in the neonate they are partial motor, whereas in the rest of the first year of life they begin as partial and soon evolve into infantile spasms or, less often, other types of generalized seizure. In the majority of

TSC patients, infantile spasms have their onset in the first 6 months and less often in the seventh through ninth months of life. The infantile spasms are commonly associated with two other features of the West syndrome: arrest or regression of psychomotor development and an EEG pattern of hypsarrhythmia. The median age at onset of infantile spasms in the West syndrome for all etiologies, including idiopathic, is the fourth month of life. Some authors have reported two peaks at ages 3.5 and 5.5 months,[10] which would suggest seizure type heterogeneity, including perhaps infantile spasms and bilateral massive myoclonic seizures reported together under the label of infantile spasms. Infantile spasms was the presenting complaint in 69% of TSC patients in one large study,[11] in 68% of patients in Hunt's study,[12] and in two thirds of TSC patients seen at the Mayo Clinic. The majority of these patients developed mental handicaps.

By the first birthday patients with TSC have either partial or generalized seizures of any variety except pure absences (classic petit mal). Infantile spasms may persist if treatment was unsuccessful or recur after a successful treatment. The seizures may be tonic, clonic, tonic-clonic, atonic, bilateral massive myoclonic, atypical absence, partial motor, or complex partial.

It is a common belief that seizures of TSC patients are generalized from their first appearance and "centrencephalic" in origin.[13] It is more logical that they originate in the cortical tubers as partial complex and become generalized as they invade the basal ganglia, perhaps the caudate nucleus in particular. If by "centrencephalic"[13] is meant "pure absences" with bilateral synchronous 3-Hz spike-and-wave discharges in the EEG, I have not found a single example among more than 400 TSC patients. Critchley and Earl,[14] in their classic paper, mentioned an 8-year-old girl with TSC who for many years had "petit mal" seizures and no gross mental defect. She later developed intracranial hypertension with bilateral papilledema but without localizing neurologic features. At postmortem examination there were cerebral changes typical of TSC, including an intraventricular tumor the size of a walnut. No clinical description of the seizures is given in this brief report. Nonetheless, the authors commented that, in TSC, "all types of attacks may be encountered, grand mal, petit mal, and Jacksonian." I suspect the patient had complex partial rather than petit mal seizures. Gastaut et al.[15] reported a coincidental association of TSC with petit mal in a patient who had inherited a disorder from each parent. Overall, complex partial seizures with or without secondary generalization are the most common seizure types in TSC patients from their first year of life through childhood.

Clinical-Neuroimaging Correlation

There is an inverse relation between the number of cortical tubers in a patient and the age at the onset of seizures. The likelihood of the first seizure being an infantile spasm increases in parallel with the total number of tubers ($p < .001$).[16] The frequency and severity of the epileptic seizures are also

greater when the number of tubers is higher. In a study of 75 patients with TSC who had sequential head MRIs, the 29 patients who had infantile spasms harbored more cortical tubers than the 26 patients who had other types of generalized seizures.[16] The average number of tubers in patients with infantile spasms was 5 in the frontal lobes and 5.6 in the parietal lobes, whereas patients who had only partial seizures averaged 1.3 tubers in the frontal and 2.3 in the parietal lobes. Remarkably, TSC patients who never had seizures had an average of 3.4 tubers in the frontal and 3 tubers in the parietal lobes. Partial seizures are associated with tubers in any of the cerebral lobes except the frontal. Myoclonic seizures or febrile seizures are associated with tubers in any lobe except the parietal.[16] No correlation has been found between the presence of uncalcified or calcified periventicular hamartomas and the development of partial or generalized seizures.[16]

Since the introduction of the fluid-attenuated inversion recovery (FLAIR) method for head MRI, a greater number of tubers have been detected in TSC patients than with MRI done without FLAIR. Consequently, the correlation of number and location of tubers with symptoms and prognosis of cerebral TSC must be determined once more using this new technique (see Chapter 8).

Treatment of Seizures

Treating the seizures of TSC patients is frequently a challenge for several reasons: (1) the seizures may be difficult to recognize as such or it may be difficult to determine their type; (2) the seizures are often refractory to AEDs; (3) increasing the doses of AEDs to the tolerable maximum may lead to signs of toxicity and mental deterioration; (4) persistent seizing may cause postictal obtundation, which may be mistaken for toxicity, and vice versa; (5) rapid withdrawal of seemingly ineffectual AEDs may cause exacerbation of seizures; and (6) adding newer AEDs may complicate the pharmacodynamics and cause seizure increment or chronic intoxication.

For many years it has been my advice to avoid pertussis immunization, with the old type or cellular diphtheria-tetanus-pertussis vaccine, in any infant with TSC with or without infantile spasms or any other type of seizures. This advice has been amply debated in recent years, and now it is the prevailing belief that the pertussis immunization does not cause, trigger, or aggravate seizures in infants with TSC. There are no clinical data to support the empiric recommendation. Nonetheless, it is prudent not to re-administer the cellular pertussis vaccine to infants who developed an immediate acute reaction with fever, irritability, refusal to eat, or lethargy after an injection of pertussis antigen. The acellular vaccine has been proven to be much safer.

Treatment of Infantile Spasms in TSC Patients

From 1958 until recently, the preferred therapy for infantile spasms was either intramuscular adrenocorticotropic hormone (ACTH), hydrocortisone,

or oral synthetic corticosteroids (prednisone or dexamethasone). The AEDs were second-line treatment of infantile spasms because of the greater effectiveness of ACTH over AEDs. My experience as well as that of others[17] is that treatment with intramuscular ACTH injections is more effective and rapid acting than prednisone or any of the other synthetic corticosteroids. Until recently hormone therapy was more effective than the then-available AEDs. However, the advent of vigabatrin provided an effectual AED for treating infantile spasms that has taken the place of intramuscular ACTH and oral steroids. Because of the serious side effects of ACTH and corticosteroids, they should not be used unless vigabatrin fails to stop the infantile spasms. The undesirable side effects of hormonal therapy are Cushing syndrome, hypertrichosis, acne, obesity, arterial hypertension, intracranial bleed, electrolyte imbalance with sodium retention, hypokalemia, osteoporosis, cataracts, peptic ulcers, reversible or irreversible cerebral atrophy[9] or pseudoatrophy, insomnia, irritability, hyperactivity, and unprovoked, prolonged, inconsolable cry. To these complications must be added the high incidence of seizure recurrence when ACTH is being reduced, even if slowly tapered. The choice of ACTH or synthetic hormones, the optimal dose, the duration of treatment, and the method of withdrawal have been controversial. The failures and complications have provoked so much skepticism about all forms of treatment of infantile spasms that some physicians question the wisdom of treating infants with TSC and infantile spasms at all. In my view it is a mistake not to attempt to stop the infantile spasms. Today the treatment of choice is vigabatrin; if it fails, ACTH should be tried, taking all necessary precautions and watching closely for complications. If hormonal therapy is used, it should not be for a period of time longer than 16 weeks, including the tapering period.

Vigabatrin (Sabril), an inhibitor of γ-aminobutyric acid (GABA) aminotransferase, the principal catabolic enzyme of GABA, has been found to be more effective for controlling infantile spasms than any AED, particularly for the symptomatic variety and particularly when the etiology is TSC. Vigabatrin is freer of side effects than ACTH or valproate. Because vigabatrin is more effective and safer than ACTH in patients with infantile spasms caused by TSC, it is now the drug of choice. ACTH is recommended if vigabatrin fails to control the infantile spasms. Valproate and lamotrigine are used as add-on medications for the treatment of infantile spasms if vigabatrin alone meets with failure.[18] Valproate therapy, effective in as many as 50% of patients when given in large doses, is also potentially unsafe because it may cause anorexia, nausea, vomiting, leukopenia, hyperglycinemia, elevation of liver enzymes, hepatic failure, reversible or irreversible cerebral atrophy, dementia, pancreatitis, mild hair loss, alopecia, hand tremor, hyperammonemia, Reye syndrome, thrombocytopenia, increased appetite, obesity, depression, behavioral changes, inappropriate antidiuretic hormone secretion, and enuresis, among other side effects.

Patients successfully treated with ACTH or vigabatrin who no longer have infantile spasms may have recurrence of these seizures or develop

complex partial seizures during the withdrawal period. Valproate may be beneficial to prevent recurrence of seizures. In this situation vigabatrin may not be the best choice of AED if it was ineffective at first. Lamotrigine may also be useful to prevent the onset of complex partial seizures. Nitrazepam, clonazepam, clobazam, phenytoin, phenobarbital, or gabapentin may have little or no effect in preventing complex partial seizures, or their efficacy may be of limited duration. Felbamate has been effective in partial seizures in patients with TSC but it has not been properly tested because of the high risk of causing aplastic anemia, leukopenia and thrombocytopenia, or hepatic damage. There have been numerous fatalities and yet, because it has been effective in patients with the Lennox-Gastaut syndrome, its use is acceptable when all other AEDs have failed. There is at least one report of felbamate being effective in the treatment of infantile spasms.[19] Seizures of patients with TSC may become refractory to all AEDs, including lamotrigine. Polytherapy may be the next option to control partial seizures. In a selected small proportion of patients, lesionectomy or surgical removal of the cortical tuber causing the partial seizures may be an option (see below).

Many epileptic patients who do not have TSC or any other recognized structural lesion may suffer frequent partial seizures but no focal epileptogenic lesion can be identified in the EEG. Paradoxically, some patients with cerebral TSC having many cortical tubers identified by MRI may never have seizures. It is unknown what causes some patients with cerebral TSC to start having seizures, but once seizures start they tend to continue coming, and the longer they go on uncontrolled the more difficult is to prevent them with AEDs. The number and strategic location of the tubers seem to be important factors. Since the introduction of MRI, and more recently with the FLAIR technique, I have found that a higher number of cortical tubers in an infant's brain correlates with onset of seizures early in life and with the probability of having severe generalized seizures, including infantile spasms. This danger, and the chances of suffering a severe mental handicap, are greater if the tubers are located in the frontal or the temporal lobes.[16,20] Furthermore, Bolton and Griffiths[21] have proposed that patients with epileptogenic tubers in the left temporal lobe are susceptible to the development of autism or autistic like behavior. The same attribution has been proposed for frontal lobe tubers.

When medical treatments, particularly vigabatrin and ACTH, have failed to stop the infantile spasms, a surgical approach should be considered.[22] The clinical data, video-EEG recording, and neuroimaging (including MRI, single-photon emission computed tomography, or positron emission tomography) must indicate that there is an area of hypoperfusion or hypometabolism interictally corresponding to the clinical-electrographic event emanating from a cortical tuber. The location of the lesion away from primary motor areas will permit a surgical tuberectomy. At the Mayo Clinic in the period between 1986 and 1991, only nine patients underwent a tuberectomy; of these, four are totally seizure-free while taking AEDs, two are seizure-free without taking any AEDs, two had greater than 80% seizure reduction, and

one experienced only an initial temporary reduction in seizure frequency.[23] Unfortunately the number of patients who should be surgically treated is very small. It is apparent that the patients who are medically intractable are the ones with the largest numbers of tubers by neuroimaging and also with more generalized seizures and epileptiform abnormalities in the EEG.

Mental Handicap

Mental handicap is, after seizures, the most common neurologic symptom of patients with TSC. In my experience, when there is mental handicap, generalized seizures or partial seizures secondarily generalized are always associated. Mental handicap is never present in TSC patients who have not had these types of seizures. Approximately 50% of the patients with TSC seen at the Mayo Clinic had mental handicap ranging from very severe to borderline. Among the TSC patients with average intelligence, there are some who infrequently had seizures only of the partial motor type. All the patients with a severe mental handicap started to have generalized seizures in the first years of life and continued to be subject to them for a few months or years. Some of these patients are severely mentally handicapped youngsters, unable to speak or to understand spoken language or gestures, to follow directions, or to perceive danger. Many remain nonambulatory for years, continue to have bouts of generalized seizures, and seem to be specially vulnerable to seizing during infections. Accidental physical trauma occurs with falls during seizure exacerbations. Self-induced injuries are seldom a problem, and then only in the very severely mentally handicapped. Even after reaching puberty, the survivors persist in being fully dependent on their parents or caregivers; they live precariously, without education, socialization, or enjoyment of their existence.

Certain TSC patients who have few cortical tubers are seizure-free, or their seizures begin late in life. As shown in Table 4.1, all mentally retarded youngsters had or have had seizures. Table 4.2 and Figure 4.1 show that, when generalized seizures begin in the first 2 years of life, a great majority of the subjects are mentally handicapped, whereas, if the seizures begin late

TABLE 4.1. Mental Status in Relationship to Seizures

	No. of Patients		
Mental Status	Without Seizures	With Seizures	Total
Average intelligence	19	40	59
Mentally retarded	0	89	89
Not known	1	11	12
Total	20	140	160

TABLE 4.2. Mental Status in Relationship to Age at First Seizure

	Mental Status (No. of Patients)		
	Average Intelligence	Mentally Retarded	Not Known
Age (yr) at Onset of Seizures			
0–1	7	72	11[a]
2–4	13	9	
5–9	6	3	
10–14	2	1	
15–19	6	2	
>20	5		
Unknown	1	2	
Total Patients			
With seizures	40	89	11
Without seizures	19		1
Overall	59	89	12

[a]Too young for testing.

in life, the great majority of the subjects have average mental development. As a rule, an infant with TSC whose MRI shows a large number of cortical tubers begins having seizures in the first months of life, and the seizures are likely to persist for months or years despite treatment. In the case of infantile spasms, there are frequent recurrences even after successful treatment with ACTH or during treatment with vigabatrin. Some patients who do not respond to any medication fare even worse. This is particularly so in children with a large number of cortical tubers (greater than 10) the majority of which are located in the frontal or temporal lobes.[16]

Patients with TSC who have never had seizures, and those whose seizures were infrequent or short lived, or had their onset after childhood, may attain normal mental development. Those less fortunate infants and children who began having seizures before the age of 4 years and whose seizures were frequent, generalized, or partial with secondary generalization and were untreated or intractable, are at a high risk of suffering developmental stagnation or regression of their psychomotor development. This phenomenon is equivalent to a dementing process that may last months or years, or as long as the young patient continues having seizures. When the generalized seizures originate in a frontal or temporal lobe in a patient under the age of 2 years, the risk of a dementing process leading to mental handicap, autism, or both is very high.[16]

Spastic Paralyses

A small proportion of patients with TSC have upper motor neuron signs. The majority of these patients have suffered partial motor seizures with or

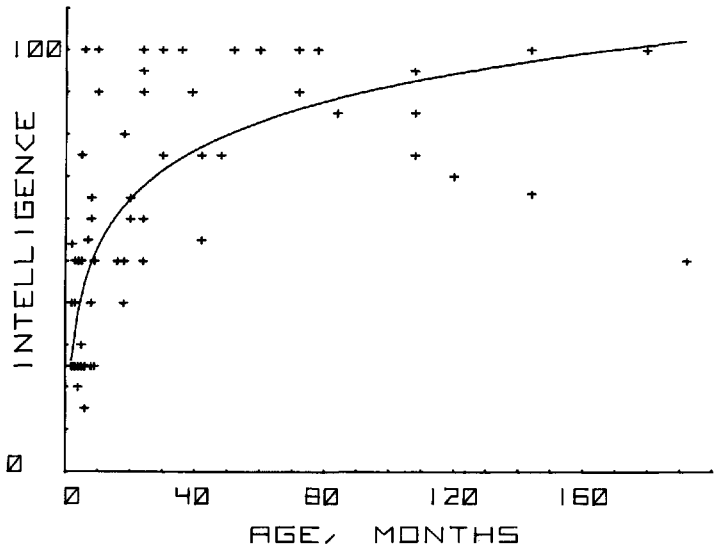

FIGURE 4.1. Computer-derived regression curve of mental status versus age at first seizure. The patient's age at seizure onset is plotted on the x axis and the estimated intelligence at the time the patient was last seen on the y axis.

without secondary generalization. A mild spastic hemiparesis may appear in the wake of daily transient episodes of postictal hemiparesis indicative of a prominent and very active cerebral tuber in the motor cortex. A double hemiplegia, usually asymmetric, may result from bilateral tubers in the motor cortex. Very severely affected patients with spastic diplegia may have associated severe mental handicap. Critchley and Earl also described monoplegia, paraplegia, and tetraplegia in their classic monograph,[14] and two of their patients had "atonic diplegia" with absent reflexes, flaccidity, and planovarus deformity of the feet. I have not found any patient with TSC whose muscle stretch reflexes were absent. Hypotonia and hyporeflexia are often features of TSC patients with persistent infantile spasms or other generalized seizures refractory to treatment.

Purposeless Movements

Involuntary movements have rarely been described in patients with TSC. I found athetosis in one of my patients. Chorea has been reported once as a "continuous, jerk-like, irregular, unsustained, involuntary movements" of hands and feet.[24] The head CT scan of this patient demonstrated periventricular calcifications, including a large calcified lesion in each caudate nucleus, undoubtedly SENs or SEGAs. Furthermore, this subject had facial angiofibromas, poliosis, and periungual fibromas and, undoubtedly, TSC. I

have seen transient chorea in an infant with TSC that appeared when ACTH was being tapered and vigabatrin introduced.

A curious symptom found in the severely retarded or demented patient is purposeless, bizarre motions of the hands, which adopt unusual postures.[14] These movements are apparently no different from the "mannerisms" found in other severely retarded patients who do not have TSC. Some severely handicapped patients with TSC have the habit of striking themselves repetitively and rhythmically with a finger, or with one or both hands, about their face, forehead, teeth, or other body parts. Other patients display rhythmic head movements or move the entire upper trunk in a rocking fashion, which can be violent enough to cause the head to strike a wall or the floor. Although these are purposeless movements, they should not be classified as involuntary because they can be stopped at will.

Considering the extensive involvement of the basal ganglia and cerebral cortex in some patients, it is remarkable that there are so few cases reported to have athetosis or chorea[24] and that the most common abnormal movements that are not localizing neurologic signs are those seen in the severely handicapped patients.

Cerebellar Symptoms and Signs

Cerebellar findings are seldom reported, although cerebellar tubers occur in as many as 15% of patients who have cerebral TSC. One of the 29 patients reported by Critchley and Earl[14] had cerebellar signs, and one with cerebellar signs reported by van Bogaert et al.[25] demonstrated a cerebellar lesion at postmortem examination. Among the patients seen at the Mayo Clinic who had cerebellar tubers detected with head radiography, CT scan or MRI, none had signs or symptoms attributable to cerebellar involvement of TSC. However, TSC patients have developed transient ataxia from taking large doses of anticonvulsant medication.

Intracranial Hamartomas

Subependymal Nodules

The SENs are hamartomas that vary in size between 2 and 10 mm or more, are partially or completely calcified, and are located on the lateral wall of the lateral ventricles along the terminal sulcus. Some nodules protrude into the ventricle and others are embedded into the basal ganglia region. After cortical tubers, SENs are the next most frequently found lesion in patients with cerebral tuberous sclerosis. SENs are well visualized on the head CT scan without injection of contrast material if they have become calcified or after injection of contrast if they are uncalcified. Not all patients with ce-

rebral TSC harbor this lesion, but the great majority of patients with TSC do, many of them remaining free of neurologic symptoms.

There is no clear gross or histologic distinction between SENs and SEGAs.[26,27] It is only by clinical convention that a subependymal hamartoma that grows sufficiently to cause symptoms is designated a tumor; also, by neuroimaging convention, a contrast-enhancing subependymal lesion on CT scan is called a tumor or a SEGA.[25]

Subependymal Giant Cell Astrocytomas

In a series of 345 patients with TSC from a large medical center, 6.1% of patients had at least one SEGA. This high figure is due to the selection of patients with intracranial tumor for neurologic or neurosurgical referral.[27] There was no patient with a SEGA who did not have additional findings of TSC.

The SEGA grows by multiplication of dysplastic cells with characteristics of both neurons and glial cells (see Chapter 18). It is thought that these cells arise from the SENs situated in the lateral ventricular walls, perhaps as a result of the loss of a further tumor suppressor gene. Why this hamartoma grows preferably during childhood or adolescence of TSC patients but not in adulthood, as do angiomyolipomas, is still unknown. The median age for TSC patients to develop signs of intracranial hypertension from a ventricle-obstructing SEGA is 9 years (range 1 to 27 years).

Symptoms of SEGAs are consequent to increased intracranial pressure. The essential clinical features are headaches, vomiting, and bilateral papilledema. Between 40% and 50% of these patients will also have retinal astrocytic hamartoma(s), which confirm the diagnosis of TSC and SEGA even before neuroimaging. The head CT scan will demonstrate dilatation of one or both lateral ventricles and a tumor mass obstructing one or both foramina of Monro. SEGAs are almost exclusively intraventricular and may obstruct the anterior portion of the third ventricle, or grow from one ventricle into both lateral ventricles without attachment to the ventricular walls, thus forming a cast in one or both ventricular cavities. If SEGAs are left untreated, the patient succumbs to the intracranial hypertension.

The treatment of a SEGA is surgical. In an advanced stage of intracranial hypertension, the patient may benefit from decompression with a shunt. In patients not acutely ill, tumorectomy, ideally by computer-assisted stereotactic approach, is the procedure of choice; if this is not available, resection by a transcallosal approach may be done. About one third of the patients who develop a SEGA will develop a second tumor in the other hemisphere. SEGAs may also develop within the white matter of the hemisphere, apparently from nests of dysplastic and heterotopic cells that failed to differentiate and migrate to the cerebral cortex.

Aneurysms of Cranial Arteries

There have been at least seven cases of intra- or extracranial aneurysms reported in patients who had TSC. Convincing evidence that this is not coincidental is based on two facts. First, six of the seven reported patients were young adults or children (ages between 6 and 26 years)[28–35]; only one was much older (53 years).[33] Second, aneurysms of the aorta or other large arteries resulting from a congenital defect of the arterial wall have been recognized on many occasions (see Chapter 16). One of the reported cases, a 12-year-old boy, had a fusiform dilatation of both internal carotid arteries in the region of the siphon.[28] Two other patients, ages 6 and 17 years old, had a unilateral aneurysm of the supraclinoid portion of internal carotid.[31,33] The 6-year-old, in addition to the fusiform internal carotid aneurysm, had another fusiform aneurysm of the A1 and A2 segments of the anterior cerebral artery and has been twice reported.[33,35] The 17-year-old, in addition to the internal carotid aneurysm, had another aneurysm of the left anterior communicating artery.[31] Another 7-year-old boy had a giant suprasellar aneurysm that arose from the A1 segment of the right anterior cerebral artery.[32] A 24-year-old man died after a subarachnoid hemorrhage from a ruptured saccular aneurysm of the right middle cerebral artery.[29] A 26-year-old woman who died of a massive hemorrhage in a kidney at autopsy was found to have an unruptured aneurysm of the choroidal plexus of the right lateral ventricle.[30] The 53-year-old man had a giant aneurysm of the supraclinoid portion of the left internal carotid artery.[34]

In summary, at least seven individuals with TSC each had one or two aneurysms of the internal carotid, posterior communicating, middle cerebral, or anterior cerebral arteries.

Epileptic Pseudodementia in TSC

Infants with TSC who suffer frequent generalized seizures at a time when their brains are still growing and undergoing structural changes are vulnerable to cognitive impairment. Although the mechanism is not well understood, infants and children whose seizure onset is at a very young age suffer impairments, possibly through distortions of the cortical assemblage that could include defects in neuroblast differentiation and migration, dendritic spine formation, dendritic pruning, apoptosis, orientation of growth cones to navigate toward specific axonal destinations, synapse formation, and establishment of neuronal circuits. It seems plausible that relentless, abrupt, and repetitive bizarre electroclinical activity originating in the cortical tubers and generalized as infantile spasms could disrupt the establishment of an effective neuronal circuitry and create misdirected and obsolete false paths in a developing brain. The result would be further recurrence of seizures, resistance to AEDs, impairment of cognition, and a developmental decline or standstill with arrest or regression of language acquisition and decline of the

social advances that in its more severe form is childhood autism. In short, the many cortical tubers, through their electroclinical behavior, are able to create additional chaos. Supporting this view is the fact that, in all my patients, severe mental deficit, cognitive and behavioral disorders, and autism occurred only in those patients who had suffered frequent, severe, and persistent generalized seizures in the first 3 or 4 years of life. These handicaps did not occur in patients with cerebral TSC who never had epileptic seizures. Because these abnormalities are always associated with the seizures emanating from cortical tubers, it behooves us to recognize and bring the seizures under control as soon as possible (see also Chapter 5).

Behavior Disorders

Impaired social behavior and communication skills, obsessions, and hyperactivity have long been recognized in children with TSC who had infantile spasms, as Hunt and Dennis reported.[36] Other behavior disorders of difficult management are impulsivity, aggressiveness, attention deficit, and sleep disorders. However, of all the syndromes of mental dysfunction found in early childhood, the most puzzling to the neurologist and the neuropathologist are the so-called psychosis of childhood syndromes, including childhood schizophrenia and autism.

In my experience, mental dysfunction in patients with TSC is always associated with seizures. There is sufficient evidence to support the concept that cumulative damage occurs from repeated infantile spasms or convulsive status epilepticus, and it is not necessary that the patient have multiple growing tumors; however, there must be multiple cortical tubers.

Childhood Autism

Autism is a syndrome with a variety of possible etiologies that chiefly consists of disturbed cognition, communication, and social interactions. As described by Rimland,[37] "The first awareness of any problem is often the observation that the child fails to make the anticipatory movements prior to being picked up. . . . "Between the fourth and eighteen months several disturbing symptoms will have begun to appear . . . prolonged rocking and head banging in the crib, apathy and disinterest in the surroundings, obsessive interest in certain toys or mechanical appliances, highly repetitive and ritualistic play, insistence on being left alone and that the physical environment remain unchanged." One of the most disturbing of the symptoms is what has been called autistic aloneness: "The child's attention cannot be attracted by calling his name or speaking. Most autistic children act as if other people did not exist. . . . After the child has reached the second birthday not only [has] the child's development become of concern, he has become exceedingly difficult to live with." The lack or the fleetingness of attention and

the lack of speech makes the parents suspect deafness. The prognosis of autism is closely related to the development of language. Speech itself usually is not related. Comprehension of spoken language is crucial for expecting a favorable prognosis. Kanner and Lesser[38] note that "The prognosis of early infantile autism has not been influenced by any form of therapy."

The actual percentage of autistic children who have cerebral TSC is not known. The cited number is probably an underestimate in part because not all autistic children are examined in depth to detect signs for diagnosing TSC. Greenstein and Cassidy[39] noted that current genetic literature fails to mention autism as a possible manifestation of TSC. The same authors stated that "it is unnecessary and wasteful to perform all the usual ancillary tests including renal ultrasound and cardiac echocardiogram to rule out TSC in every autistic child as a rare cause of childhood autism."[39] It is my view that autistic children with a history of infantile spasms or any variety of seizures *should* be investigated for signs of TSC because there is high probability that TSC is its cause.

Hunt and Dennis[36] reported a prevalence of autism of 51% in a group of 90 children with TSC gathered from the Great Britain Tuberous Sclerosis Association. In a study carried out in Sweden, 61% of patients met the criteria for autism and 86% for a "more broadly defined form of pervasive developmental disorder."[40] This very large proportion in this series of patients may indicate that the category is poorly defined and probably includes other types of mental handicap. (See Chapter 5 for additional information.)

Dementia in the Adult

Another aspect of the development and progression of mental symptoms in patients with TSC who were subject to seizures was reported by Critchley and Earl:[14] "a combination of intellectual defect proper with a primitive form of . . . catatonic schizophrenia, though the exact form of reaction varies with the level of the mental development at which it occurs." These authors also observed that "the depth of psychosis is independent of the degree of intellectual defect, although the two processes are so inextricably intertwined as to render impossible any accurate estimation of the part played by either in any given case." Confusing the issue is the effect that the unfavorable environment of a home or asylum for the mentally handicapped may have played on these authors' patients. These extrinsic factors can obscure any signs of mental deterioration.

Harrison and Bolton[41] reported on a small group of TSC patients who manifested signs of specific developmental disorders such as dyspraxia, dyscalculia, visuomotor disturbance, and speech delay. Because these are common disorders in the general population, it is difficult to make a good case from these important observations.

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Psychiatric and Psychological Aspects

Introduction

Tuberous sclerosis complex (TSC) is one of the most common genetic conditions and one associated with some of the most problematic behaviors in children, in that both autism and hyperactivity can occur against a background of mental handicap and intractable epilepsy. Adults also are known to develop hallucinations and delusions, high levels of anxiety, and obsessive behaviors, although there is no reported evidence for dementia occurring without seizures. Mental handicap has been found in prevalence studies to affect less than 50% of those with tuberous sclerosis,[1] but it remains true that all those with this disability have had seizures at some point in their lives. The earlier seizures begin, particularly infantile spasms, the greater is the likelihood of later severe mental handicap,[2(pp28,31)] although this does not inevitably happen. However, although infantile spasms are associated with autism, this is not always the case in tuberous sclerosis.[3,4] There are case reports of autistic behavior in tuberous sclerosis without a history of seizures[5] or mental retardation.[4,6] It is therefore likely that, although mental handicap or epilepsy plays a role in the abnormal behavior found in TSC, the underlying cause is directly related to the brain pathology. Language difficulties can compound the autistic or socially impaired behavior, and, where the child has little understanding of accepted social behavior and is obsessed with a restricted range of unusual activities, aggressive or destructive behavior can result.

Although physicians are concerned with the control of the epileptic seizures, for many families it is the behavior problems that cause major disruptions. The stress experienced by parents and siblings can be helped by family counseling as long as the contribution of the neurologic dysfunctions of the person with TSC are not ignored. With the advent of new techniques in computed tomography (CT), magnetic resonance imaging (MRI), and

other brain scanning modalities, it will become possible to understand how the brain lesions and current seizures are involved in the behavior, and this information should lead to more tailored methods of intervention.

Background

Early descriptions of TSC come from physicians such as Bourneville, workng in mental institutions where much abnormal behavior would be assumed to be part of mental handicap. "Epiloia" as an alternative name for the condition was proposed by the psychiatrist E. B. Sherlock in 1911,[7] but, because it means "epilepsy and anoia" (mindlessness), it is not nowadays an acceptable term.

Some of the best descriptions of behavior in tuberous sclerosis were published in 1932 in Critchley and Earl's[8] report of autistic behavior in tuberous sclerosis, predating Kanner's use of the term *autism* but using other psychiatric terms then current for classification. These descriptions are quoted as an introduction to behavior in TSC because, as recent studies become more controlled, they tend to lack any descriptions of actual behavior in favor of median test results. Although this gives more accurate information on the relationship of behavior in TSC to that in other psychopathology, such as autism or hyperactivity, it detracts from consideration of how the actual behavior in TSC could be related to the causes of the condition or to social circumstances. Critchley and Earl studied 29 residents of mental institutions and noted that "often the family history shows no evidence of tuberose sclerosis but bears a strong psychopathic taint" (p. 314), with three of the study children having parents in asylums. They stated that "The essential feature of the psychology of epiloia is a combination of intellectual defect proper with a primitive form of psychosis. The intellectual defect is always pronounced, all our cases falling into the categories of imbecility and idiocy. The psychosis always resembles a primitive form of catatonic schizophrenia, though the exact form of the reaction varies with the level of mental development at which it occurs. . . . The depth of psychosis is independent of the degree of intellectual defect, although the two processes are so inextricably intertwined as to render impossible any accurate estimation of the part played by either in any given case" (p. 320).

Those with more severe mental handicap were said to have absent speech but often have screaming fits. Allowing for the language of the 1930s, there are descriptions of "dissociation" and complex obsessive stereotypies that can be seen nowadays in those diagnosed to be autistic. "All manner of bizarre attitudes and stereotyped movements occur, and these are most striking in the hands and fingers. Somewhat similar hand movements sometimes occur in simple aments of the idiot grade, but they are uncommon and are very rarely complex, whilst in the low-grade epiloic they are a constant feature, frequently indulged in, and often of an amazingly complicated nature. These movements appear to have some significance for the

patients; they occur when the appearance of dissociation is most marked, and the eyes usually fixate the moving hand. Thus one boy has been seen with rapt expression keeping up a highly complicated rapid movement of his right hand and slow and entirely different movement of the left, completely indifferent to his neighbour who was hitting him vigorously on the head with his open hand'' (p. 322) "[I]t is impossible even to guess at their subjective processes, but from the sudden fixation of their gaze upon nothing and from their air of listening—again to nothing—intently and with a strange and alien smile, it seems possible that they undergo some crude hallucinatory experiences'' (p. 323). Crutchley and Earl[8] tested five subjects with psychometric tests but commented that behavior under test showed obvious indications of psychosis in all cases. "One boy who had kept up an amazing series of attitudinizations and elaborate movements of his hand, suddenly ceased, and filled in the Wallin pegboards quickly and neatly, in thirteen and fourteen seconds respectively, to return at once to his movements, whence no further stimulation could arouse him'' (p. 325).

A more complex picture is described in some of those patients with mild mental handicaps with a deterioration in adolescence. This deterioration is now far rarer, so that it is possible that most cases would nowadays be classified as a response to institutional life, undiagnosed brain or kidney tumors, or untreated nonconvulsive seizures or even status epilepticus. Critchley and Earl[8] noted that "This is first seen at or after the fifth year, occurring as periods of hours or days during which the patient appears vague and preoccupied and is mentally inaccessible'' (p. 321). At or before puberty, "the states of preoccupation are longer and more frequent and the patients grow solitary, silent and apathetic and liable to sudden brief outbursts of motiveless excitement similar to those seen in catatonic schizophrenics. They usually resent interference and become dangerous to other patients and sometimes to themselves, though the self-injury is always slight and suicidal attempts do not occur. Speech which may have been fairly fluent now falls off, they use very few words and may show echolalia and short perseverative repetition of phrases, or may become entirely mute'' (p. 321).

After Critchley and Earl there were no further systematic studies of behavioral problems in TSC during the following 50 years, but there were occasional small studies, and 50 cases have been collected and reviewed by Smalley and colleagues.[6] Case reports cannot give any indication of the prevalence of problems, but three problems predominated: autism or autisticlike behaviors (36%), hyperactive or impulsive behaviors (26%), and aggressive or uncooperative behaviors (48%). The majority had seizures (90%) and mental retardation (64%).

More systematic studies began again in the 1980s with Hunt's[9] collection of data on 97 people with TSC, with an age range from 1 to 51 years, through an initial postal survey of members of the parents' support group in the United Kingdom. Hyperactivity was reported in 22%, destructive or aggressive behavior in 18%, screaming in 14%, and temper tantrums in 9%. The most pressing problem for 35% of parents was the behavior, including

the communication problems, as opposed to 24% of parents finding the epileptic seizures the biggest worry. Information on autistic behavior was not sought in this study.

In Hunt's later postal survey[10] of 300 people with TSC, with an age range from 6 months to 74 years, 80% of the sample had mental handicaps and 93% had a history of epileptic seizures, 63% with current seizures. Speech was present in 54% but only assessed as normal in 31%, with echolalia in 22%. There were 232 people with mental handicaps over the age of 2 and, although only 11% were unable to walk, 55% had no speech at all, a very high level of communication problems. Self-care skills were also limited, with over one quarter unable to feed themselves, over half being incontinent, and over two thirds unable to dress or wash themselves. This level of dependency adds to the time taken by parents in caring for their children as well as increasing the likelihood of back injuries and fatigue.

From parental reports,[10] 56% had shown behavior that caused problems to others at one time, and this varied from 18% of those with no mental handicap to 64% of those with learning difficulties. One of the underlying causes of disruptive behaviors can be hyperactivity, and 28% of the whole sample were considered to be more physically active than would be expected for their age; this included 35% of those with mental handicaps as opposed to 2% of those without. Uninhibited behavior in public was reported for 55% of those with mental handicap and 7% of those without. Noncompliant behavior when learning a new task was present in 55% of those with mental handicaps but also in 40% of the rest. Sleep problems were reported for 66%, 60% having difficulty falling asleep and 62% waking in the night and not settling easily. There were problems of anger control in 33% of the whole group, and again the majority of people with aggressive behavior were learning disabled, with 96% of rages, 95% of attacks on others, and 97% of self-injurious behaviors occurring in this group.

The basic criteria for autism or pervasive developmental disorder include an impairment of interactive social behavior, communication problems, and obsessive or ritualistic behaviors. Aloof behavior and a lack of easy eye contact were reported by Hunt[10] for 41% of those with mental handicaps, with 44% described as "in a world of their own," although only 16% objected to physical contact. However, the aloof behavior was also reported in 10% of those with normal intelligence. Obsessive behaviors posed problems in those with mental handicaps; routines were an obsession with 31%, unusual objects with 46%, 44% had repetitive and odd play, and 50% had the unusual routines of gestures called stereotypies. In contrast, these kinds of behaviors were reported for less than 7% of the rest of the sample.

Although over 50% of children with tuberous sclerosis are within the normal range of intelligence, many will have problems during their schooling, mainly problems associated with specific language delays and retrieval of words from memory. Abstract concepts such as time can be difficult to grasp, as can other relationships expressed in mathematics. The specific

communication problems of 14 children with TSC with normal intelligence (IQ >70) were reported by Baltexe.[11] Nearly three quarters had delayed language development and half had extra help for reading, writing, math, or language within normal schools, but for the majority of this group the problems were mild. The severe language problems of the more disabled children have never been studied, but the milder problems may give some indication of the areas affected.

There was impaired use of interactive language for social communication in 87% of the children studied, but gesture, body movements, and interpretation of nonverbal facial communication were normal. Eye contact was normal apart from two children who were later found to have oculomotor apraxia. Receptive language was generally good, particularly if associated with visual input, but there were receptive problems associated with the construction of any kind of relationship, particularly temporal or spatial. Expressive language showed far more problems, with extreme word retrieval difficulties; even when presented with two words these children showed long hesitations. This also occurred in the social use of language. Making inferences was a high-functioning area, but abstract language and concepts were difficult and metaphorical expressions rarely used or understood. There were problems with auditory language processing (accurate repetition), which could mean that instructions would be heard and understood but not retained long enough for action. It is easy to see how such working memory problems could cause difficulties between the children and their parents or teacher.

Autistic Behavior in Tuberous Sclerosis

As noted earlier, autistic behavior was described in children with tuberous sclerosis by Critchley and Earl[8] even before Kanner first named the syndrome. A recent review by Rutter and his colleagues[12] of medical conditions that could be associated with autism concluded that tuberous sclerosis had probably the best evidence for a significant association. The first systematic study to assess autism in TSC was that of Hunt and Dennis[13] using interviews and medical records for 90 children, the majority of whom had mental handicaps. The interviews were undertaken in the early 1980s, before there were established checklists for autistic behavior, but questions on behaviors were included that fulfilled Rutter's criteria for autism, that is, a profound failure to develop social relationships, language retardation, and ritualistic or compulsive behavior that showed onset before 30 months of age. Assessing all the children at 5 years of age, there were 46 children (51%) who were classed as autistic, all of whom were learning disabled. At the time, many people believed that the autistic behavior in TSC was the result of the early infantile spasms, and 69 children in the study had a history of these seizures. Forty of the autistic children, 58% of those with infantile spasms, had indeed had this form of epilepsy, but there were also six children who had no medical records of infantile spasms, although other seizures were

recorded. Therefore, infantile spasms themselves could not be the only cause of the behavior in these children.

There were further studies supporting an association of autism and TSC that was higher than in the general population. In Finland Riikonen and Amnell[13] had been following a group of 192 children with infantile spasms from all causes and had found that 24 (13%) were autistic, so that the 58% in the Hunt and Dennis[13] study of children with TSC represented a far higher proportion with this outcome. Riikonen and Simell[15] assessed the behavior of 24 children with TSC from a group with infantile spasms and mental retardation and found that 17% were autistic and 4% had poor social contact and extreme shyness. Curatolo and colleagues[16] studied the electroencephalograms (EEGs), MRIs, intelligence, and behavior of 23 children with TSC over the age of 5. Six (26%) fulfilled the diagnostic criteria for autism, all of whom again were mentally retarded. Although autism was reported to be associated with TSC in these three studies, it ranged from 17% to 58% because of the various sources from which the samples were drawn. The question also remained whether autism in TSC only occurred in the group with mental handicaps and/or seizures. The presence in the Hunt and Dennis study of the 42% of children who had infantile spasms but who were not autistic, and in particular of the 9% who were also of normal intelligence, was a strong indication that neither of these factors causes the autism in TSC.

Smalley and colleagues[6] also analyzed seven systematic studies of autistic populations that included people with TSC in the sample. The frequency of children with TSC in the autistic population ranged from 0.4% to 3%, whereas TSC at that time was thought to occur in 0.01% of the general population. Among those in the autistic populations known to have seizures, the frequency of TSC was higher at 8% to 14%. By then reliable behavior checklists for autism had been developed and these researchers were able to compare the behavior of 13 children with TSC with that of 14 autistic children without TSC. Six of the 13 had an IQ greater than 70, none had an IQ in the range of 51 to 70, and seven had an IQ less than 70. Smalley et al.[17] found seven (54%) of the TSC-affected sample to meet the criteria for autism of the *International Classification of Diseases*, 10th revision (ICD-10)[18]; six of these seven had severe mental handicap. On the Autism Diagnosis Interview, the autistic children with TSC rated similar to the autistic children without TSC on the Social and Verbal and Nonverbal Communication domain (with a trend to higher scores), but lower on the Repetitive Rituals domain. Fewer of the autistic children with TSC were reported to have shown preoccupations, compulsions, or unusual attachments to objects, although there were similar frequencies of hand and finger stereotypies and unusual sensory responses. Although the sex ratio among TSC subjects was approximately equal, the study found significantly more autism among the males ($p = .002$). All seven autistic children with TSC had seizures but, although six of the seven were learning disabled, even the pres-

ence of one autistic child with normal intelligence indicates that the behavior is not the result of the mental retardation per se.

Epidemiologic studies are required to ascertain the frequency of autism or other disorders in TSC. Populations drawn from clinics or selected from parent groups are open to bias toward more severe problems. There have been two systematic prevalence studies, one in Scotland by Hunt and Shepherd[3] and the other in Sweden by Gillberg and colleagues.[4] The prevalence study of the behavior of 21 children ascertained to have TSC in the west of Scotland found 24% of children who fulfilled the criteria for autism described in the *Diagnostic and Statistical Manual of Mental Disorders*, third edition, revised (DSM-III-R)[19] and a further 19% who showed an autisticlike condition, a total of 43% with such socially impaired behaviors. Unlike the preponderance of males in the Smalley study, three were males and six were females. Of the 12 children with moderate to profound mental handicaps, five were autistic, four had some traits, and three had no disorder. The nine children of average or near-average intelligence included one boy with "odd" social interaction and jumbled speech but eight who were not socially impaired. Seizures were not always current for the autistic children, having stopped up to 8 years previously for one 10-year-old girl. There were also four children with current seizures who had normal intelligence and no autistic behavior. The Swedish study[4] found 32 cases of children with TSC under the age of 20 in the western part of Sweden, 28 of whom took part in the study. Eighty-six percent of children had some autistic behavior according to DSM-III-R criteria, with 61% fulfilling the full criteria for autism, but there was a lower percentage of children in the normal IQ range than the 44% in the Scottish study. There was no significant association of TSC autism with the sex of the child. The 19 children with mild to profound mental handicaps (IQ <70) included 14 with autism, four with an autisticlike condition, and one who was not psychiatrically examined. There were 11 children with near-average or average intelligence (IQ >70); 3 were autistic, 2 had autisticlike conditions, 1 had Asperger syndrome, 1 was described as "odd," 3 had no disorder, and 1 was not examined. All the seven children with moderate mental handicap who were examined were rated to have autism, as were three of the four with mild mental handicaps, the fourth having autistic traits. Only 14 of the 24 children with autistic behavior had a history of infantile spasms, and one of these had no other history of seizures. The authors suggest that the clinical triad of severe mental retardation, epilepsy and autistic behavior under the age of 5 years has a stronger correlation with TSC than the classic diagnostic triad of severe mental retardation, epilepsy, and skin problems.

These three studies found that there was a very high occurrence of autism in TSC and that, although mental handicap was not inevitably concomitant with autism, it was strongly associated. The three studies collected data on epileptic seizures and, again, although seizures are very common in people with TSC, similar histories were found in those with and without autism. This strongly suggests that the autistic behavior in TSC stands as a

symptom of the condition in its own right, and that TSC should be borne in mind as a diagnosis whenever there is a history of epilepsy in an autistic child, because the condition has implications for other family members.

One of the main criteria for autistic behavior is an inability to relate well with one's peers; therefore, many of the children with moderate mental retardation may find integrated education a great strain because they cannot relate to the activities around them unless specifically directed. The presence of this behavioral syndrome implies the need for very structured learning programs in the education of these children, even for those with severe mental handicaps. They may derive little benefit from being taught as part of a group, ignoring instructions not specifically directed to them, a problem compounded in TSC by additional attention deficits. Unawareness of social behavior also makes teaching self-care skills difficult. Behavior learned and practiced in one setting may not be transferred to another. These problems, taken together with deficits of language and communication, can sometimes lead to frustration and outbursts of aggression in these children, or sometimes to withdrawal into their own interests. Their behavior can also seem odd to their peers because those interests may not be the same as the prevailing group interest, and this can lead to teasing and bullying.

The autistic behavior in tuberous sclerosis may be related to the position of the brain lesions. Curatolo and colleagues[16] reported that severely retarded children with autism, hyperkinesia, and severe sleep disorders showed large bifrontal and posterior tubers on MRI, as opposed to nonretarded children with hyperkinesia and aggressive behaviors, who had isolated cortical parietal or posterior tubers. A third group with moderate retardation and hyperkinesia but no autism had frontal tubers that were single and unilateral. Curatolo et al. concluded that, because hyperkinesia was present in all three groups, it may not be as directly related to the position of the tubers as the autistic behavior.

Hyperactivity in Tuberous Sclerosis

Brain damage, from whatever cause, is associated with hyperactive behavior, so it is no surprise that the other major behavior disorder in children with TSC is hyperactivity, but this has been less studied than autism. However, if it is associated with an attention deficit, as in hyperkinetic disorders (ICD-10) or attention-deficit hyperactivity disorder (ADHD; DSM-III-R), this will produce not only learning difficulties at school but disruptive behaviors at home, at school, and in public. In the early Hunt postal study,[9] 22% of children were reported by parents to be more active than would be expected for children of their age. In the fuller interview study,[13] 59% of children had problems recorded, with 41% of the children being both autistic and hyperactive, but the majority of children in the study were learning disabled. In the review of 50 cases by Smalley and colleagues,[6] hyperactive

or impulsive behavior was reported for 10 people (20%), with three (6%) also showing autistic behavior. Hyperactivity was not part of the further study comparing 13 children with TSC with 14 autistic children.

In Hunt's later study of 300 cases of TSC,[10] 28% of the whole sample were considered to be more physically active than would be expected for their age. Thirty-five percent of people with learning difficulties were reported to be overactive, as opposed to only 2% of people with normal intelligence. In the same way, 30% of people who had an epileptic seizure were overactive, as opposed to 5% of those without seizures. The criteria for attention-deficit disorders were not sought, but uninhibited behavior in public was reported for 55% of those with mental handicap and 7% of those without. Noncompliant behavior when learning a new task was present in 55% of those with mental handicaps but also in 40% of the rest, and 64% of those who were overactive also showed noncompliant behavior.

Hyperactivity is only one component of an attention-deficit disorder, but in the prevalence study of behavior in all children ascertained to have TSC in the West of Sweden,[4] 46% of the children examined were rated to have ADHD as diagnosed from five items: activity level, power of concentration, attention, ability to sit still, and vigilance; all of these children had some autistic behavior. Attention-deficit disorders can cause learning problems in ordinary classwork, and half the children who had IQs in the range of 51 to 85 had attention-deficit disorders, although none of the children with average intelligence did. In contrast, although not specifically looking at hyperactivity, in the prevalence study of autism in Scotland,[3] parents reported 52% of the children, almost half of whom were of normal intelligence with no autism, to be overactive.

These studies looked at children of varying ages with a variety of questions but in total do point to an association of hyperactivity and attention-deficit disorder in children with tuberous sclerosis, not a surprising finding because any brain damage, such as the brain lesions in tuberous sclerosis, may make children more vulnerable to hyperactivity.[20] Over a third of the children with tuberous sclerosis and learning difficulties were overactive, and a quarter had the overactive and impulsive behavior that can disrupt both family and school or center life. This area of behavior in TSC merits further study in relationship to the autism, epilepsy, and mental handicaps in the condition. An attention deficit can lead to learning difficulties, which in turn can lead to lower self-esteem in a child who is aware of the problem but unable to control the behavior, so that grossly disruptive conduct can result. Attention deficits are common in the general school population, and medications are prescribed to increase concentration, but there is a need to study whether the use of this medication is appropriate in children with TSC, who may also have epilepsy. Intervention with behavioral programs to aid concentration and advice to parents on how to help with acceptable behavior in the home should also be an early concern of physicians who have children with TSC in their care. Too often such advice is only given after the behavior

is so disruptive that a referral to a psychiatrist becomes necessary and the ingrained problems are more difficult to tackle.

Sleep Disorders

Sleep disorders are rarely inquired about in routine clinical practice but can sap family morale when they persist over a period of weeks, months, and even years. Because two thirds of the 300 people with TSC in Hunt's study[10] had been reported to have had sleep problems, a further, more controlled interview study was undertaken by Hunt and Stores.[21] Forty children with TSC were compared with three groups: their siblings to control for family variations, age/sex-matched children without TSC, and the published results from a study of sleep problems in children with mental handicaps in general.[22] The Quine Sleep Questionnaire[22] was used to assess sleep problems, and detailed information on seizures was gathered and pervasive developmental disorder was assessed from the Developmentally Delayed Children's Behaviour Checklist (DDCBC).[23] At night, the severe settling problems and disrupted sleep in the group of children with TSC were far greater than those found in siblings and children of normal intelligence, and even greater than found in children with mental handicaps in general. There was a highly significant association between the presence of uncontrolled epilepsy and these problems ($p < .001$), but no significant association with autistic traits. There was also a significant positive correlation between the number of sleep problems of individual children and their Total Behaviour Problem scores on the DDCBC, where the mean score for the children with severe mental handicap was within the range of DDCBC scores for children rated by child psychiatrists as psychiatric cases.

The sleep is disturbed sometimes from active seizures, but also from epileptic activity disrupting brain wave patterns normally found during sleep. This has been demonstrated by Bruni and colleagues,[24] who found in an EEG and polysomnographic study that the sleep architecture of 10 children with tuberous sclerosis was very abnormal. Compared with controls, the TSC group showed a shorter total sleep time; a reduced sleep efficiency, which was higher than 90% in only one case; a higher number of awakenings and stage transitions; and a decrease in rapid eye movement sleep. There were overt nocturnal seizures in three children, but interictal epileptiform activity was found in all cases. There was a consistent improvement in sleep architecture in the children who had a second polysomnographic recording after a seizure-free period. Sleep alterations were more evident in the children who showed large bifrontal and temporal tubers on MRI than in those who had isolated cortical parietal or posterior tubers. Only one very disabled child had a marked disturbance in circadian rhythm.

From these studies it would appear that the disturbed sleep is related to the epileptic activity arising from the brain lesions and not to psychological problems found in children without TSC. Behavior modification programs

for sleep have sometimes been successful, but parents may need more support over a longer time for these to succeed. There have been anecdotal reports that melatonin dramatically reduces sleep problems in some children. This was the subject of a controlled study in the United Kingdom reported by O'Callaghan et al,[25] employing analysis of the level of a melatonin metabolite in urine. There was a slight improvement in initial settling time, sufficient for a further study to be undertaken. Given the correlation between sleep problems and daytime behavior problems in the children, improvement in sleep, whether produced by melatonin or better seizure control, would not only improve sleep for both the children and their families, but would also lessen the daytime problems, thus reducing overall parental stress.

Aggressive Behavior

Aggression is probably the behavior that will bring children with TSC to the attention of psychiatrists and psychologists, particularly those children receiving mainstream education, where such behavior is less tolerated. Adults may find themselves in court because of such behavior. Hunt and Dennis[13] found aggressive or destructive behavior reported for 13% of children, but Hunt reported 33% in her study of 300 people.[10] Attacks on other people occurred in 28% of Hunt's sample and self-injurious behavior was reported for 29%, with people biting or hitting themselves or banging their heads. Only 5% attacked both other people and themselves. The majority of people with aggressive behavior were mentally handicapped. Ninety-six percent of temper tantrums, 95% of attacks on others, and 97% of self-injurious behavior occurred in this group, which indicates that given the inability of half of this group to express their needs in speech, aggression may be a learned mode of communicating their distress.

In Smalley et al.'s review of 50 reported cases,[6] 48% showed aggressive or destructive behavior, which is higher than in the other studies. However, in an unpublished study reported at the International Tuberous Sclerosis Symposium in October 1994, the same group had used information from the American TSC Registry and a survey of parents belonging to the American support group (the National Tuberous Sclerosis Association). This information was compared with the Overt Aggression Scale, which measures both verbal and physical aggression at four levels of severity. Aggression was recorded for 54% of the 353 people in the survey, but in the majority of cases it was assessed to be only mild.

The triggers for aggressive behavior vary with individuals, but the high percentage of occasional aggression in TSC can be readily understood given the epilepsy and potential side effects of medication, mental handicaps, lack of communication skills, autism, and attention deficits all associated with the condition.

Other Psychiatric Problems in Adults

There are very few published reports of other psychiatric problems in TSC, and those that exist tend to be reports of single cases of serious psychoses. However, given the high levels of both cognitive and psychiatric problems in the children with mental handicaps, it would be naïve to suppose that no problems occurred either in children who had normal intelligence or in adults when faced with the normal stresses of later life. Concrete thought processes and obsessive interests that are accepted as "odd" in a child in mainstream education will be less tolerated in a wider adult society, causing unexpected social isolation and consequent depression. This is an area that needs further research because there is some evidence that tuberous sclerosis could make adults more vulnerable to psychiatric problems. The fact that TSC is a genetic disorder means that adults who are aware they have this dominant trait must consider whether to have children or wait until tests become available, a factor that can cause depression and stress in relationships.

In a study of an extended family with 74 members, there were psychiatric evaluations of 16 relatives without TSC and 17 relatives with TSC in whom the physical expression was mostly mild.[17] The overall rate of psychiatric disorder was 76.5% in those with TSC and 25% in nonaffected relatives ($p = .009$). These problems were mainly anxiety disorders in 58.8% of those with TSC and 12.5% of those unaffected, and mood disorder in 35.3% of those with TSC and 6.2% of those unaffected. Critchley and Earl,[8] as previously quoted, described the psychosis in TSC as a "primitive form of catatonic schizophrenia" with profound "disassociation." Gomez[2(p32)] observed that mental dysfunction in patients with TSC is always associated with generalized seizures or increased intracranial pressure. Cumulative damage can occur from repeated and frequent convulsions, and mental retardation in TSC is not commonly associated with the growth of the tumors. A deterioration of behavior and ability is not normally a feature of TSC but does occur in individual cases following an onset of intractable seizures in adolescence or early adult life. Zlotlow and Kleiner[26] reported a patient who graduated from high school at 18 and developed seizures at 19, when a gradual personality deterioration began with demanding, childish, and aggressive behaviors. He entered a mental hospital at 21, and 10 years later he was diagnosed to be a chronic schizophrenic, unable to care for himself, with totally incoherent and irrelevant speech.

Heckert and coworkers[27] provide a useful review of cases of psychosis in TSC reported to 1972; cases of autism, hyperactivity, and aggression are included. In addition, there are 11 cases from various sources with diagnoses of schizophrenia, paranoia, hallucinations, or delusions: eight of the cases had seizures recorded with onset as late as age 30; two were not recorded as epileptic but had episodic outbursts of violence, irritability, or rage; and the final person had changed from absences with amnesia (in the patient's words, "his blood seemed to stand still . . . and he would forget") at age 18 to somnolent and disinterested behavior at age 37. The authors them-

selves reported a 55-year-old-patient who became overtly psychotic at age 20 with auditory and visual hallucinations. Intercurrent episodes of excitement with loudly voiced anger, probably provoked by delusional and hallucinatory experiences, alternated with episodes of seclusiveness, mutism, anorexia, and negativism. His condition did not follow the usual regression pattern of schizophrenia and, at age 54, when severe thought disorder was apparent, his EEG was within normal limits, as was his intelligence. In the cases reviewed, the authors noted the high incidence of complex partial seizures in those patients reported not to have generalized epilepsy, and although they could not establish this in their own patient, they noted his episodic disturbance in taste perception.

A further case of epilepsy and psychosis in TSC was briefly reported by Clarke et al.[28] with delusions "to send the earth back into the sun" and auditory hallucinations of God's voice. In the years leading up to admission, generalized seizures had increased to as many as 10 per day. A similar case of paranoid delusions that family members had been replaced by imposters, along with auditory hallucinations and episodic olfactory hallucinations, was reported by Holschneider and Szuba[29] with EEG evidence of complex partial seizures originating from a right frontal lobe focus. There are now sufficient reports of such behaviors in adults with TSC to merit further controlled studies to understand the bases of the behavior and prevent inappropriate medications.

Cerebral Pathology and Behavior in TSC

Although the length of life can be nearly normal, the quality of life of people with the severer problems of TSC can be very restricted. The cortical tubers cause epilepsy and mental handicaps and are also implicated in the autism and hyperactive behavior that are a prominent feature of the disease. These lesions are present even before birth and usually do not calcify. They can be detected in late infancy with MRI but not with a CT scan.

Frontal and parietal cortical tubers were found in over 80% of 75 cases of TSC studied at the Mayo Clinic,[30] with mesial or temporal lobe lesions in around 70%. Infantile spasms were statistically significantly related to the total number of tubers ($p < .001$) and not to the number in any one area of the brain, with 86% of these children having mental handicap. Children with partial seizures had fewer frontal lobe tubers than patients with infantile spasms, and fewer were learning disabled. Sulcal islands, gyral cores, and migration lines or wedges are thought to consist of neuronal dysplasia, heterotopia, and white matter hypomyelination. A similar study in Europe[31] has been done to evaluate the neuropsychiatric performances of 23 children, as well as the relationship of their performance to epilepsy and to the number and location of cortical tubers detected on MRI. The six children with autism all had intractable seizures, severe mental handicap, and more than four cortical tubers.

Research is just beginning into the relationship between behavior and lesions in TSC. Work was reported by Weber and colleagues[32] on a longitudinal study of 30 children using neuropsychological tests, positron emission tomography, and functional MRI scans. Preliminary results found that, although many patients did not have cerebellar lesions, the number of these lesions was positively related to the rating on the Childhood Autism Rating Scale, as was the number of subependymal lesions rather than the number of cortical tubers. In comparing the group with cerebellar lesions to the group without, there was no significant difference in IQ but those with cerebellar lesions were significantly more autistic than the group without them. One hypothesis on the cause of autistic behavior[33] is that the cerebellum controls the integration of cognitive resources as well as motor coordination. Its impairment could disrupt normal development of attentional processes early in life, and these attention deficits could later prevent the normal development of certain cortical areas of the brain.

However, there are different theories of autism. Bolton and Griffiths[34] produced evidence for temporal lobe involvement in autism in TSC. The brain scans for 18 children with TSC were compared blind to their clinical diagnoses according to ICD-10 criteria for the three brain regions (frontal, temporal, and cerebellar) previously implicated in autism. Eight of the nine children with autism or atypical autism had tubers in the temporal lobes, compared with none of the children with other psychiatric disorders (p = .0009), and there were no significant associations with frontal or cerebellar lesions.

At the TSC International Research Symposium in 1996, Harrison reported an unexpected and specific deficit he had found in 36% of 22 adults with TSC of normal intelligence. This was an inability to shift an attention set in rule acquisition in a visual task, a task that the group performed with less ability than a control group with Alzheimer's disease. Perhaps this was the basis of the noncompliant behavior when learning a new task reported in 40% of those with normal intelligence in Hunt's survey.[10]

These and similar studies that combine neuropsychological data with MRI and other scans should in future lead to a deeper understanding not only of behavior in TSC but of how the human brain functions. In a review of epilepsy, language, and behavior, Tuchman[35] suggested that tuberous sclerosis might be a good model for the understanding of relationships between such disorders.

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Barbara F. Westmoreland, M.D. 6

The Electroencephalogram in Tuberous Sclerosis

Previous publications on the electroencephalographic (EEG) findings in tuberous sclerosis complex (TSC)[1-5] note a high incidence of EEG abnormalities in patients with TSC. My experience confirms this opinion. The EEGs of 361 patients with TSC were analyzed to determine the relationship between the EEG findings and seizures, age at onset of seizures, mental retardation, and other clinical concomitants of TSC. The patients included 201 males and 160 females with ages ranging from 2 days to 63 years at the time of the EEG recording.

EEG Findings

Two hundred eighty-two patients (78%) had epileptiform abnormalities on their EEGs, 37 (10%) had slow-wave abnormalities, and 42 (12%) had normal EEGs. Of the 282 patients with epileptiform abnormalities, 98 (35%) had focal spike or sharp-wave discharges, 94 (33%) had multifocal epileptiform abnormalities, 63 (22%) had a hypsarrhythmic pattern, and 27 (10%) had generalized spike-and-wave discharges.

Focal Epileptiform Abnormalities

Ninety-eight patients (55 males and 43 females) between 2 months and 58 years of age had focal epileptiform abnormalities (Table 6.1). Fifty-six patients had onset of seizures before 2 years of age. Nine of these patients had infantile spasms previously but were having predominantly focal seizures at the time of the EEG recording. Fourteen patients had onset of seizures between 2 and 4 years of age, 25 had onset of seizures after 5 years of age, and 3 did not have seizures.

TABLE 6.1. Predominant Seizure Type and EEG Findings

EEG Finding	Seizure Type[a]				
	IS	TC	Partial	AA	Total
Hypsarrhythmia	63				63
Multifocal	55	12	24	3	94
Generalized spike-wave		22	3	1	26
Focal		21	74	0	95
Slow-wave abnormalities	1	16	8	5	30
Normal		15	7	3	25
Total	119	86	116	12	333

[a]IS = infantile spasms; TC = tonic-clonic; AA = atypical absences.

Sixty patients had focal spike or sharp-wave discharges over the temporal regions. These were unilateral in 47 patients and bilateral in 13 patients. Forty-two of these patients had complex partial seizures, 8 had focal motor seizures, and 10 had generalized tonic-clonic seizures.

The other 38 patients had focal spike or sharp-wave discharges over the frontal region (16 patients), the centroparietal region (15 patients) (Fig. 6.1), the occipital region (5 patients), and the central vertex region (2 patients). Twenty-two of these patients had focal motor seizures, 11 had generalized tonic-clonic seizures, 2 had complex partial seizures, and 3 did not have seizures.

Sequential recordings were performed in 27 patients: 18 patients continued to have focal epileptiform abnormalities, 2 developed a generalized spike-and-wave discharge, and 7 no longer exhibited epileptiform abnor-

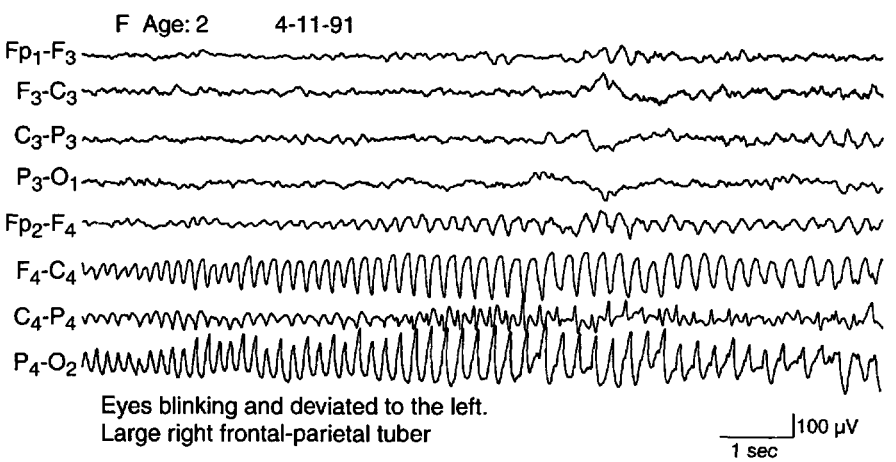

FIGURE 6.1. Focal seizure discharge arising from the right centroparietal regions in a 2-year-old girl who has a large right frontoparietal tuber.

malities. Fourteen of the patients continued to have seizures; 13 of the patients, including 4 whose seizures stopped after surgery and 3 in whom the epileptiform abnormalities had disappeared, became seizure-free.

Multifocal Epileptiform Abnormalities

Ninety-four patients (52 males and 42 females) with ages ranging from 5 weeks to 37 years had multifocal epileptiform discharges. Eighty-two patients had onset of seizures prior to 2 years of age, 9 had onset of seizures between 2 and 4 years of age, and 3 had onset of seizures after 5 years of age. Fifty-five of these patients had infantile spasms. Twenty-four had partial seizures, 12 had generalized tonic-clonic seizures, and 3 had atypical absence seizures (Table 6.1).

Forty-five patients had sequential recordings. Thirty patients continued to show multifocal epileptiform abnormalities on their EEGs: 27 of these patients continued to have seizures and the other 3 stopped having seizures. Nine patients had focal spike discharges on follow-up tracings, and seven of these patients continued to have seizures. Two patients had generalized discharges and both continued to have seizures. Three patients in whom the epileptiform abnormalities were replaced by mild slowing stopped having seizures. One patient whose EEG became normal continued to have seizures.

Hypsarrhythmia

Sixty-three patients (37 males and 26 females) from 2 months to 8 years of age had hypsarrhythmia (Fig. 6.2). All 63 patients had the onset of infantile spasms (West syndrome) between 1 and 18 months of age (Table 6.1). Although hypsarrhythmia is usually seen in patients less than 4 years of age, four patients showed a modified hypsarrhythmic pattern on their EEGs between 4 and 8 years of age.

Thirty-five patients were seen in follow-up: 12 patients had generalized tonic-clonic seizures, 15 had partial seizures, 6 had atypical absence seizures, and 2 had stopped having seizures. Follow-up EEG tracings obtained in these 35 patients showed a persistent hypsarrhythmic pattern in 5 patients, multifocal epileptiform abnormalities in 14, focal spike or sharp-wave discharges in 6, generalized slow spike-and-wave discharges in 5, slow-wave abnormalities without epileptiform features in 4, and a normal EEG in the one who stopped having seizures.

Generalized Spike-and-Wave Discharges

Twenty-seven patients (15 males and 12 females) between 11 months and 49 years of age had generalized spike and sharp-wave discharges. Eighteen of the patients had onset of seizures prior to 2 years of age; five had onset

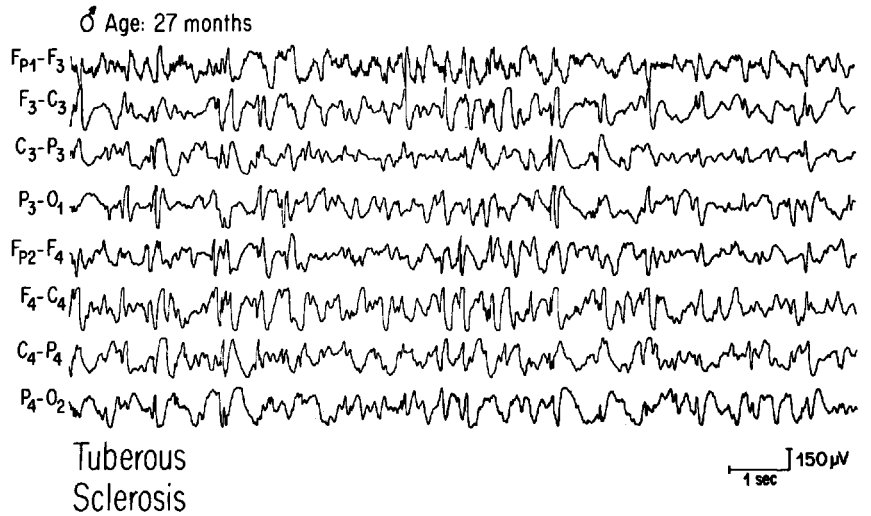

FIGURE 6.2. EEG showing hypsarrhythmic pattern in a 27-month-old boy with tuberous sclerosis who was having frequent infantile spasms.

of seizures between 2 and 4 years of age, and four had onset of seizures after 5 years of age. Twenty-two patients had generalized tonic-clonic seizures, one had atypical absence seizures, three had partial seizures (Table 6.1), and one did not have seizures. Nineteen patients had generalized atypical spike-and-wave discharges, and eight patients with the Lennox-Gastaut syndrome had slow (2.0 to 2.5 Hz) spike-and-wave discharges (Fig. 6.3).

There were no patients with TSC who had typical absence seizures (petit mal) clinically or whose EEG contained the classic 3-Hz spike-and-wave pattern.

Six patients had sequential recordings. Three patients continued to show generalized spike-and-wave discharges and seizures. In three patients the epileptiform abnormalities were replaced by slow-wave abnormalities, and two of these patients continued to have seizures.

Electrographic Seizure Discharges

One hundred eight patients had electrographic seizure discharges during one or more recordings. This consisted of electrodecremental seizure discharges in 25, generalized spike-and-wave discharges in 23, and focal seizure discharges in 60 patients. The electrodecremental seizure discharges were associated with infantile spasms in 23 patients and generalized tonic seizures in 2. Generalized spike-and-wave seizure discharges were associated with generalized seizures in 21 patients and no clinical accompaniment in 2.

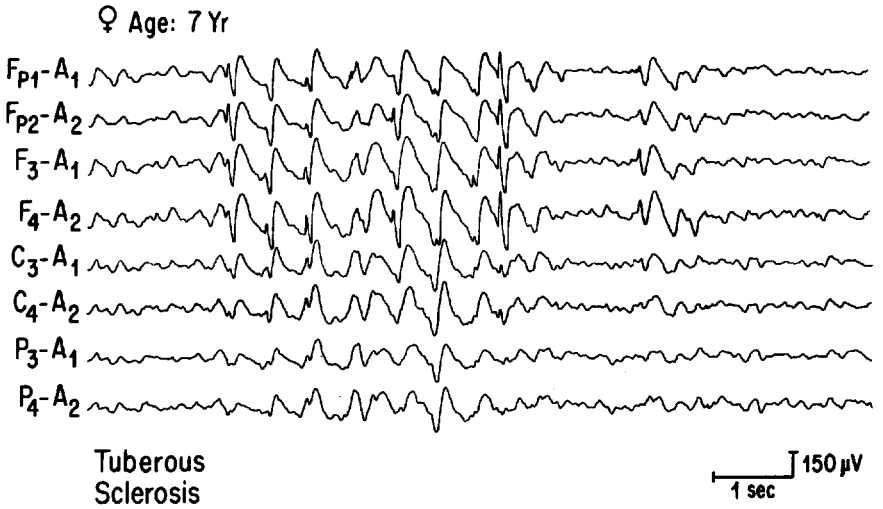

FIGURE 6.3. Generalized sharp- and slow-wave complexes (slow spike-and-wave pattern) in a 7-year-old girl with generalized tonic-clonic seizures.

Sixty patients had focal seizure discharges. Thirty-three patients had focal seizure discharges arising from the frontocentroparietal regions (Fig. 6.1). These were associated with partial motor seizures in 21, a complex partial seizure in 1, a generalized seizure in 1, and no clinical accompaniment in 10. Sixteen patients had temporal seizure discharges that were associated with complex partial seizures in 12. Subclinical temporal electrographic seizure discharges occurred in the other four patients during sleep. Nine patients had occipital seizure discharges that were associated with complex partial seizures in four and no clinical accompaniment in five. Two patients had central vertex discharges that were associated with partial motor seizures and posturing (i.e., supplementary motor seizures).

A finding seen in several patients was the presence of focal spikes occurring in a periodic or quasiperiodic fashion throughout the recording and unassociated with any clinical symptoms (Fig. 6.4).

Slow-Wave Abnormalities

Thirty-seven patients (19 males and 18 females) between 9 months and 63 years of age had slow-wave abnormalities on their EEGs. Thirty patients had seizures, and the other seven patients had other complications of TSC. Twelve patients had onset of seizures before 2 years of age, 11 between 2 and 4 years, and 7 between 5 and 20 years. Sixteen of the patients had generalized tonic-clonic seizures, eight had partial seizures, five had atypical absence seizures, and one had infantile spasms (Table 6.1). Three of those

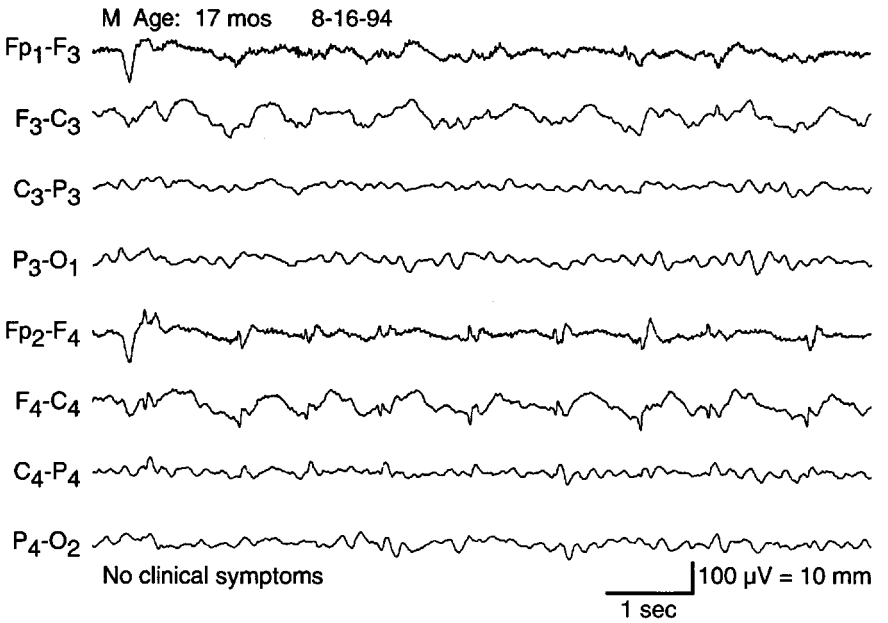

FIGURE 6.4. Focal periodic spike discharges over the right frontal region unassociated with any clinical symptoms in a 17-month-old boy.

patients had follow-up EEGs that became normal or showed a decrease in the slow-wave abnormalities.

Of the 7 patients who did not have seizures, three had obstruction of the cerebrospinal fluid circulation secondary to an intraventricular tumor, and their EEGs showed intermittent, generalized, and semirhythmic 1- to 2-Hz slow-wave abnormalities. Two patients had right frontal astrocytomas with focal slowing over the frontal region and continued to show slowing after surgery, although their seizures stopped. One patient who had had an astrocytic hamartoma of the left frontal lobe removed several years previously later developed a left sphenoidal ridge meningioma, and her EEG showed focal delta slowing over the involved area. One patient experienced a syncopal episode during the EEG and showed generalized 1- to 2-Hz delta slowing during the syncopal episode.

Normal EEGs

Forty-two patients (22 males and 20 females), between 2 days and 56 years of age, had normal EEGs. Twenty-five of these patients had seizures: 12 had onset before 2 years, 1 between 2 and 4 years, and 12 between 5 and 28 years of age. Fifteen patients had generalized tonic-clonic seizures, seven had partial seizures, and three had atypical absence seizures (Table 6.1). Eighteen of the patients stopped having seizures prior to the EEG recording.

Three patients who remained seizure-free had follow-up tracings that remained within normal limits. The other 17 patients with normal EEGs were asymptomatic.

Sleep Recordings

Two hundred fifty-four patients had sleep recordings. Two hundred eighteen patients had epileptiform activity during sleep, and 36 patients had normal sleep recordings. In the patients with epileptiform activity during sleep, the interictal activity was increased in 84, activation occurred only during sleep in 34, activation of other types of epileptiform abnormalities occurred in 32, and there was no change in 52. Ten patients had electrographic seizure discharges during sleep, and six patients had seizure discharges upon arousal. Sixteen patients had an asymmetry of the spindle activity. Patients who had hypsarrhythmia and frequent multifocal spike discharges showed little in the way of sleep activity. Several of these patients showed the emergence of more normal sleep activity in later recordings when there was a decrease in the epileptiform activity.

Sequential EEG Recordings

One hundred twenty-three patients had multiple recordings. One hundred fourteen of these patients had epileptiform abnormalities on their initial EEGs. Eighty patients continued to show similar but less prominent epileptiform abnormalities on subsequent recordings. Fifteen patients who initially had hypsarrhythmia or multifocal epileptiform abnormalities showed more focal epileptiform abnormalities on later tracings. Nineteen patients showed a disappearance of epileptiform abnormalities on later recordings, although nine continued to have seizures.

Six patients had slow-wave abnormalities on their initial EEGs. Three patients showed a decrease in the slow-wave abnormalities on subsequent tracings but continued to have seizures. One patient showed an increase in focal slowing in association with the recurrence of a tumor. Two patients had surgery for focal mass lesions and continued to show focal slowing over the involved area; however, they stopped having seizures.

Three patients who had seizures and normal EEGs remained seizure-free and continued to show normal EEGs.

In general, the most severe abnormalities, particularly with regard to epileptiform abnormalities, were seen in young children with early onset of seizures. The abnormalities tended to decrease in amount and degree with increasing age.

EEG Findings in Relation to Mental Retardation

There was a good correlation between the degree of EEG abnormality and the degree of mental retardation, with the most severe EEG abnormalities

occurring in patients with moderate or severe mental retardation. In general, patients with moderate or severe mental retardation displayed the most severe and prominent epileptiform abnormalities. Patients with infantile spasms and hypsarrhythmia showed a greater degree of mental retardation than patients with other types of seizures or EEG abnormalities. Patients with generalized and multifocal seizures and generalized or multifocal epileptiform discharges were usually more severely retarded than patients with focal seizures and focal discharges. Almost all of these patients had onset of seizures before 2 years of age. Most of the patients with focal or generalized spike-and-wave discharges who had onset of seizures after 2 years of age were only mildly retarded or had normal intelligence.

Almost all of the patients with normal EEGs and most of the patients with only mild EEG abnormalities had normal intelligence.

The EEG and Neuroimaging Studies

Patients with large tubers or giant cell astrocytomas showed focal or lateralized abnormalities on their EEGs corresponding to the side or region of the mass lesion (Fig. 6.1). Patients with obstruction of the foramen of Monro or ventricular systems showed generalized slow-wave abnormalities. Most of the patients with multifocal cortical tubers had multifocal epileptiform abnormalities on their EEGs; however, some patients had more focal epileptiform abnormalities. Patients with subependymal or periventricular tubers showed multifocal, focal, or generalized epileptiform abnormalities. Several of those patients, however, had normal EEGs, and most of these patients were asymptomatic and did not have seizures.

Surgery

Twenty-eight patients had intracranial surgery for removal of mass lesions or control of seizures. Twenty patients had surgery for resection of tubers, hamartomas, or giant cell astrocytomas involving the frontal region,[14] the parietal region,[4] and the occipital region.[2] Two patients had temporal lobectomies for seizure control; one was found to have gliosis and one cortical dysplasia.

Nine patients became seizure-free following surgery. Four patients showed a significant reduction in the number of seizures following surgery. Eight patients continued to have seizures with persistent epileptiform abnormalities on their EEG after surgery. One patient died.

Six patients had surgery for giant cell astrocytomas obstructing the foramen of Monro or third ventricle. These patients showed generalized and focal slow-wave and/or epileptiform abnormalities on their preoperative recordings. Three patients stopped having seizures after the surgery even though one patient continued to show frontal spike discharges. One patient

continued to have seizures and showed generalized spike-and-wave discharges. Two patients died subsequent to the surgery.

The EEG in Family Members with TSC

There were 12 families with TSC in which more than one family member had an EEG. Five families had a parent and a child with TSC. All of the children had seizures with focal or multifocal abnormalities on the EEG. Three of the parents had seizures with focal discharges on their EEGs. Two families had a brother and sister with TSC who had focal seizures and focal spike discharges on their EEGs.

Five sets of twins had TSC. One pair of identical twins had infantile spasms and hypsarrhythmia on their EEGs. Another set of twins also initially had infantile spasms.[6] One of the twins continued to show focal spike discharges on his EEG but became seizure-free. The second twin had multifocal discharges on his EEG and, although he became seizure-free, was mentally retarded. In the third set of twins, one twin had focal spike discharges but no seizures. The other twin showed multifocal and generalized epileptiform abnormalities, had multiple types of seizures, and was mentally retarded. In the fourth set of twins, one twin had a normal EEG and no seizures and was of normal intelligence. The other twin had complex partial seizures, focal right frontotemporal spikes, and a giant cell astrocytoma obstructing the third ventricle. A sister of this set of twins also had TSC. She had focal motor seizures and an EEG showing generalized slowing. In the fifth set of twins, one twin had multifocal spikes and complex partial seizures. The other twin had right frontal spikes, complex partial seizures with secondary generalization, and surgery for removal of a right giant cell astrocytoma. The twins also had a sister with TSC who had right temporal sharp waves, complex partial seizures, and craniotomy for removal of a right temporal lobe tuber. Their mother also had TSC.

Discussion

Review of the EEG data reveals a high incidence of EEG abnormalities in patients with TSC. Three hundred nineteen (88%) of 361 patients having EEGs had abnormalities on their EEGs. Epileptiform abnormalities were found in 282 (78%) and slow-wave abnormalities were seen in 37 (10%).

The most common type of epileptiform abnormality was focal spike or sharp-wave discharges, which were present in 98 patients. Seventy-four of these patients had partial seizures, 21 had generalized tonic-clonic seizures, and 3 did not have seizures. Focal discharges were present over the temporal region in 60 patients, the frontal region in 16, the centroparietal region in 15, the occipital region in 5, and the central vertex region in 2 patients.

Multifocal epileptiform abnormalities were the next most common type of epileptiform abnormality. This was present in 94 patients. Fifty-five of these patients initially had infantile spasms, 24 had focal motor seizures, and 15 had generalized seizures.

A hypsarrhythmic pattern was seen in 63 patients with infantile spasms (West syndrome).

Twenty-seven patients had generalized sharp and slow-wave discharges. Nineteen had atypical spike-and-wave and eight had slow spike-and-wave discharges in association with the Lennox-Gastaut syndrome. Twenty-three of these patients had generalized seizures, three had partial seizures, and one did not have seizures.

Thirty-seven (10%) had slow-wave abnormalities on their EEGs; 22 of these patients had generalized seizures, 8 had partial seizures, and 7 had no seizures. The patients who did not have seizures showed slow-wave abnormalities reflecting other complications of the disease, including focal mass lesions or ventricular obstruction.

Forty-two patients (12%) had normal EEGs. Eighteen of these patients had generalized seizures and seven had partial seizures. The other 17 patients were asymptomatic. Most of the patients who previously had seizures had stopped having them by the time the EEG was performed.

The EEG findings in this group of patients are similar to those groups described by others, in which a majority of the patients with TSC had abnormal EEGs with varying types of epileptiform abnormalities. No one specific pattern was noted, with hypsarrhythmia and multifocal or focal epileptiform discharges being the most frequent types of epileptiform discharges described.[1-5,7-11]

A total of 333 patients (92%) had seizures (Table 6.1). The most common type of seizure was infantile spasms, occurring in 119 patients (36%). Pampiglione[12] was the first to emphasize the high incidence of infantile spasms and hypsarrhythmia in patients with TSC. Pampiglione and Moynahan[13] reported that, of 100 patients with TSC, 69% had infantile spasms. The high incidence of infantile spasms in TSC has been confirmed by others.[7,8,11,14] Conversely, TSC is the most frequently recognized pathologic entity in patients with symptomatic infantile spasms, and the incidence of TSC in patients with infantile spasms has been reported to be between 20% and 25%.[15] Of the other types of seizures, partial seizures were seen in 116 patients (35%), generalized tonic-clonic seizures in 86 patients (26%), and atypical absence seizures in 12 patients (3%) (Table 6.1).

The most severe EEG abnormalities were seen in the infant or young child with an onset of seizures before 2 years of age. This group included all of the patients with infantile spasms and approximately 55% to 60% of the patients with focal and generalized seizures. The most severe EEG abnormalities were seen in the patients with infantile spasms; less severe abnormalities were seen in patients with generalized and focal seizures. As expected, patients with frequent seizures had more frequent discharges and a greater degree of abnormality on their EEGs than those patients with in-

frequent seizures. Most of the patients who stopped having seizures had either mildly abnormal or normal EEGs. In general, the epileptiform abnormalities decreased in severity in the older child or adult. In patients who had sequential recordings, the EEG improved with increasing age. Of 114 patients who had epileptiform abnormalities on their initial EEGs, 80 patients continued to show similar but less prominent epileptiform abnormalities on follow-up tracings. Fifteen patients with multifocal abnormalities showed more focal epileptiform discharges, and 19 patients no longer exhibited epileptiform activity on subsequent recordings.

As neuroimaging studies have advanced over the past years, there has been a better correlation with the EEG findings.[16,17] Most of the patients with focal mass lesions (giant cell astrocytomas or large tubers) on magnetic resonance imaging or computed tomography scans showed corresponding focal or lateralized abnormalities on their EEGs. Patients with multifocal cortical tubers were more likely to show multifocal abnormalities on their EEGs. Patients with periventricular or subependymal lesions showed various types of epileptiform abnormalities or normal EEGs.

Of the patients who had surgery, 12 had cessation of seizures following surgery, 4 had a significant decrease in seizure frequency, and 9 continued to have seizures. In carefully selected patients with single or multiple lesions, removing the primary epileptic focus associated with a cerebral lesion can help decrease the tendency toward seizures or result in a complete resolution of the seizures.[18]

There was also a good correlation between the degree of the EEG abnormalities and the degree of mental retardation, with the most severe EEG abnormalities occurring in patients with moderate to severe mental retardation. Conversely, almost all of the patients with normal or mildly abnormal EEGs had normal intelligence.

Twenty-eight of the patients with TSC did not have seizures. Seventeen patients did not have any neurologic symptoms of the disease and had normal EEGs. Four patients had focal or generalized spike discharges but did not have seizures. The other seven patients showed slow-wave abnormalities reflecting some other underlying pathology of the disease, such as focal mass lesions and ventricular obstruction.

Summary

Patients with TSC have a diversity of EEG abnormalities, reflecting a focal or multifocal cerebral disorder. Although epileptiform abnormalities are the most common type of abnormality, no one specific type of EEG pattern was seen in patients with TSC. Instead, the EEG abnormalities relate more to the age at onset of the seizures, the type of seizure, the degree of mental retardation, the age at which the EEG was recorded, and the course of the disease process. In general, the EEG is a good indicator of the severity of

cerebral dysfunction and is a useful means of detecting and documenting the various neurologic problems associated with TSC.

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7

Magnetoencephalography

One of the most significant advances in the last few years in the clinical study of localization-related symptomatic epilepsies has been the search for topographic correlation between the seizure types and the epileptogenic area. Prompt detection and precise localization of lesions may lead to surgical treatment and improved seizure control.

Cortical tubers detected by magnetic resonance imaging (MRI) represent the epileptogenic foci of tuberous sclerosis complex (TSC). Video-electroencephalography (EEG) recording techniques have led to significant progress in the classification of seizures associated with TSC, demonstrating that they have focal or multifocal origin in the vast majority of cases.[1] However, correlation between cortical tubers detected by MRI and epileptogenic areas is far from definitive. One problem is that the subtle focal onset of secondarily generalized seizures may not be recognized and seizures may be incorrectly diagnosed as generalized at their onset. The risk of making an incorrect diagnosis is high with seizure types that are by definition generalized, such as infantile spasms, atypical absences, and atonic seizures.[2] In such cases, accurate topographic localization by visually inspecting the EEG tracings may be difficult in the presence of seemingly bilaterally synchronous abnormalities. Spike mapping of EEG tracings may allow prompt recognition of the focal onset of apparently generalized bursts, revealing small interchannel time differences.[3,4] Identification of the source of interictal spikes has proved to be more accurate both in time and in space than visual inspection of EEG tracings. Patients with epileptic foci that are not easily defined by surface EEG data generally require invasive EEG monitoring with intracranial electrodes.

An improvement in functional localization can be obtained by combining EEG and magnetoencephalography (MEG) signals. Whereas scalp EEG detects both tangential and radial sources (i.e., activity in the sulci and in the gyri), MEG selectively measures tangential sources (i.e., activity in the

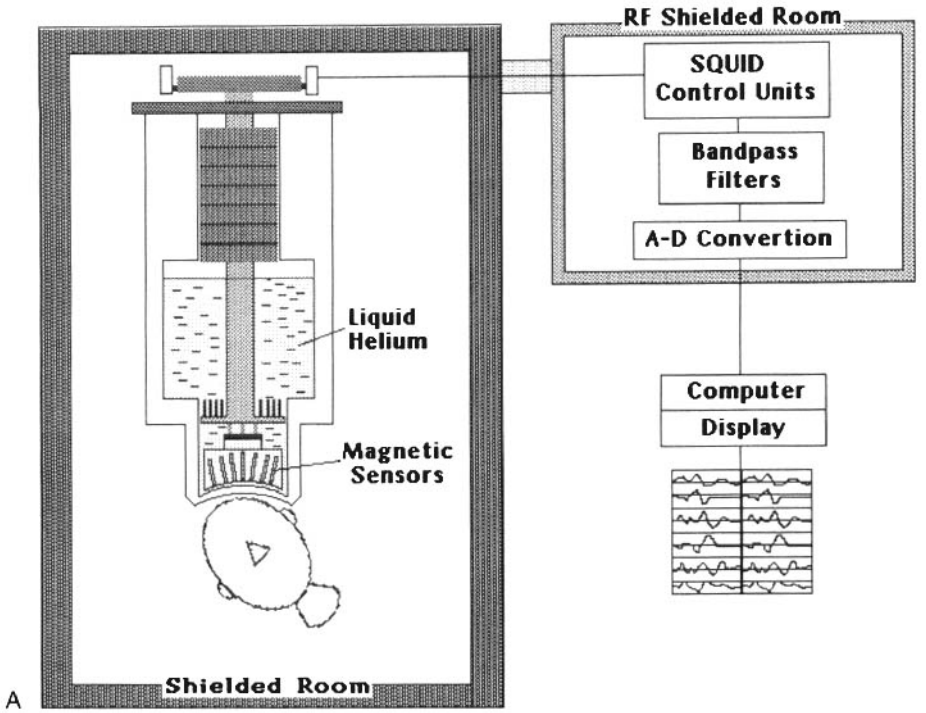
sulci). MEG measures magnetic fields that are primarily associated with intracranial currents. The intracranial currents that pass into the skull and scalp contribute only partially (about 5%) to extracranial measured magnetic fields; by contrast, the EEG signal is more affected by the volume currents and the interposed tissues. The small value of 5% intracranial currents in the MEG means that it may be possible not to include the skull and scalp within the source of localization. For this reason, spatial resolution of MEG is about one third better than EEG, because the magnetic field is not distorted by resistive properties of the tissues.

Instrumentation

The recent availability of large-array neuromagnetometers has given significant impulse to the study of epilepsy by neuromagnetic method. The capacity of this technique for identifying sources of cerebral activity was initially tested when investigating the functional organization of the living brain. It was soon clear to the people involved in this field that the imaging possibilities of this new method might become extremely advantageous in clinical practice for several reasons: (1) MEG is safe and absolutely noninvasive; (2) MEG could provide information directly related to the source of pathophysiologic activity, with minimum interference from different tissues intervening between the source and the sensor; and (3) MEG might prove to be a powerful tool to achieve functional imaging with a time resolution of less than 1 millisecond and a spatial resolution of the order of 1 mm.

All available large-scale neuromagnetometers require a magnetically shielded room for proper operation. The ambient magnetic field is attenuated inside a typical room by a factor of 100 to 1000 at direct current (dc) and more than 10,000 at the line frequency, whereas the available space is sufficient for clinical studies. The rejection of disturbing fields is improved by using superconducting detection coils wound in a gradiometric configuration that makes the coils insensitive to uniform fields (first-order gradiometer) and, if needed, also to the first spatial gradient of the field (second-order gradiometer). The partial reduction of sensitivity that accompanies the adoption of a gradiometric detection coil is compensated by the intrinsic sensitivity of the detector used to measure the magnetic field, namely the Superconducting Quantum Interference Device (SQUID), to which the detection coil is inductively coupled. The overall performances of the latest generation of systems based on dc-biased SQUIDs are indeed extremely satisfactory (Fig. 7.1).

In order to overcome the problems associated with the use of small systems, a few research groups have started the development of "total-head" systems, able to detect magnetic signals from all over the scalp. In this helmetlike system, the sensors are positioned inside a cryogenic dewar in order to cover all the scalp, including the portion below the inion, on the



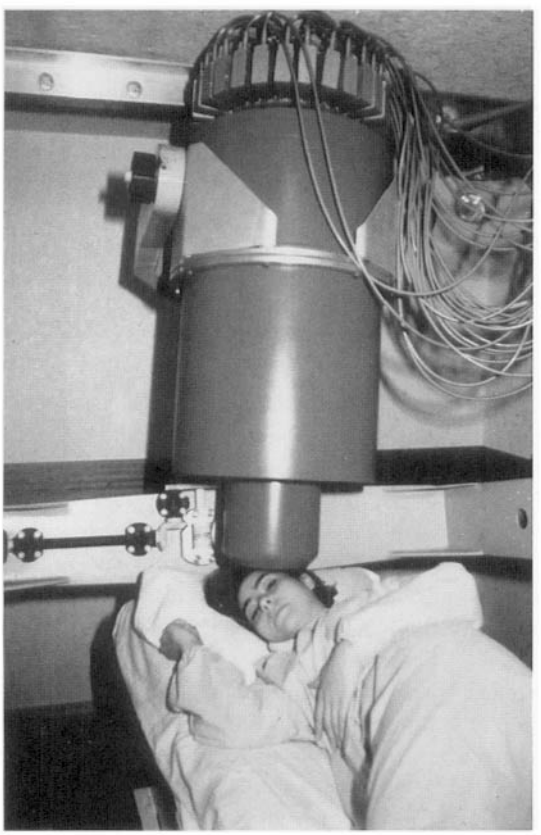


FIGURE 7.1. Schematic drawing (*A*) and photograph (*B*) of the 28-channel neuromagnetometer system operating at the Istituto di Elettronica dello Stato Solido–CNR in Roma.

back of the head, and part of the cheeks. The reason for such a wide coverage on the side of the head is because magnetic activity from epileptic foci in the temporal lobe often expands well below the ear. It should be remarked that the magnetic field associated with a dipolar source in the cortex has the shape of concentric circles: consequently, it leaves and enters the scalp at sites symmetrically apart from the projection of the source on the scalp itself. All the neuromagnetic systems provide integration with MRI data.

The helmetlike configuration seems ideal for source localization studies, even when the probable location of the pathologic source, or when sources from both hemispheres have to be investigated.

Procedures for Localization of Epileptic Activity

The difficulty of localizing the source of epileptic spikes produced by a relatively concentrated neural volume, as in the case of focal epilepsy, favors a magnetic approach. This problem can be resolved by simply measuring the spatial distribution of the epileptic signal over the scalp and then fitting this pattern with the one generated by a hypothetical model source over the outer surface of a spherical medium with homogeneous conductivity, accounting for the head of the patient.

We have used a template analysis for an automatic selection of epileptic complexes. The analysis has been carried out on EEG recordings during interictal periods and has permitted the construction of isofield maps illustrating the distribution of the magnetic field over the scalp in correspondence to the selected complex. The procedure followed for the template analysis was executed in two steps. First, a search for a pathologic complex was performed by the operator on the raw EEG signals from a specific derivation. A sequence of "similar" complexes was selected by eye and then averaged to get the template waveform. Clearly, this step—which uniquely depends on the operator's skill—is crucial because the whole subsequent procedure relies on this choice. Second, the correlation between the template and all the EEG recordings (from the same derivation) is evaluated and, if its value is larger than a preset threshold (typically 0.85 to 0.9), the corresponding MEG epoch is averaged. In this way a set of averaged MEG signals is obtained that correspond to all, or part of, the measuring sites to be used for investigating the field distribution over the scalp relative to the selected pathologic complex. The same procedure may be repeated for other significant pathologic events.

The field distribution obtained by this technique can be used for three-dimensional source localization. Combining MEG data on brain function with MRI data on brain structure is even possible to localize an equivalent current dipole (ECD) in a zone corresponding to involved cortical areas. A head-based three-dimensional coordinated system allows computer merging

of MEG signal ECD localizations with MRI multiplanar images, a process termed *magnetic source imaging* (MSI).

Neuromagnetic Study of Focal Epilepsy

Previous observations have shown that MEG localization estimates are consistent with the localization of the seizure focus as shown by simultaneous surface EEG recordings and by separate examination via depth electrodes, computed tomography scan, and MRI.[5,6] The Italian experience in cases of focal epilepsy assessed by means of MEG[7] has shown that the morphology of the spike pattern is often variable in the same patients and during the same recording session. One of the more important differences between EEG and MEG recordings is observable in the case of concurrently active multiple sources. MEG's selectivity to tangential currents and the more localized field pattern significantly facilitates the data interpretation. Differences between MEG and EEG signals are often in favor of magnetic measurements when multiple discrete sources of activity are to be resolved.[8] Furthermore, MEG findings show that, in some patients affected by focal epilepsy, more than a single generator of pathologic complexes is present. In particular, as compared to EEG, MEG showed a large number of complexes from the focal area, giving a clearer dynamic view of the epileptic activity. Specifically, the frequently observed rapid sequence of dipolar maps, slightly varying in position and orientation, suggests that different neuronal groups act in the same focal district, and provides hints on the real extension of the epileptogenic zone. Figure 7.2 represents a sequential field mapping obtained from a selected epileptic spike in a patient with focal epilepsy showing a dynamic changing over time (every 6 milliseconds) of the epileptic activity.

The MEG recordings of patients with TSC affected by partial epilepsy showed isolated spikes, sometimes preceded by attenuation of the background activity. In these patients the MEG spikes were more polyphasic than the EEG signal, and sometimes the epileptic complex was not present on the EEG traces.[9] For instance, in one subject we have observed at least three different epileptic patterns on MEG traces. The representation of the corresponding magnetic field on the scalp showed a "quadrupolar" map. This pattern can be the result of two distinct generators of opposite sense with simultaneous activation. The ECD localization of this pattern showed an activation of a wide left temporo-occipital region, in topographic concordance with a cortical tuber detected by MRI (Fig. 7.3). This relatively large activated area suggests that this zone can correspond to the "epileptogenic target area." There are several reasons for these findings. Interictal activity in partial epilepsy is a complex phenomenon in which relatively large areas of neocortex and archicortex can be simultaneously or consecutively activated through three mechanisms: (1) directly by fast-association fibers, (2) by fast-association fibers that trigger local phenomena that in turn give way to sharp waves or spikes, and (3) by fast propagation along the

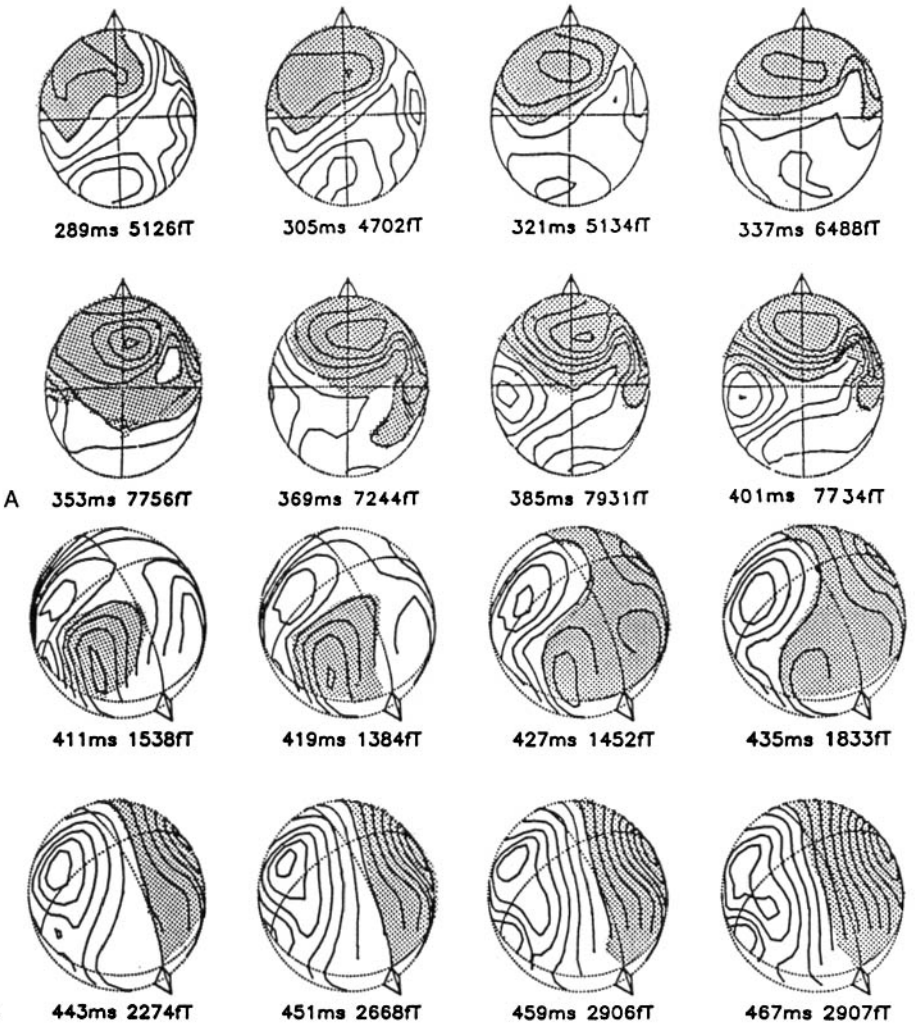


FIGURE 7.2. Sequence of field mapping in patients with right parietotemporal epilepsy (*A*) and right temporal epilepsy with secondary generalization (*B*).

cortex through corticocortical fibers. Also, there must be a generic mechanism by which widespread areas of the cortex generate certain patterns in response to epileptiform events (Fig. 7.4). A simultaneous activation of large areas of the neocortex in a generic form can simulate electrical activity distributed over a wide solid angle similar to that generated by a deep single electrical source. However, in this context distinction between electromagnetic and neuronal propagation of the signal can be difficult because the existence of multiple solutions of the inverse problem complicates modeling in the presence of multiple sources. For instance, it can be shown that, the deeper the source, the wider is the surface area where it is detected.

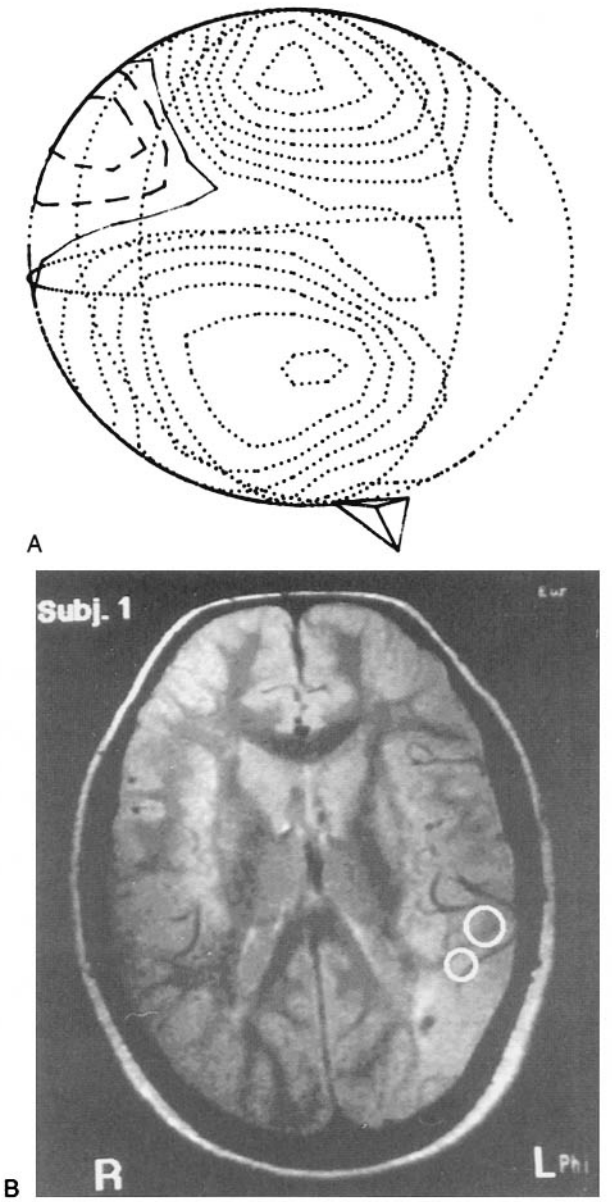


FIGURE 7.3. Isofield contour map in a 13-year-old girl (*A*). MSI localized the seizure focus to the left posterior temporal lobe (*B*).

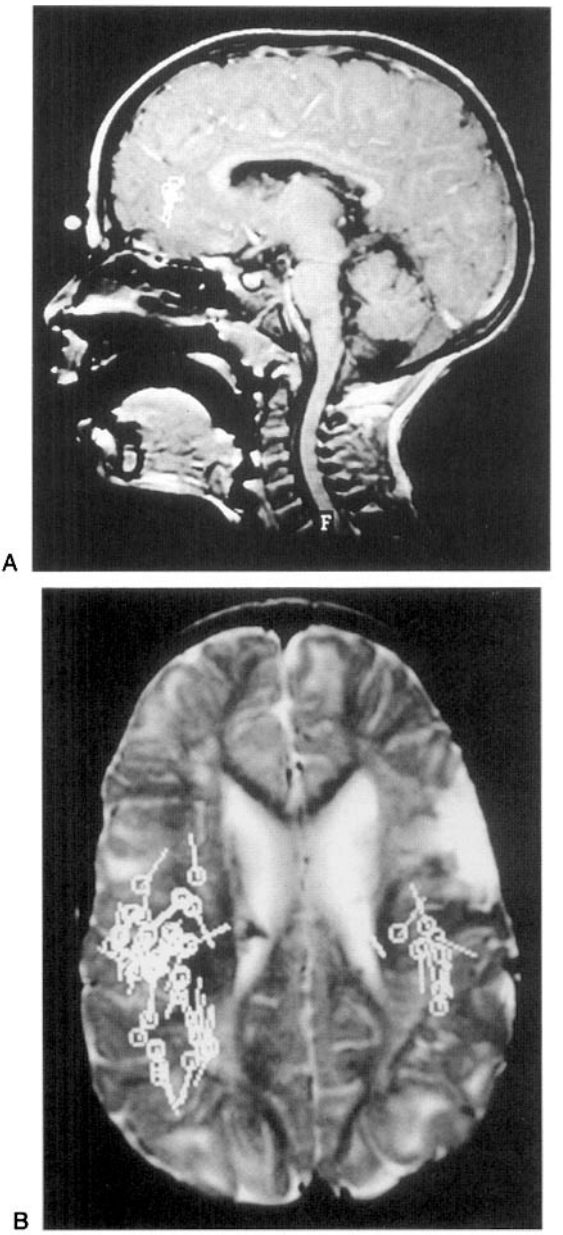


FIGURE 7.4. Three-dimensional MSI localized a focus in the left and right temporal and parietal areas. This 3-year-old girl with intractable partial seizures had a previous resective surgery of the left anterior temporal lobe. (Measurement obtained at MEG Laboratory, Scripps Institute, La Jolla, CA.) (*A*) Sagittal section; (*B*) Axial section; (*C*) Coronal section. *Illustration continued on opposite page*

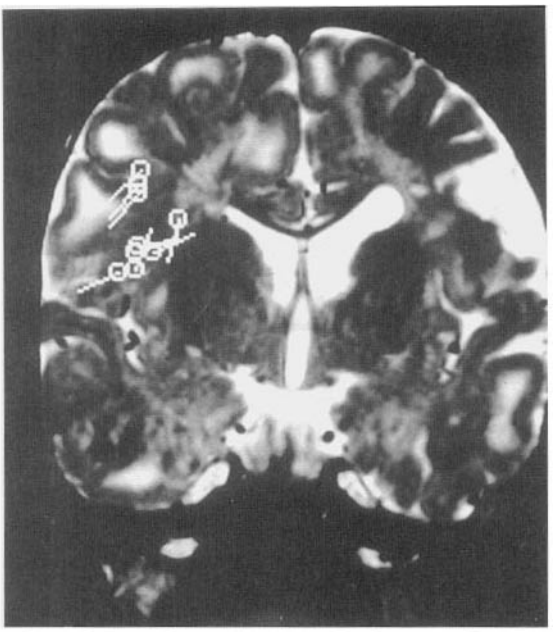


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FIGURE 7.4. (*continued*)

Therefore, it could be difficult to distinguish scalp electromagnetic fields generated by an extended neocortical source from those generated by a single deep source on the basis of the spatial distribution of external electromagnetic fields.[10]

Limitations and Future Directions

The most important component in treatment of TSC patients affected by epilepsy is the possibility of identifying the epileptogenic "trigger" and the epileptogenic "target" areas responsible for the generation and propagation of epileptic seizures. In TSC, which is often characterized by many brain lesions, it is difficult to identify the epileptogenic area only on the basis of anatomic information and scalp EEG. We suggest that MEG, combined with EEG, may be a suitable technique to fulfil this task. At the present time it may be concluded that findings gathered with EEG and MEG are not mutually exclusive, but rather complementary. These techniques are the only noninvasive methods with a time resolution less than 1 millisecond available for studying the physiology and the pathology of the central nervous system.[11]

MSI, a technique that combines MEG data on brain function with MRI data on brain structure, can provide both functional and structural information with a good time and space resolution and in particular additional spatial

data in TSC patients with convexity foci. In the future, mapping the results of dipole localization sources on a three-dimensional MRI reconstruction could allow more accurate localization of the zone of the cortical focal abnormalities. However, it remains unclear to what extent MSI could be of assistance in avoiding invasive studies in surgery candidates and in helping the surgeon to perform individualized and conservative resections in children with intractable seizures associated with TSC.

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8

Brain Imaging in the Tuberous Sclerosis Complex

Tuberous sclerosis complex (TSC), one of the hamartomatoses, affects multiple organ systems. The skin, kidney, and central nervous system (CNS) are commonly involved in this disorder. Basic CNS pathologic findings in TSC include subependymal nodules (SENs), cortical/subcortical tubers, subependymal giant cell astrocytoma (SEGA), heterotopic dysplastic neurons in white matter, and ventricular enlargement. Intracranial or extracranial vascular disease may also occur but is rare. Most of these pathologic findings are readily imaged with current technology.[1-8] This chapter contains a review of the neuroimaging findings that characterize this congenital disorder.

Advances in radiologic imaging over the last 25 years have dramatically altered the diagnostic approach to innumerable diseases and provided an essentially noninvasive window into the human body. Modern computed tomography (CT) and magnetic resonance imaging (MRI) are the two principal computer-generated imaging modalities used in the evaluations of the CNS. Ultrasound plays a limited role in vascular and neonatal CNS imaging. CT and MRI rely on different physical properties of tissue to display cross-sectional anatomy. The CT image reflects the relative attenuation of an x-ray beam by different tissues, while the magnetic resonance image depends on a complex interaction between paramagnetic hydrogen nuclei, radiofrequency pulses, and sampling times. When the basic physics of one computed imaging modality, such as MRI differs from that of another computed modality, such as CT, it is easy to understand why their sensitivity for detecting pathology is not apt to be the same. CT possesses greater sensitivity for bone disorders and soft-tissue mineralization, while MRI possesses superior soft-tissue contrast and multiplanar capabilities. Each technique will have advantages and disadvantages.

As computer-generated imaging begins to unravel intracranial disorders, it is obvious that many of the more common pathologic processes, such as infarcts, hemorrhage, and tumors, possess a well-defined temporal profile,

similar MRI features, and sometimes a common biologic behavior. Recognition of the temporal profile of cerebral infarcts that evolve rapidly and stabilize within a month is easy. Brain hemorrhage requires a longer period of time but is complete in a few months. The profile for intracranial tumors is more variable, but its duration is intermediate and may span several months to a few years. In contrast, the pace of evolution in some hamartomas is so slow that a decade or a generation may elapse before their profile is evident on imaging studies. Neurofibromatosis and TSC fall into this latter category.

The similarity in MRI appearance of the more common hamartomatoses—neurofibromatosis 1 (NF1) and TSC—has become obvious as the literature has accumulated.[9–13] Also, hamartomas of NF1 must have been quite subtle because they were not mentioned until high-field-strength MRI magnets became generally available. MRI has revealed focal lesions that are characterized by increased T_1 and T_2 signals in infants and children who have either disorder. In NF1, the focal areas of increased T_1 signal have been limited to the globus pallidus while the areas of increased T_2 signal are localized to the cerebellum and brain stem. These lesions appear during the first decade and have been reported to disappear during the second decade of life.[14] Altered myelin has been mentioned to explain these findings, but the pathology of these lesions is uncertain. In contrast, the focal areas of increased T_1 signal that have been found in patients with TSC have occurred during early infancy, are located in the cerebrum, and are probably early manifestations of tubers.[15,16] In our experience, recognition of increased T_2 signal in the tuber is possible by 12 months of age.

As CT and especially MRI have continued to evolve over the last decade, the images have improved and additional MRI scanning sequences have been discovered. CT not only identifies more intracranial calcifications than plain head radiographs but also accurately localizes the lesions to the region of caudate or cerebral mantle. Furthermore, CT also permits the detection of many uncalcified SENs and some tubers in the myelinated brain. MRI using spin-echo imaging sequences has been less fruitful than CT in demonstrating calcification; however, these sequences have excelled in the detection of tubers to such an extent that MRI has become the examination of choice for the evaluation of TSC. Initially, spin-echo MRI had seemed so sensitive that several authors tried to find a definitive relationship between the tuber burden of the brain and clinical symptoms. A few studies have addressed the question of whether the prognosis of TSC patients (in regard to cerebral dysfunction and seizure activity) can be accurately predicted from the quantitative assessment of parenchymal tuber burden.[17–19] These studies, including a review and meta-analysis of previous work, do indeed show that the cortical tuber count is a biomarker that can reasonably predict future cerebral dysfunction in TSC patients.[20] Despite spin-echo MRI and pathologic evidence to the contrary,[21] when evaluating patients with seizures for potential surgery, the hope is that a tuber might actually be solitary.[23] Similarly, geneticists must have been tempted to reassure potential parents whose

clinical and spin-echo MRI examinations did not reveal evidence of TSC.[23] Progress in MRI has at least partially solved these two problems.

Software seems just as important as hardware. It is difficult to comprehend how just tweaking a few computer dials and buttons on the same MRI magnet could yield recent advances such as fluid-attenuated inversion recovery (FLAIR)[24] and magnetization transfer imaging sequences.[25] Neither of these techniques has been extensively evaluated clinically. Regardless, FLAIR imaging sequences are demonstrating a greater capacity to disclose cortical tubers in the myelinated brain than had been noted by spin-echo MRI sequences,[26] but their sensitivity for the detection of TSC in the developing and still-unmyelinated brain has yet to be determined. To date, we do not have any experience with magnetization transfer imaging of TSC.

Computed Imaging Findings

Subependymal Nodules

SENs are hamartomatous growths that are typically located along the outer walls of the lateral ventricles, nearly always adjacent to if not embedded within the caudate nucleus. These SENs, found in 80% of patients, are often multiple and generally small (1 cm or less).[2] SENs must develop during fetal life and grow in proportion to the remainder of the brain. In the absence of a metabolic disorder, calcification occurs only in dead tissue, so SEN degeneration begins during late fetal life and continues throughout childhood. MRI of SENs shows these lesions to generally have a signal isointense with white matter on T_1 and iso- or hypointense with white matter on T_2-weighted sequences (Fig. 8.1*A*,*B*). When calcified, their MRI signal and intensity vary depending on the type and degree of calcium apatite within the SENs. If the mineralization is predominantly caused by calcium phosphate, the SENs will show increased T_1 signal.[27] If the predominant mineralization is calcium carbonate, the SENs will show decreased signal, particularly on the T_2-weighted sequences. Some SENs enhance following intravenous gadolinium administration.[28] Because SEGAs also enhance, this enhancement may be important, but the incidence of SEGA is low. CT depicts SENs well, especially if calcified, as is commonly the case (Fig. 8.1*C*). This calcification can occur at any age, including prenatally. Calcified SENs are the single most diagnostic CT feature of TSC, although SENs are asymptomatic. SENs seldom enhance appreciably after administration of iodinated contrast. Any SEN near the foramen of Monro that enhances is usually a SEGA, particularly if the lesion is large or growth has been evident on serial imaging studies.

Subependymal Giant Cell Tumors

SEGAs typically lie adjacent to the foramina of Monro.[4,29] Pathologically SEGAs are unique to TSC and histologically identical to SEN.[30] In fact, it

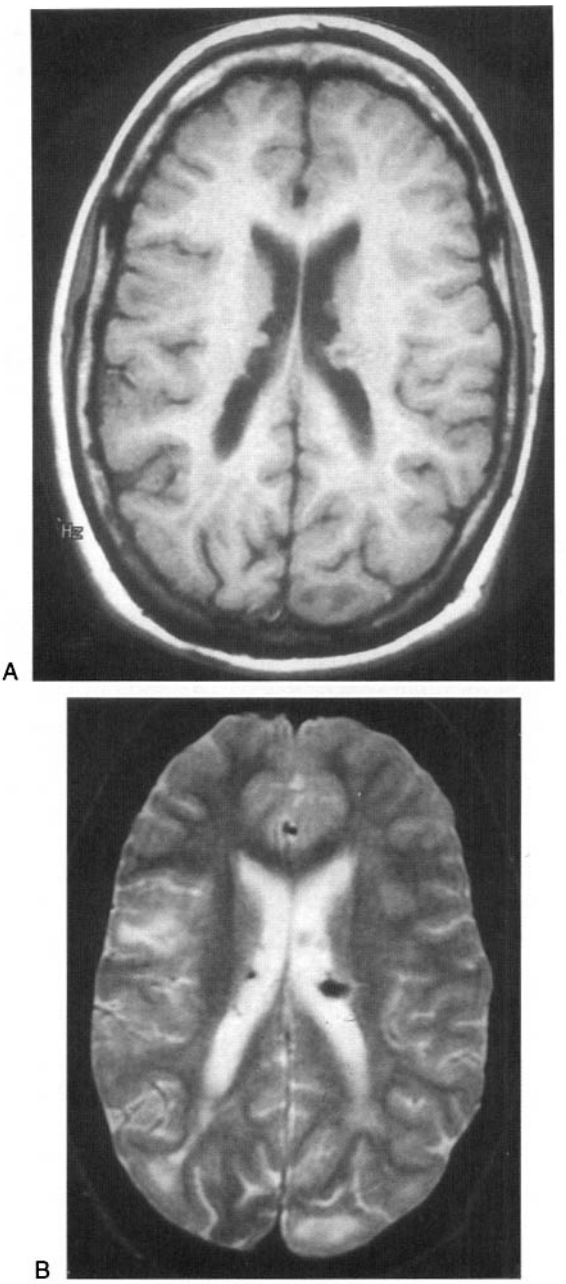

FIGURE 8.1. *A*, T_1-weighted axial MRI with and without gadolinium demonstrates multiple subependymal nodules (SENs) along the outer walls of both lateral ventricles, some showing mild enhancement. *B*, Comparable T_2-weighted images demonstrate the SENs, some of which show very low T_2 signal, indicating calcification. Also note the multiple cortical tubers. *Illustration continued on opposite page*

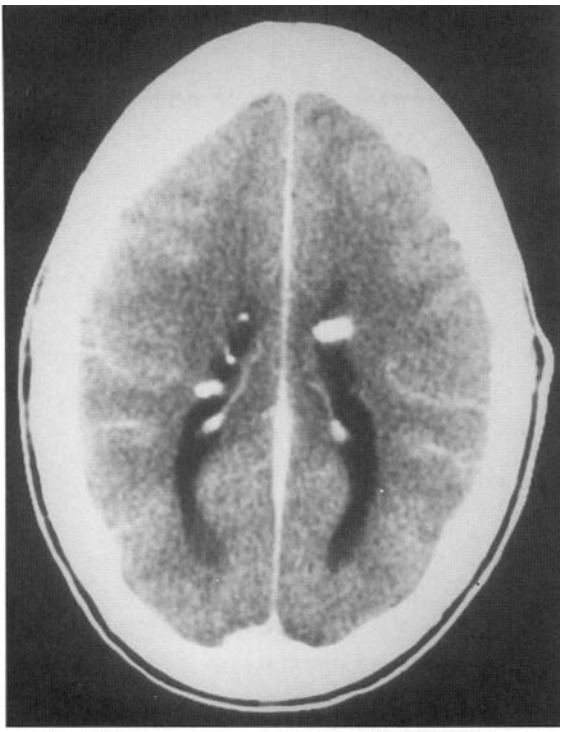

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FIGURE 8.1. (*continued*) *C*, Contrast-enhanced CT scan of the same patient demonstrates the very conspicuous calcified SENs. Cortical tubers are not well seen.

is generally accepted that SEGAs evolve from SENs, are typically located in the head of the caudate nucleus near the foramina of Monro, and differ from SENs only in their propensity to grow. Growth continues during late childhood and adolescence.[4] Clinically most patients present with hydrocephalus[31] from ventricular obstruction at one or both foramina of Monro. A few present as a growing lesion found on serial imaging studies. MRI of SEGAs often shows a heterogeneous mass that signals less than normal white matter on T_1 and more than the adjacent white and gray matter on T_2 exam (Fig. 8.2*A*). Hypointense regions within these tumors may be secondary to calcification, tumor vessels, or intratumoral hemorrhage.[32] Virtually all SEGAs enhance following gadolinium administration[27] (Fig. 8.2*B*). On CT, SEGAs are heterogeneous, partially calcified, large lesions at the foramina of Monro with or without ventricular obstruction (Fig. 8.2*C*). Traditionally any lesion showing enhancement on CT at the foramen of Monro following administration of iodinated contrast medium has been considered a SEGA and was either removed or monitored closely to detect enlargement. This axiom generally remains true: Progressive growth of an enhancing lesion at a foramen of Monro in a TSC patient indicates it is a SEGA.

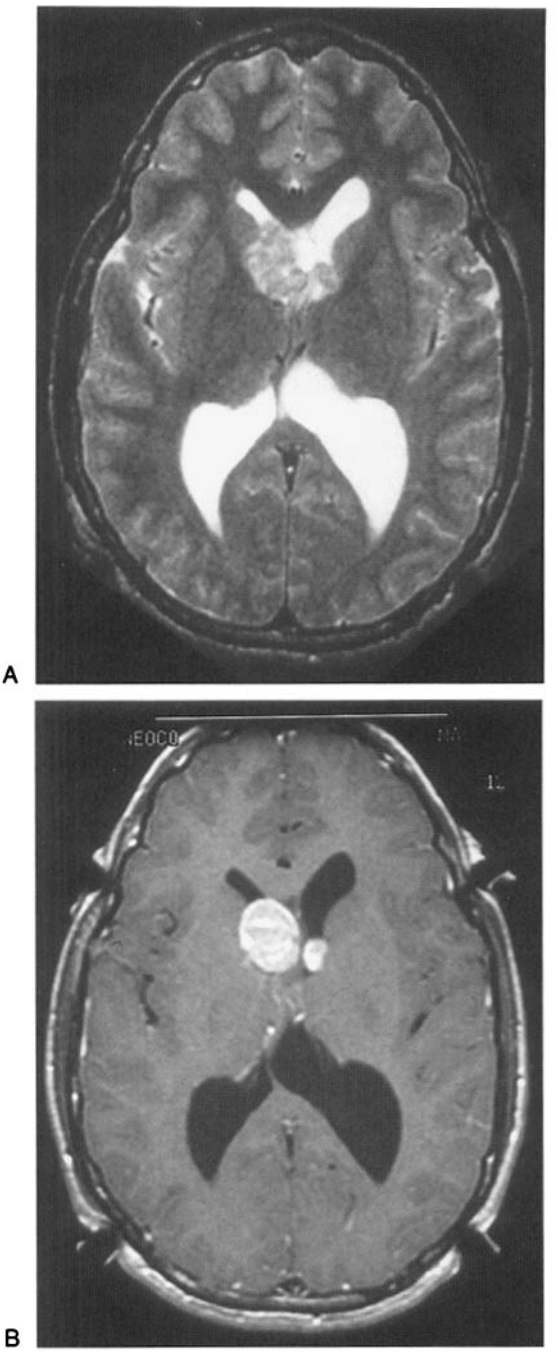

FIGURE 8.2. *A*, T_2-weighted scan at the level of the foramen of Monro demonstrates bilateral SEGAs, larger on the right, showing some heterogeneous increased T_2 signal and partial obstruction of the lateral ventricular outlet. *B*, Gadolinium-enhanced T_1-weighted image in the same patient demonstrates significant enhancement in the bilateral SEGAs. *Illustration continued on opposite page*

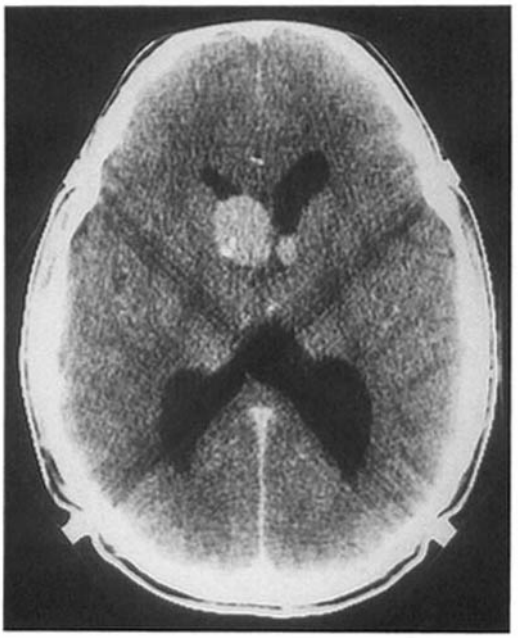

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FIGURE 8.2. (*continued*) *C*, Contrast-enhanced CT at a comparable level demonstrates significant SEGA enhancement and ventricular obstruction.

Cortical Tubers

Cortical and subcortical tubers are hypomyelinated hamartias involving mainly the cerebral cortex and underlying white matter. The cerebrum is affected in 90% and the cerebellum is also involved in 15% of our TSC patients. Brain stem lesions, although reported in the literature, were not evident in our series.[1–3] Tubers are often multiple, vary in size and cortical in location, and sometimes calcify.[33,34] Tubers may or may not be associated with underlying white matter abnormalities,[35] including occasional curvilinear bands, migration lines, or wedges extending from the periventricular region to the cortex. Although histologic proof is seldom available, it is believed that these white matter abnormalities represent hypomyelinated lesions and are pathologically similar to the subcortical part of tubers.[30] Spin-echo MRI sequences show that tubers generally enlarge the gyri and appear as regions of low T_1 and high T_2 signal unless they calcify (Fig. 8.3*A*,*B*). It has been noted that FLAIR imaging sequences are more sensitive than spin-echo sequences.[26] We concur because FLAIR identified more tubers, including small lesions in the cortex, in 9 of our 15 patients evaluated by this sequence (Fig. 8.3*C*). Gadolinium enhancement is not uncommon in tubers.[16] In the very young patient with immature white matter, the MRI appearance of tubers varies. Many tubers are not detectable. If detectable, the tubers do not show the characteristic findings noted in the myelinated brain. Instead, the tuber may appear relatively hypointense compared to the adja-

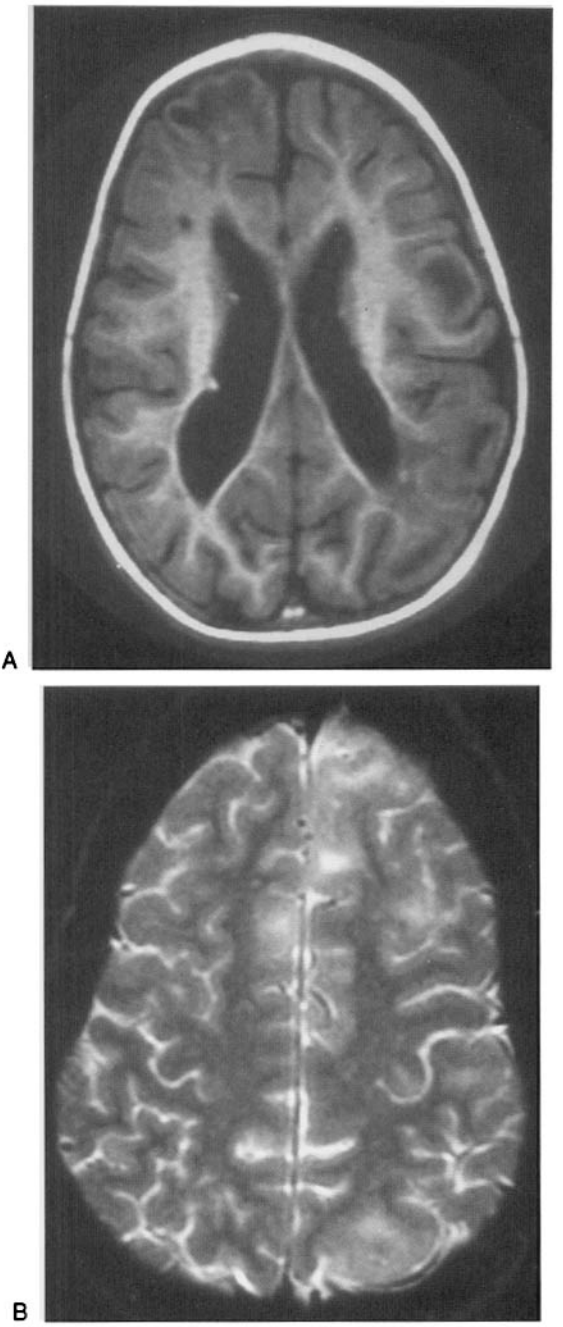
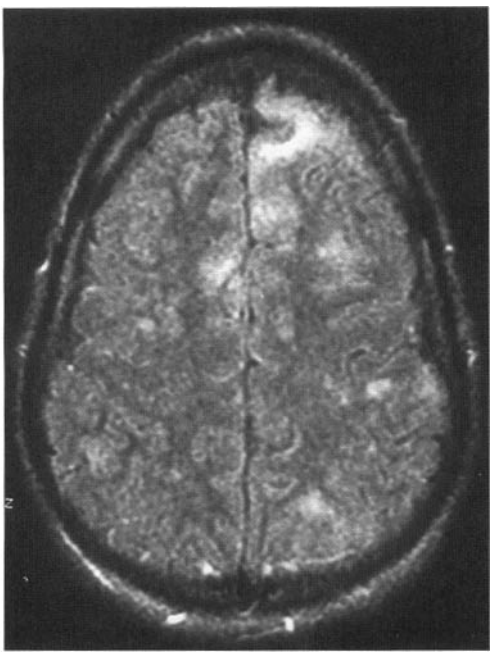


FIGURE 8.3. *A*, T_1-weighted scan demonstrates multiple cortical tubers that expand the gyri and have relatively low T_1 signal in the subcortical region. Also note the SENs and ventricular dilatation commonly seen in TSC. *B*, Proton density and T_2-weighted images near the vertex show several cortical tubers with relatively increased T_2 signal. *Illustration continued on opposite page*



C

FIGURE 8.3. (*continued*) *C*, FLAIR image in the same patient as *B* confirms the presence of multiple cortical tubers and identifies more small tubers than the standard spin-echo T_2-weighted images in *B*.

cent unmyelinated white matter on T_2-weighted sequences. Furthermore, Altman et al.[15] have even reported increased T_1 signal in lesions that probably represent tubers in unmyelinated white matter of infants (Fig. 8.4).

CT features of tubers generally demonstrate low attenuation as compared to adjacent parenchyma, may calcify, and rarely enhance with iodinated contrast (Fig. 8.5). Progressive calcification sometimes occurs and suggests persistent degeneration in these hamartias. Similarly, the disappearance of previously detected tubers over time and the absence of noncalcified tubers in adults raises the question of delayed myelination. Within the background of the unmyelinated brain of infants, CT may not detect the same hypomyelinated tuber that is obvious in the myelinated brain.

Some neuroradiologists are skeptical or puzzled about the plethora of imaging descriptions that has been used in the literature to define tubers. These include cortical tubers, subcortical tubers, sulcal islands, gyral cores, and migration lines, spindles, or wedges. All of these variations may merely support the concept of a temporal profile that reflects the degree of hypomyelination relative to whether a tuber is located in an unmyelinated, myelinating, or myelinated brain. The reason for the increased T_1 signal in tubers during early infancy is unknown.[15] Presumably this paradox reflects either the texture of the tuber or a peculiar protein concentration that creates increased T_1 signal. Only 1 (a 4-month-old infant) of our 22 patients under 2 years of age had this finding. By contrast, increased T_2 signal in tubers is

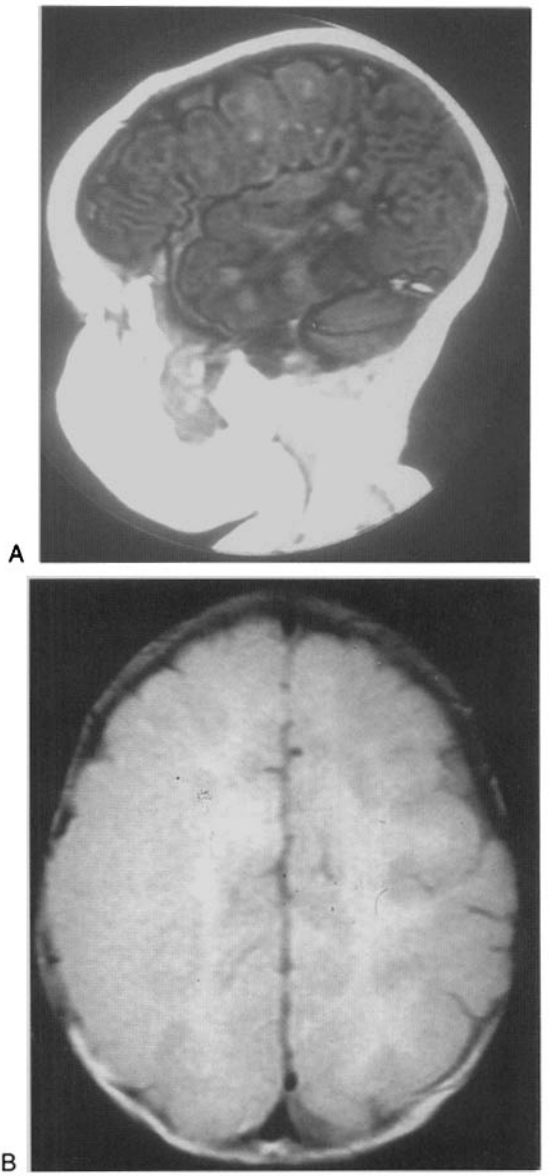


FIGURE 8.4. Four-month-old infant with TSC. *A*, Sagittal T_1-weighted image shows foci of increased T_1 signal in numerous tubers. *B* and *C*, Proton density (*B*) and T_2-weighted (*C*) images show a large, partially calcified tuber in the right frontal lobe showing mixed T_2 signal. Note the normal increased T_2 signal in the unmyelinated, immature white matter of both hemispheres. *D–F*, Same patient 2 years later with comparable images demonstrates that the increased T_1 signal seen on the initial scan is not identified. T_2-weighted scans demonstrate innumerable cortical tubers, many of which were not identified on the initial scans. Note the "sulcal island"-type tuber in the right frontal lobe and the large, calcified, right-sided tuber. *Illustration continued on opposite page*

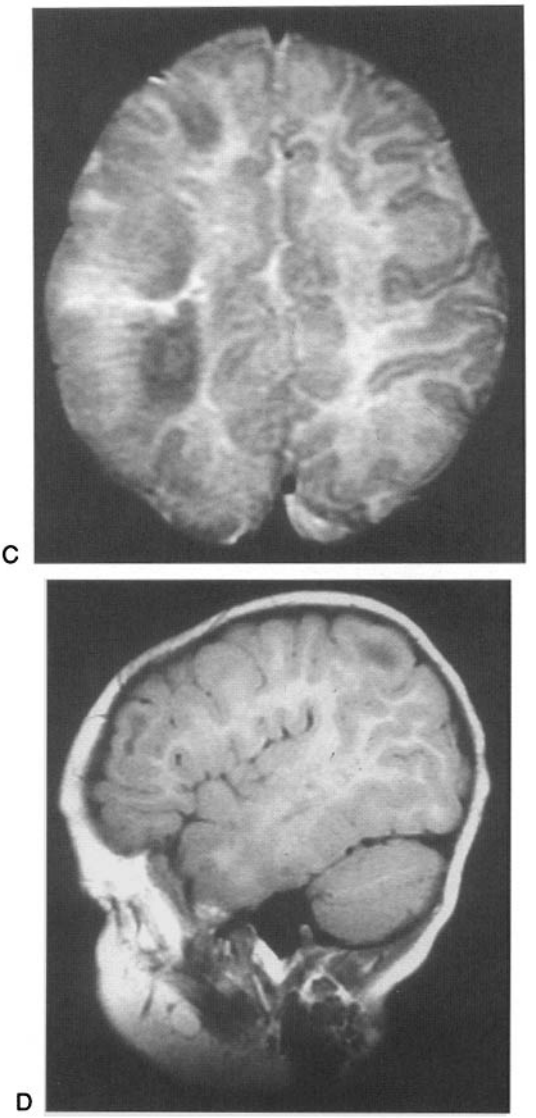


FIGURE 8.4. (*continued*)

notable in the myelinated brain of our TSC patients. If hypomyelination in the tuber is severe during childhood, decreased T_1 and increased T_2 signals may reflect a water content bordering on edema, since the volume of the intragyral white matter appears increased in most patients with gyral cores and many patients with sulcal island tubers. Most tubers are located in the cortex and extend into the gyral white matter, where the increased T_2 signal may capture the eye to such an extent that its cortical component is not

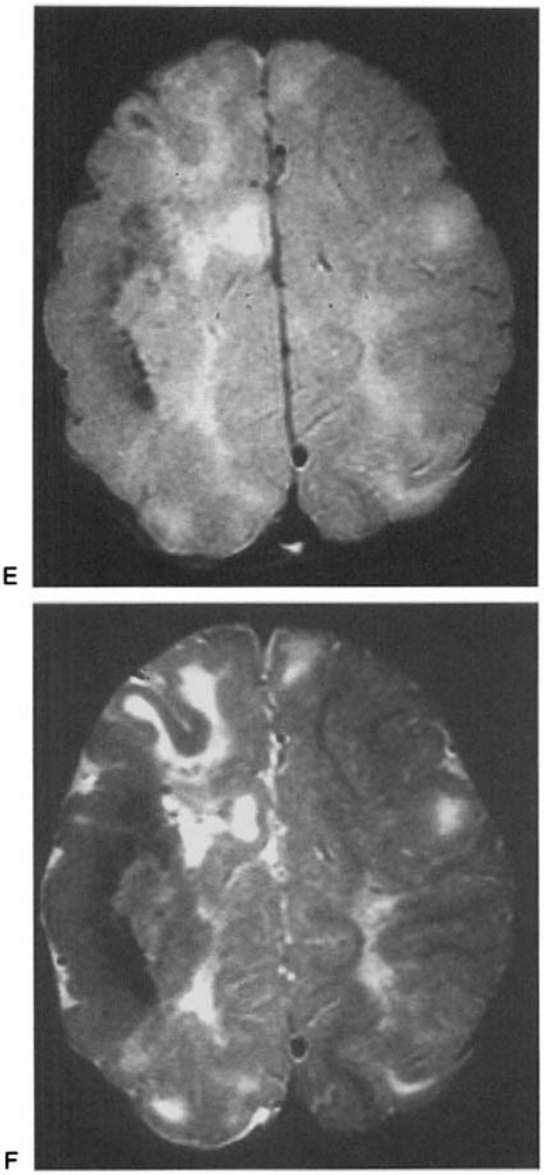


FIGURE 8.4. (*continued*)

obvious. CT has depicted tubers during the first decade of life that have disappeared or resolved by the third decade. Also, only calcified tubers have been detectable by CT in older adults. The resolution could be due to gliosis, but we believe delayed myelination is a more logical explanation, although we have no histologic proof. These findings match those in other hamartomatoses.[14]

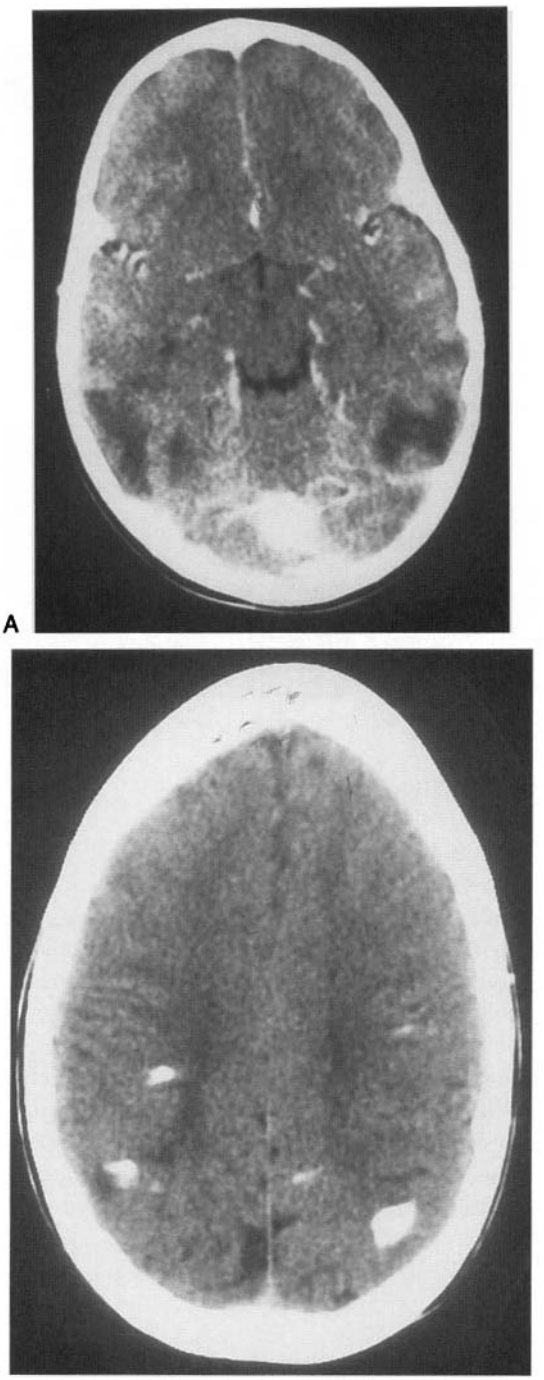


FIGURE 8.5. *A*, Contrast-enhanced CT demonstrates several low-attenuation cortical tubers, largest in the posterior temporal regions without significant enhancement. *B*, CT of another older patient shows multiple cortical tubers containing significant dystrophic calcification.

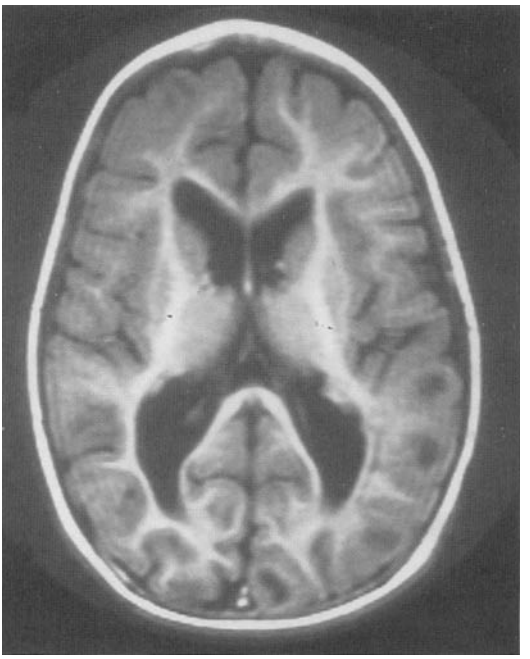


FIGURE 8.6. Axial T_1-weighted scan shows ventricular enlargement without obstructing SEGA. Note the relative paucity of white matter, multiple small tubers, and SENs.

Miscellaneous CNS Findings in TSC

Lateral ventricular enlargement occurs in 30% to 55% of patients with TSC.[1-3] The ventricular dilatation is usually mild to moderate in degree. Occasionally the dilatation is secondary to an obstructing SEGA at a foramen of Monro, but in the great majority of patients the cause is not apparent (Fig. 8.6). Previous authors have suggested this may be developmental or secondary to cerebral atrophy. MRI and CT serve equally well to assess the ventricular system and detect an obstructing SEGA.

Other rare findings associated with TSC include cerebrovascular occlusive disease and vascular ectasias or aneurysm. Magnetic resonance angiography is a reasonable screening tool for these vascular problems if involvement of large vessels is suspected. Conventional angiography remains the definitive examination.

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9

Neuropathology

Introduction

Tuberous sclerosis complex (TSC) is a genetically determined, variably expressed, multisystem disorder that manifests as hamartias (tubers and heterotopias in the central nervous system [CNS]), benign hamartomatous growths (subependymal nodules), and malignant-behaving lesions (hamartoblastomas) with many features of neoplasms. As a rule, the latter do not occur in the CNS but take the form of rarely occurring clear cell carcinomas of the kidney. Just where subependymal giant cell astrocytomas (SEGAs) fit into this scheme is uncertain in that malignant examples are very rare. The importance of CNS involvement in TSC is emphasized by the fact that the condition has retained its name for nearly a century. Derived from a single pathologic feature, namely potatolike firmness of segments of the cerebral cortex, the designation *tuberous sclerosis* has persisted despite occasional suggestions of more suitable, all inclusive terms such as *multiple system hamartosis*.[1] Indeed, the brain occasionally lacks tubers and, in rare instances, is grossly entirely normal—that is, if one accepts as TSC cases those with only isolated extraneural manifestations of the disease (*"formes fruste"*).[2,3]

All objections aside, the term *tuberous sclerosis complex* persists as a designation for all forms and variants of the disease. The fact remains that the majority of patients identified as having the disorder do experience symptoms referable to the CNS. Even in subjects without neurologic symptoms, CNS lesions are present in essentially all whose brains are adequately studied. We venture to say that, in those rare instances in which the brain is said to be anatomically normal, an exhaustive histologic search would likely reveal lesions. CNS abnormalities therefore remain the hallmark of TSC and underly its most common and clinically serious manifestations.

Macroscopic Findings

Cortical Tubers

On external examination, the typical TSC brain shows striking and virtually pathognomonic changes. Present on the surface of the cerebral and less often of the cerebellar hemispheres are cortical "tubers," lesions varying markedly in size, number, and location.[2,4] Typical examples involve gyral crests and are firm, smooth, somewhat raised, and more pale than normal surround-

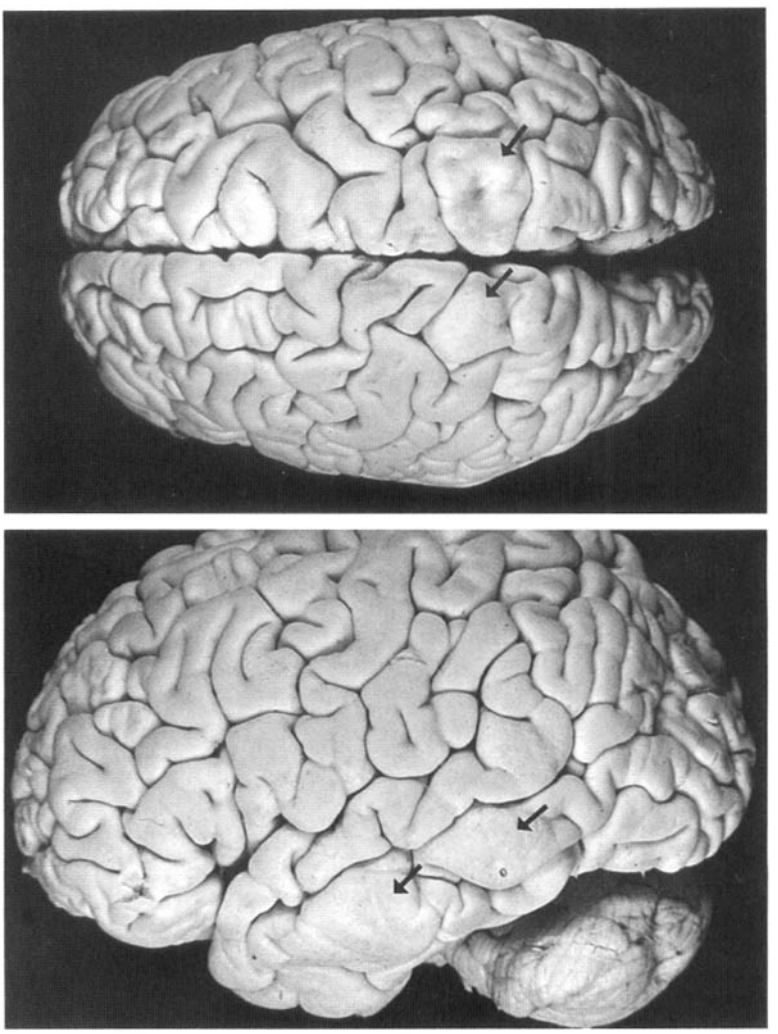


FIGURE 9.1. Cortical tuber. The appearance of tubers varies from globular, localized enlargement of gyri (*top, arrows*), often with umbilication, to widening of single, still elongate gyri (*bottom, arrows*).

ing cortex (Fig. 9.1). Most are circular or elongated in the direction of the affected gyrus (Figs. 9.1 and 9.2). At times tubers involve segments of adjacent gyri and obscure sulcal markings. In unfixed brains, small examples are more easily palpated than seen. The distinction of normal from tuberous cortex may be enhanced by viewing the brain under ultraviolet light.[5]

In any single case, the number of grossly apparent cortical tubers varies from none to greater than 40. Similarly, their size ranges from millimeters to several centimeters. Although the distribution of tubers over the brain surface is random, the frontal lobes may be somewhat more often affected.[6] Laterality is not a factor but, in individual cases, involvement of the two hemispheres is often asymmetric. Cortical tubers occasionally occur in the

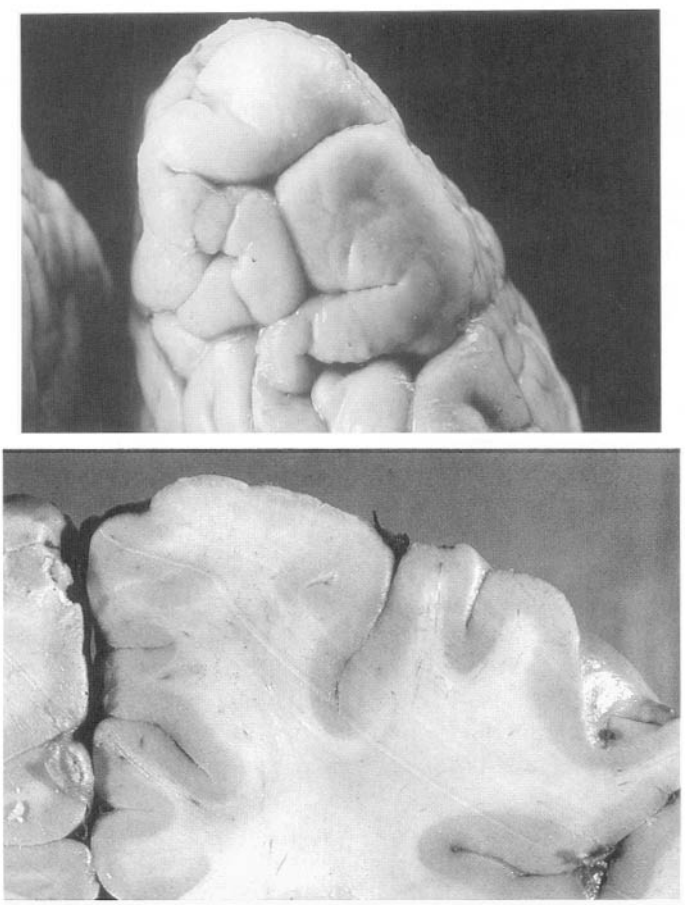


FIGURE 9.2. Cortical tuber. A close-up view (*top*) shows both central umbilication and a somewhat pebbled, reticular texture corresponding to subpial gliosis. On section (*bottom*), tubers show pallor of the cortex and obliteration of its junction with immediately underlying white matter. The latter is often discolored and may show malacia resulting from axon and myelin loss, as well as gliosis.

cerebellum[7] (Fig. 9.3). Close inspection of the surface of a cortical tuber often reveals a fine, slightly raised, reticular network caused by bands of gliosis within the molecular layer of the cortex (Fig. 9.2). Central depression or "umbilication" of tubers is often seen in older patients and is a reflection of degeneration and tissue retraction within central portions of the lesion (Figs. 9.1 and 9.2).

Sectioning the brain not only better demonstrates cortical tubers but reveals characteristic lesions within the depths of the brain and in the ventricular system (Figs. 9.2 and 9.4). Tubers are pale in appearance and are more firm than adjacent brain tissue. They are seen to have indistinct borders, blending more or less gradually into the adjacent cortical ribbon and underlying white matter. The gray and white matter junction is therefore often obscured. Additional cortical tubers, ones not apparent on the brain surface, may be found in the depths of sulci.

Neuroglial Heterotopias

Subcortical white matter, particularly beneath cortical tubers, occasionally shows small, often radially arranged gray zones representing heterotopic collections of abnormal cells. More rarely, white matter may contain firm "tuberous" lesions similar in texture to those within the cortex or the ventricular system.

Subependymal Nodules

The most striking deep lesions of TSC lie within the ventricular system (Figs. 9.4 and 9.5). Inspection of its surfaces typically reveals smooth-surfaced, round to ovoid or sausage-shaped elevations projecting into the cavity. These are firm and, because of dense calcification, often stony hard. Variable in size, subependymal nodules form rows of bosselated lesions likened in appearance to wax dripping down the side of a candle ("candle guttering") (Fig. 9.4). Although such lesions occasionally occur in the third and fourth ventricles as well as the aqueduct, the vast majority lie along the thalamostriate sulcus in the lateral ventricles. When sizeable, their deep portions may lie embedded in the caudate or thalamic nuclei (Fig. 9.5). Morphologically similar "glial hamartomas" have also been described in the olfactory lobes.[8]

When large, subependymal nodules in the region of the foramen of Monro may lead to foraminal obstruction with resultant hydrocephalus (Figs. 9.4 and 9.5). Fatal intralesional hemorrhage is rare and occurs more often in truly large examples, ones tumoral in proportion (Fig. 9.5). Transformation of subependymal nodules into so-called subependymal giant cell astrocytomas (Fig. 9.5), a gradual process, is discussed in detail below (see "Brain Tumors in TSC").

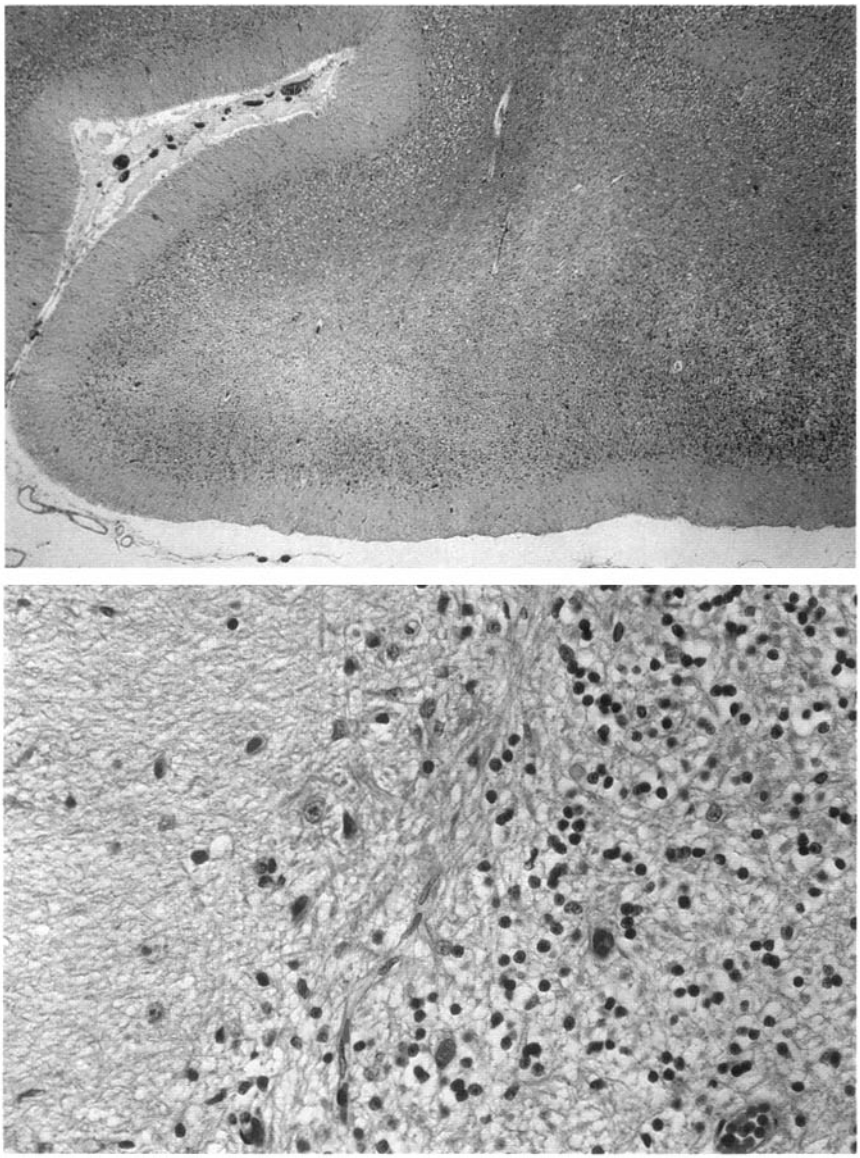


FIGURE 9.3. Cortical tuber, cerebellum. The affected folium shows focal loss of cellularity of the granular layer (*top*) and of Purkinje cells (*bottom*), as well as gliosis.

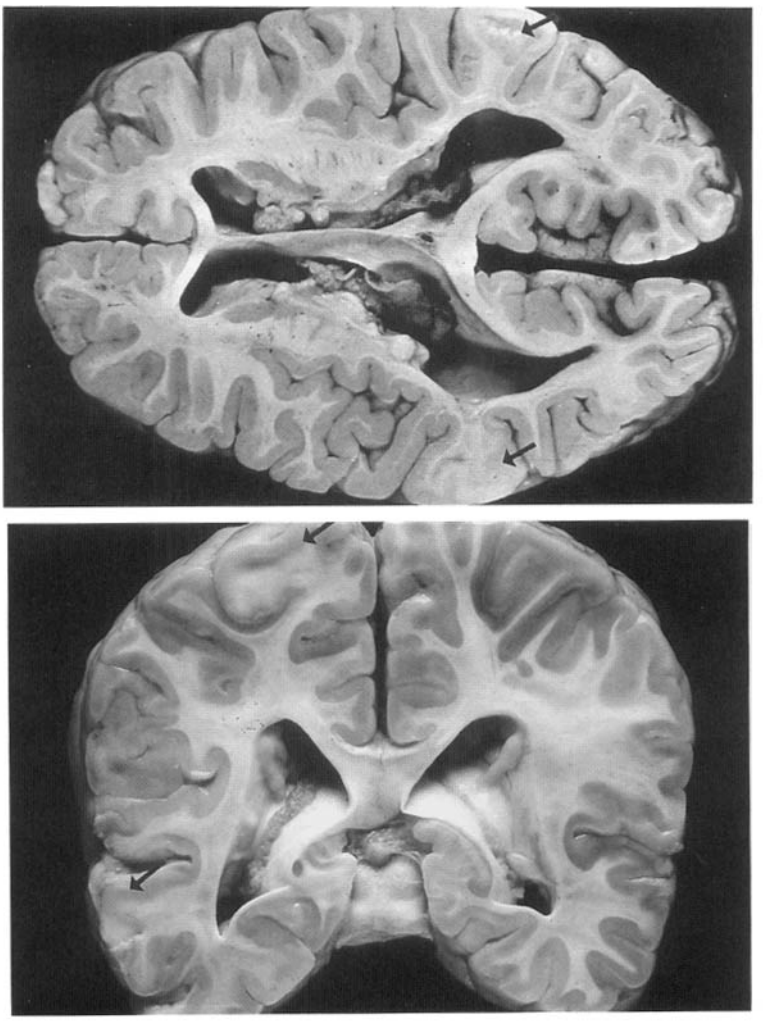


FIGURE 9.4. Subependymal nodules situated anteriorly within the ventricular system, most overlie the basal ganglia (*top*). Note accompanying hydrocephalus caused by obstruction of the foramina of Monro. Tubers are also evident (*arrows*). The "candle gutter" configuration of some subependymal nodules is seen to advantage in a coronal cut (*bottom*). Again, note tubers, showing subcortical contraction and degenerative changes (*arrows*).

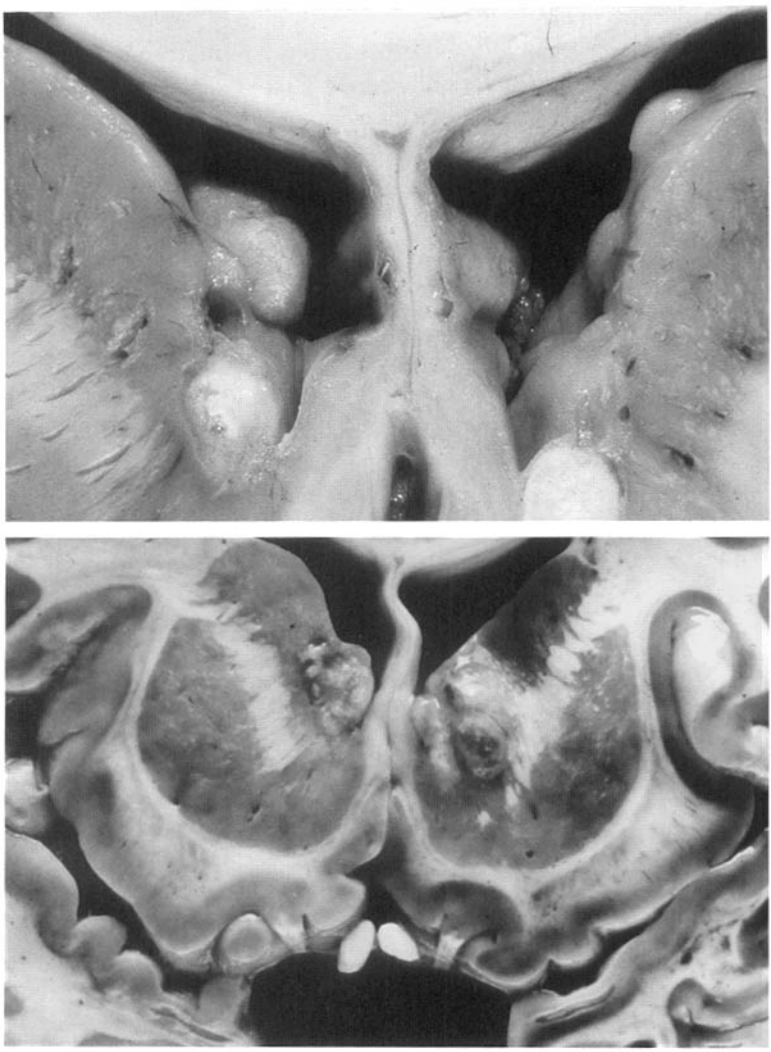


FIGURE 9.5. Subependymal nodules and SEGA. Note multifocality and demarcation of subependymal nodules from underlying brain parenchyma (*top*). With enlargement, such lesions are termed SEGAs. In this example, the tumors are bilateral and partially embedded in underlying basal ganglia tissue (*bottom*).

Miscellaneous Lesions

Other abnormalities may be grossly evident in the brains of TSC patients. Although the weight of the brain is usually within the normal range for patient age, both microcephaly and megalocephaly are rarely observed. Nonobstructive hydrocephalus and gross atrophy of the cerebellar cortex as well as of the hippocampal formation may also be seen and are attributed to the chronic effects of seizures. Intracranial aneurysms, including three cases of giant aneurysm of the intracranial carotid artery, have also been reported.[9–11]

Microscopic Pathology

Both within the brain and in viscera, the lesions of TSC are generally either hamartias, nongrowing lesions including cortical tubers and white matter heterotopias, or hamartomas (subependymal nodules), tumorlike processes that differ from true neoplasms in several fundamental ways. The latter are composed of parenchymal and supporting cells native to the tissue in which they arise but are abnormal in terms of their number, location within the organ, organization, and cellular morphology. Therefore, they appear to be the result of focally disturbed organogenesis. Unlike neoplasms, hamartomas are not autonomous growths but rather appear subject to the growth-regulating systems of their native organ. Furthermore, they often display similar reactive capabilities, including age-related changes. In contradistinction to normal tissue, hamartomas appear to have a slightly heightened tendency toward neoplastic transformation, an issue further discussed below in relation to SEGAs (see "Brain Tumors in TSC").

Cortical Tubers

This lesion, the namesake of TSC, is histologically characterized by architectural disarray, particularly lack or disruption of cortical lamination. Overall cellularity varies, being either low because of reduced numbers of neurons or significantly increased primarily as a result of astrocytes, which are often numerous (Fig. 9.6). Unlike in normal cortex, pyramidal neurons are not organized in layers but lie in dissarray, being seen within the molecular layer as well as in deeper regions. Some may be spatially disoriented with respect to the brain surface, lying horizontally or even with their apical dendrite pointed downward. A variety of degenerative changes have been described as affecting the pyramidal cells of tubers; these include shrinkage, chromatolysis, the formation of neurofibrillary tangles, the presence of Pick body–like inclusions, and excess lipopigment and/or glycogen accumulation.[12] As previously noted, tubers may also occur in the cerebellum. As in the cerebrum, they consist of disorganized cortex but contain granule and abnormal Purkinje cells.

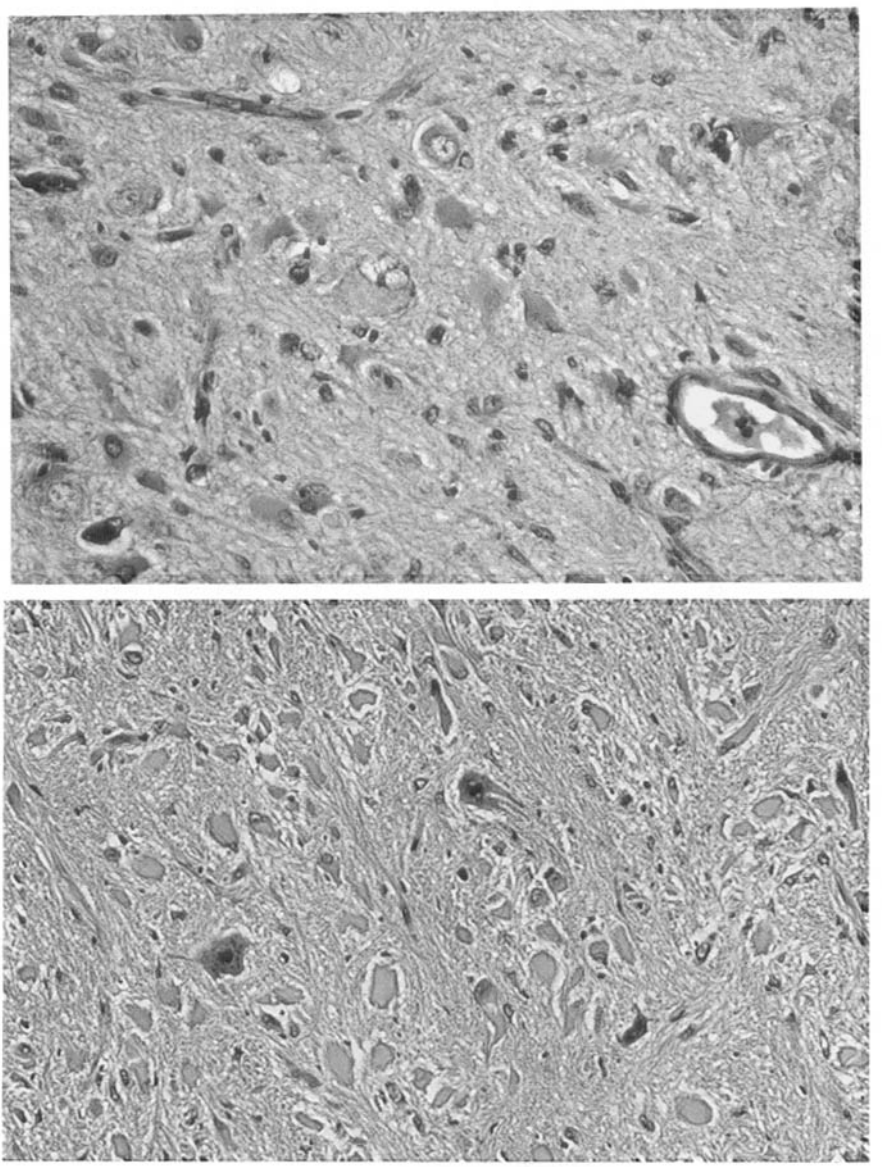


FIGURE 9.6. Cortical tuber, cerebrum. Histologically, tubers show architectural disarray, vary considerably in cellularity, and feature both neurons and large pink cells (*top*, *bottom*). The latter are a conspicuous feature in some examples (*bottom*; see also Figs. 9.7 and 9.9).

A conspicuous feature of cortical tubers, as well as of other CNS lesions in TSC, is the presence of very large cells (Fig. 9.6). Some are patently neuronal in character, with demonstrable Nissl substance on cresyl violet stain and neurofibrils on silver impregnation (Bodian or Bielschowski stains) (Fig. 9.7). Frequently dysmorphic, such neurons are occasionally found in otherwise normal-appearing cortex as well. They often show unusual patterns of immunoreactivity for neuronal markers and may exhibit staining for a variety of neuropeptides (Fig. 9.8). Despite eye-catching neuronal abnormalities, the vast majority of large cells in tubers resemble swollen astrocytes with enlarged, sometimes convoluted or multiple nuclei (Fig. 9.8). Lying singly or clustered, these often polygonal cells possess abundant pink cytoplasm and lack both the blocky Nissl substance and long argyrophilic processes so characteristic of neurons. Also distinguishing such cells from neurons is their frequent periodic acid–Schiff (PAS) positivity, and the occasional finding of PAS-positive needlelike crystals their cytoplasm[13,14] (Fig. 9.8). The cytogenesis and differentiation of these large pink cells have long been subjects of debate.[15] Despite their astrocytic appearance, immunohistochemistry and ultrastructural studies often demonstrate both glial and neuronal characteristics. A recent study of 13 tubers found glial markers (glial fibrillary acidic protein [GFAP], S-100 protein) and neuronal epitopes (neurofilament protein, class III β-tubulin) in many such cells[13,16] (Fig. 9.9). Ultrastructural studies similarly indicate that such cells show the presence of divergent differentiation (Figs. 9.10 through 9.12). The contribution of Golgi preparations as well as of immunohistochemistry to the characterization of the various cells comprising tubers is discussed in greater detail in Chapter 18.

In addition to their cellular constituents, tubers exhibit degeneration changes. Gliosis is evident both on special histochemical stains, such as the Holzer method for glial fibrils in which cell processes within the lesions are darkly stained, and on immunostains for GFAP. Sections stained by the latter method clearly demonstrate both gradual transition from involved to normal cortex and frequent extension of tubers and their secondary effects into underlying white matter. Relative lack of myelin is evident on Luxol–fast blue preparations, in which tubers and their subcortical regions appear pale as a result of lack of myelinated fibers. Glial fibers in the molecular layer may be organized in dense, tuftlike bundles (piloid gliosis) oriented in various directions; these give rise to the finely reticulated surface appearance previously described. The amount of gliosis within tubers increases with age, as do other degenerative changes, particularly loss of neurons. Accumulation of mineral deposits rarely occurs to the extent seen in subependymal nodules (see below). Settling of the loose-textured, degenerated central portions of a tuber and of its subcortical white matter (Fig. 9.4) may contribute to their often grossly evident surface depression or "umbilication."

Neuroglial heterotopias composed primarily of large pink cells of the type seen in tubers, and to a lesser degree of cells with neuronal features, may be found in subcortical white matter (Fig. 9.13). As a rule, they are not

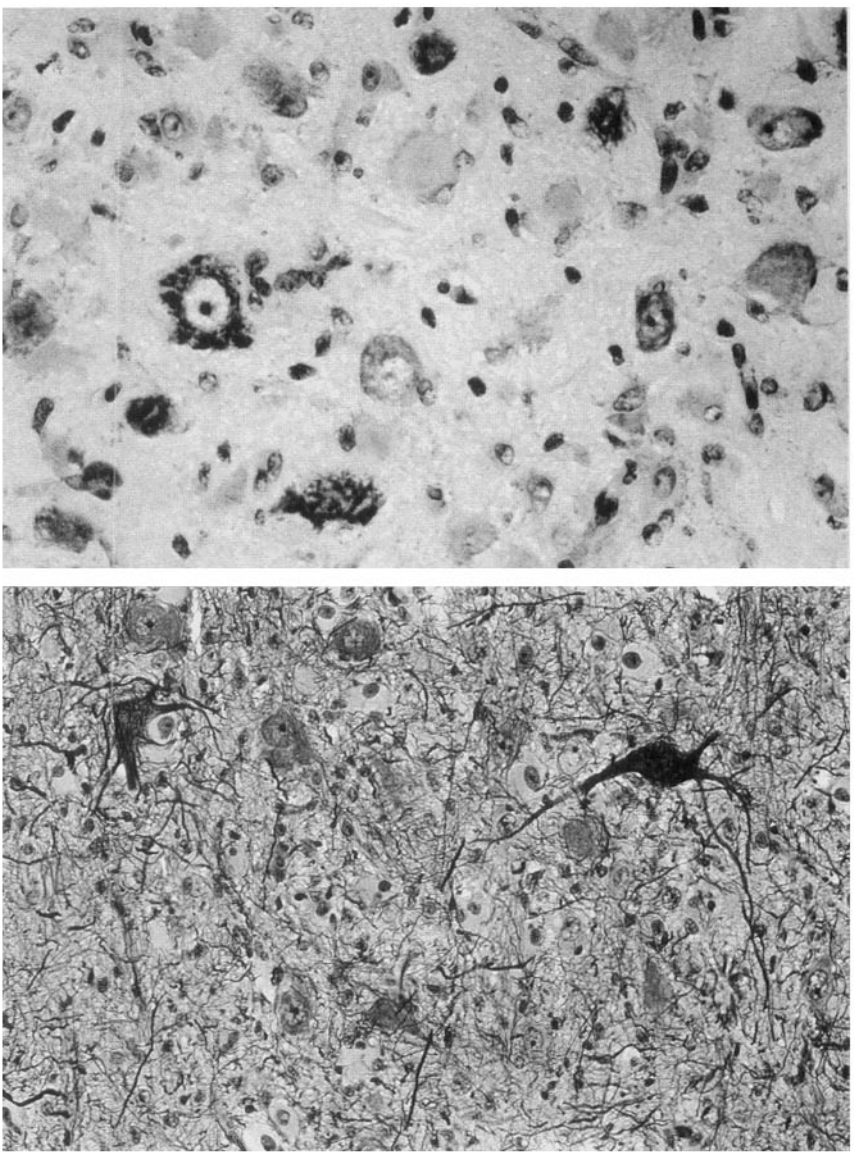


FIGURE 9.7. Cortical tuber, cerebrum. Abnormal neurons are highlighted on stains for cytoplasmic Nissl substance (*top*) and on silver impregnations (*bottom*). Note lack of Nissl substance in large pink cells (*top left*). Staining: *top*, cresyl violet; *bottom*, Bielschowsky method for axons.

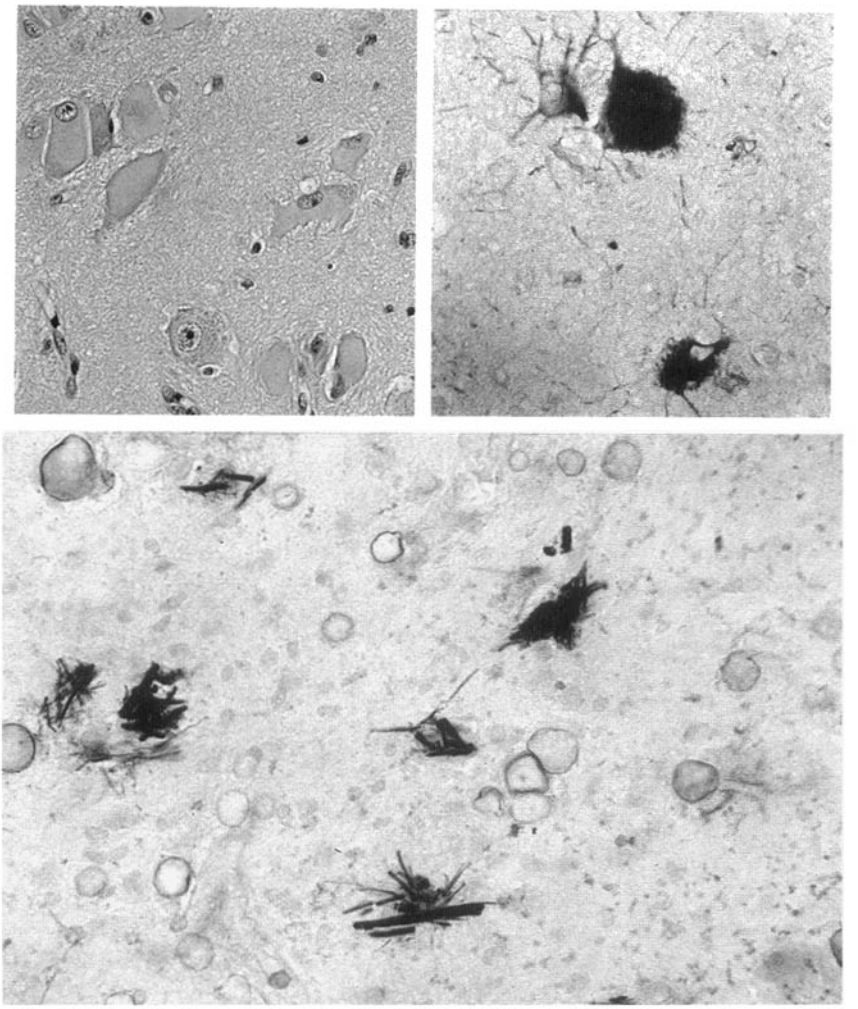


FIGURE 9.8. Cortical tuber, cerebrum. Pale pink cells typify the brain lesions of TSC. Most resemble full-bodied astroyctes with inconspicuous processes (*top left*). Note lack of Nissl substance (see also Fig. 9.7). Such cells are typically PAS positive (*top right*), occasionally containing crystalloids (*bottom*).

grossly apparent. Instead, they are microscopic in dimension, their constituent cells lying singly or clustered in perivascular sleeves. In that heterotopias are often disposed radially along a line extending from the ventricle to the tuber-affected brain surface, they are thought to result from migration arrest of cells moving from periventricular germinal matrix to the cortical zone. In the cerebellum, collections of heterotopic granule cells or large pink cells are also occasionally seen within white matter. Heterotopias containing large pink cells may rarely be seen in the brain stem or spinal cord as well.[17–19]

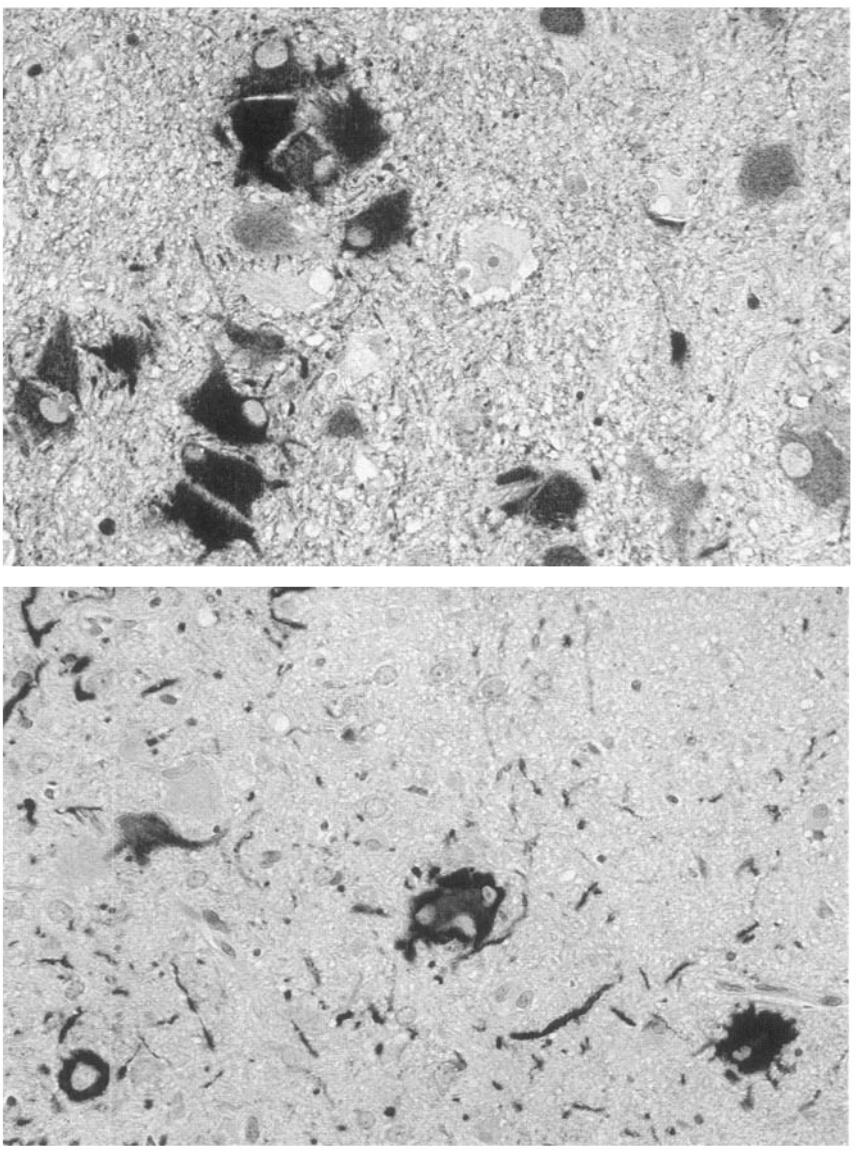


FIGURE 9.9. Cortical tuber, cerebrum. Large pale pink cells are often variably GFAP immunoreactive (*top*). Staining for neuronal markers such as neurofilament protein (*bottom*) may also be seen in such cells.

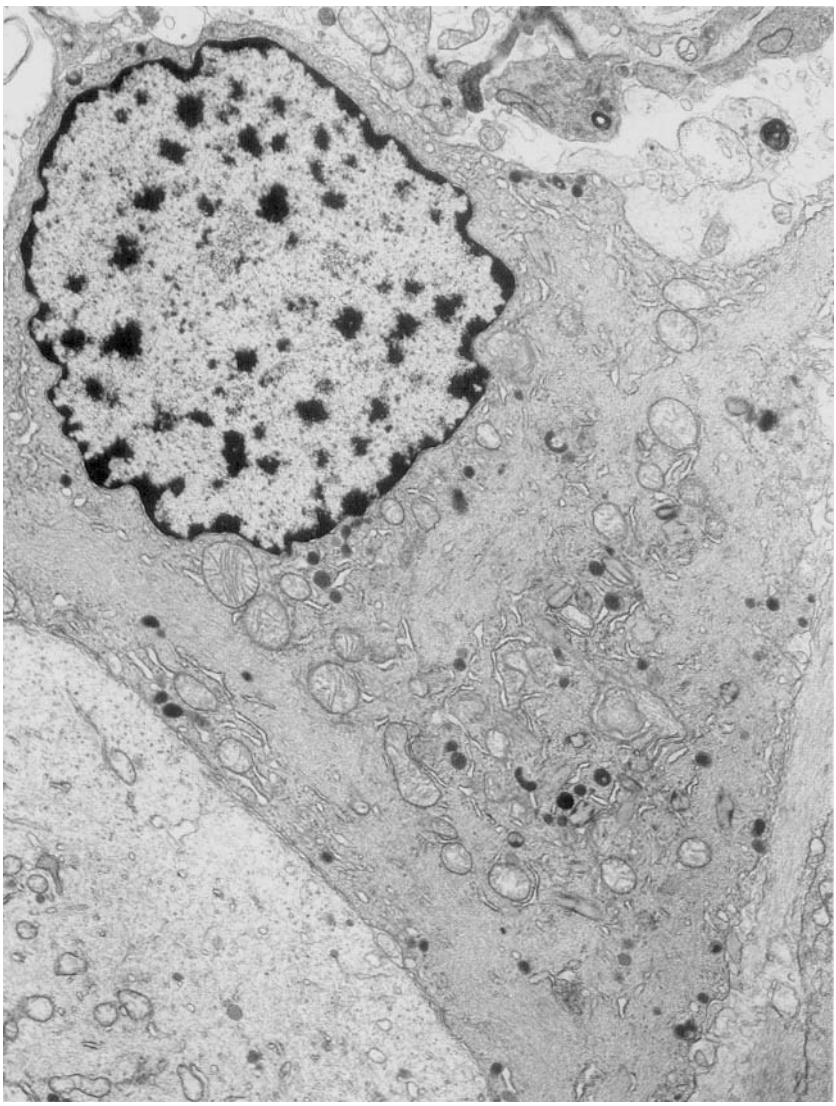


FIGURE 9.10. Cortical tuber, cerebrum. Giant cell corresponding to large pink cells on hematoxylin and eosin stain (see Fig. 9.9). Note the eccentric nucleus and abundant cytoplasm containing lysosomelike, dense bodies and numerous intermediate filaments. (×7500)

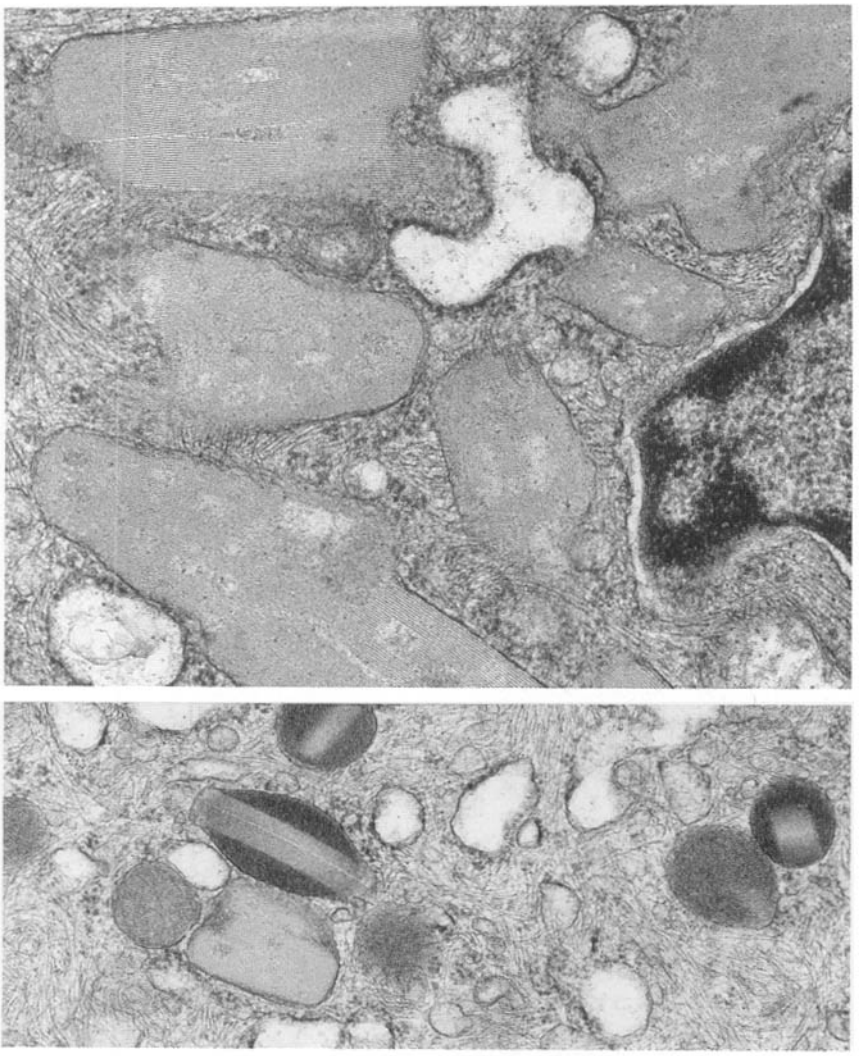


FIGURE 9.11. Cortical tuber, cerebrum. Rectangular crystalloids within a giant cell exhibit lamellar periodicity (*top*). That crystalloids arise in transition from lysosomes is suggested by the finding of structures with features intermediate between both (*bottom*). (*Top*, ×42,500; *bottom*, ×30,000)

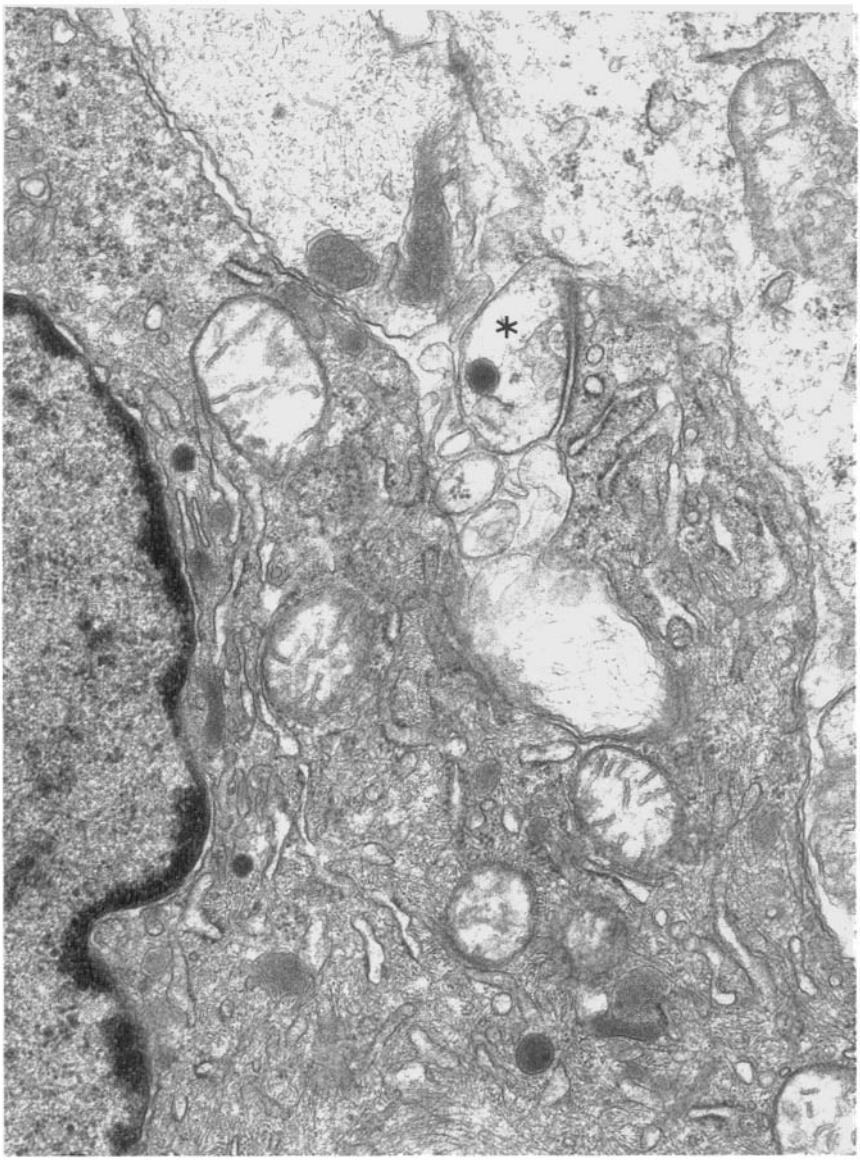


FIGURE 9.12. Cortical tuber, cerebrum. A giant cell, filled with intermediate filaments and dense bodies, is seen to be engaged in synapse formation. The synapse (*asterisk*) is seen to consist of an axonal termination containing presynaptic, clear vesicles, and a dense-core granule is attached to a short process of a giant cell. (×25,000)

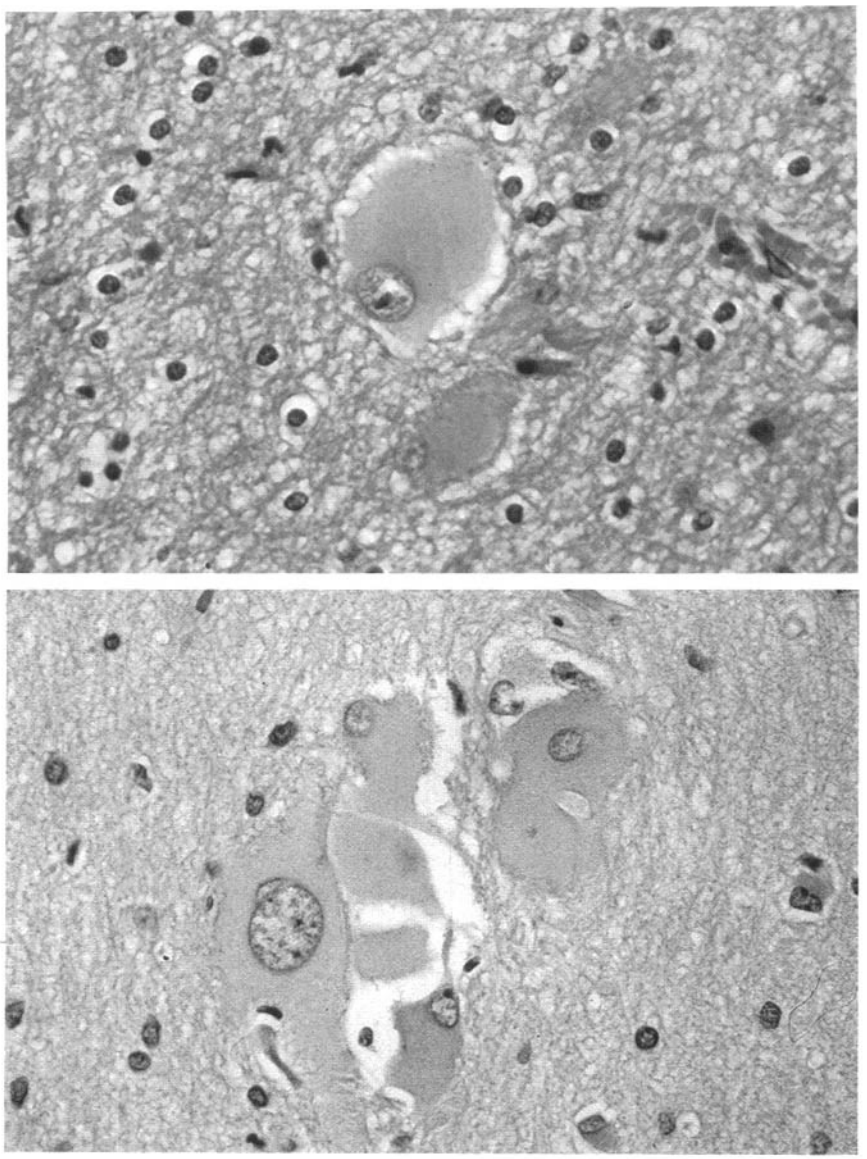


FIGURE 9.13. Heterotopia, cerebral white matter. The constituent cells of heterotopias may lie singly (*top*) or disposed in clusters (*bottom*), often in the vicinity of vessels. Such cells closely resemble the large pink cells of tubers and show the same histochemical and immunocytologic features.

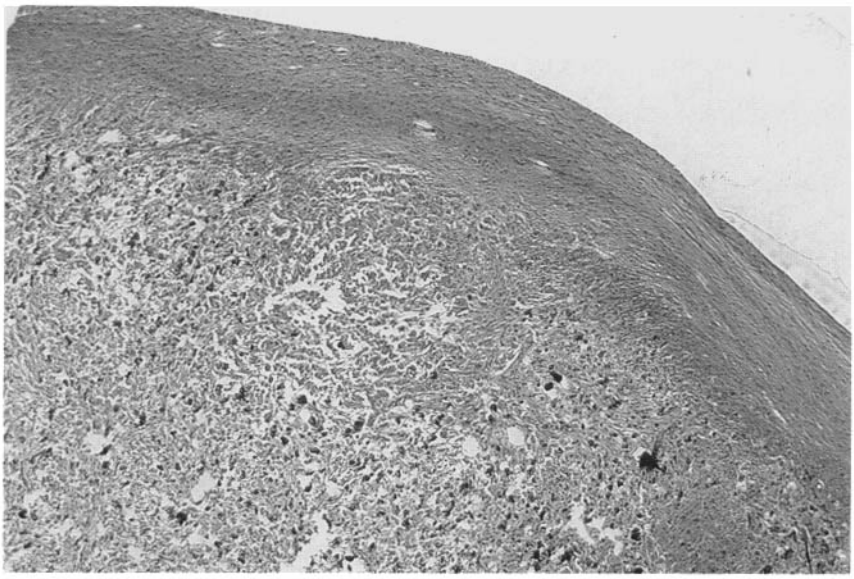


FIGURE 9.14. Subependymal nodule. Smooth surfaced, these demarcated lesions consist of spindle and epithelioid, astrocytelike cells in every way similar to those of SEGAs (see Figs. 9.15, 9.17, 9.19 through 9.25, 9.28, 9.29).

Subependymal nodules consist of an admixture of often plump, eosinophilic cells and their processes, as well as of vascular stroma (Fig. 9.14). Because of the frequent presence of dense calcific deposits, which in some instances replace all other elements, subependymal nodules may be difficult to section without prior decalcification. Architecturally solid, these demarcated, noninfiltrative lesions are covered on their ventricular surface by a layer of relatively intact ependyma (Fig. 9.15). As in cortical tubers, some cells attain near-giant proportions and contain large, convoluted, or multiple nuclei. Such large cells tend to occur at the center of the nodules, with smaller ones lying at the periphery. Their cell bodies are surrounded by a network of variously fibrillated processes that may be densely or loosely enmeshed. Stromal vasculature is often conspicuous and shows a tendency to hyalinization. Concentric spherules of calcium initially accumulate about vessel walls, later aggregating to form coarse stromal deposits. Hemorrhagic necrosis, occasionally symptomatic, has been reported to occur in subependymal nodules, particularly in large, tumorlike examples.[20]

Miscellaneous

Various other histologic abnormalities occur in the brains of patients with TSC. Of these, most are the result of repeated seizures or other insults, including trauma, infection, and nutritional deficiency.

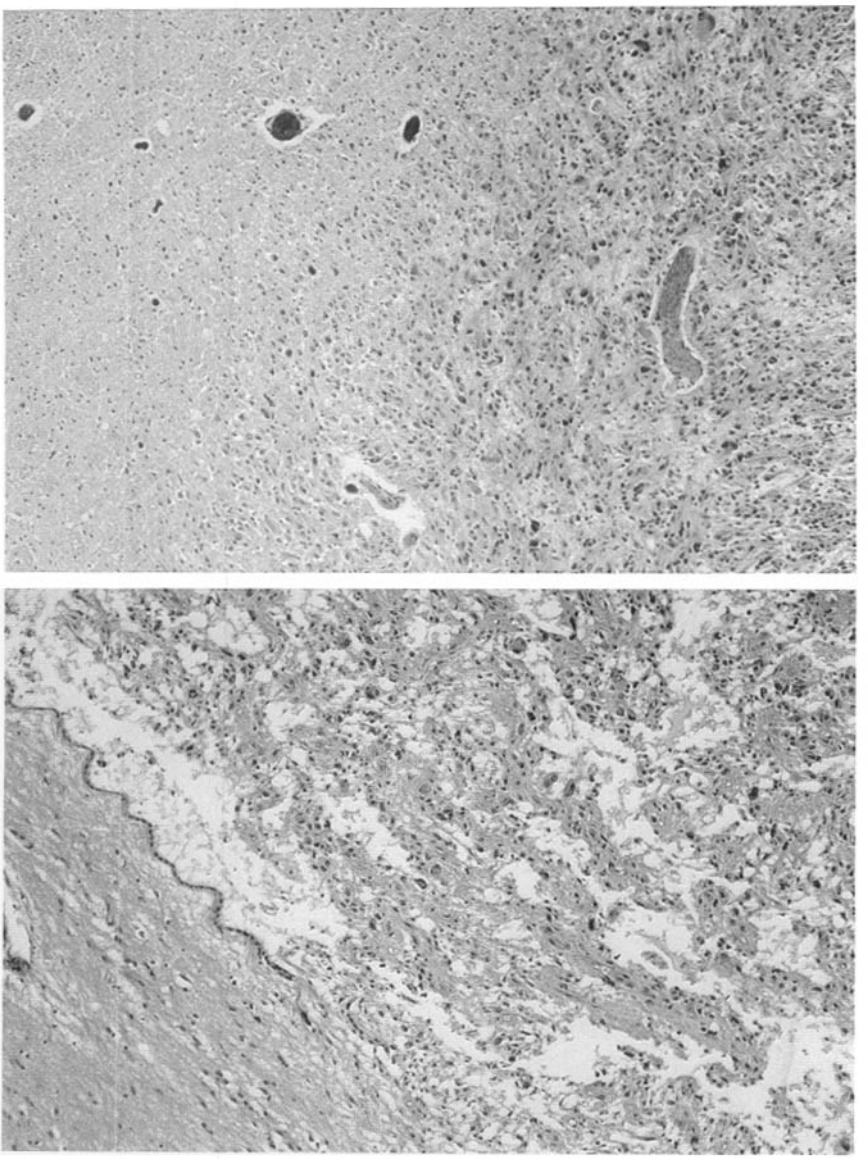


FIGURE 9.15. SEGA. Relative demarcation from underlying brain parenchyma (*top left*) is the rule. Intraventricular growth causes some to extend over intact ependyma (*bottom lower left*).

Brain Tumors in TSC

Subependymal Giant Cell Astrocytoma

The subject of brain tumors occurring in TSC is surrounded by confusion and debate. The issue relates less to the spectrum of tumors that may occur in this setting than to the nature of subependymal nodules and giant cell "astrocytomas." In part, the problem is semantic. When the term *tumor* is applied in its broadest sense as a "space-occupying lesion," most TSC lesions—short of hamartias (cortical tubers and white matter heterotopias), which do not grow—may be considered tumors. This definition would, of course, include subependymal nodules. In a more restricted clinical sense, the designation "tumor" is reserved for symptomatic space-occupying lesions having demonstrated growth potential. With regard to subependymal lesions, neuroradiologists often draw a distinction between nodules and tumors on the basis of lesion size, the cutoff being at 1 cm. Although many nodules remain small and asymptomatic, transition of subependymal nodules to SEGAs has been well documented by sequential neuroimaging studies.[21,22] From a histopathologic viewpoint, SEGAs appear to differ from subependymal nodules only in their apparently increased growth potential. Either sizeable nodules or SEGAs may obstruct one or both foramina of Monro (Figs. 9.5, 9.16, and 9.17). The result is progressive lateral ventricular dilation and increased intracranial pressure. In most instances, this continuum of ventricular lesions presents in childhood,[10,23,24] with only the largest examples being of sufficient size to be considered SEGAs. These represent

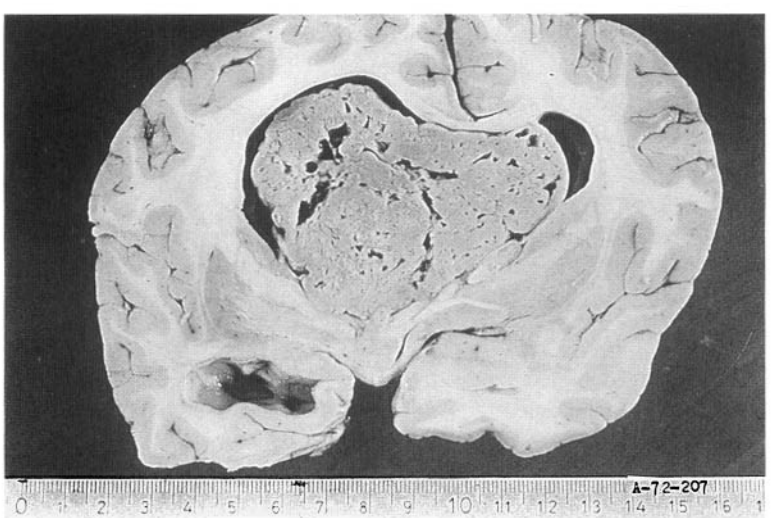


FIGURE 9.16. SEGA. This massive but otherwise typical example is well demarcated from surrounding brain parenchyma.

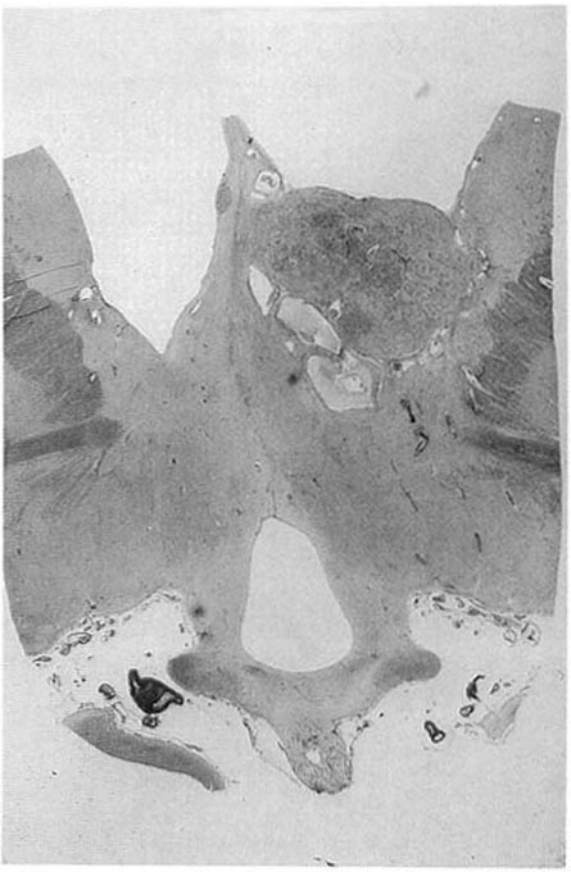


FIGURE 9.17. SEGA. This sharply demarcated example lies wedged between the septum pellucidum (*center*) and the head of the caudate nucleus (*right*). By obstructing the foramen of Monro, it produced hydrocephalus.

approximately 1.5% of pediatric brain tumors. Males and females are equally affected.[19] Congenital SEGAs are rare.[25–27] Morphologically, identical tumors may also affect the retina[28] (Fig. 9.18).

The typical SEGA exceeds 1 cm in diameter and is attached to the wall of the lateral ventricle at the opening of one or both foramina of Monro (Figs. 9.5, 9.16, and 9.17). Only occasional examples extend through the foramen into the third ventricle. The vast majority are solitary, smooth surfaced, discrete masses. Some lie partly embedded within underlying brain substance. Diffuse parenchymal invasion is not a feature of SEGA (Fig. 9.15).

The histology of SEGAs is quite variable but, in most instances, is decidely glial (Figs. 9.19 through 9.24). As a result, they have long been classified among astrocytomas.[29] The frequency of symptomatic examples in

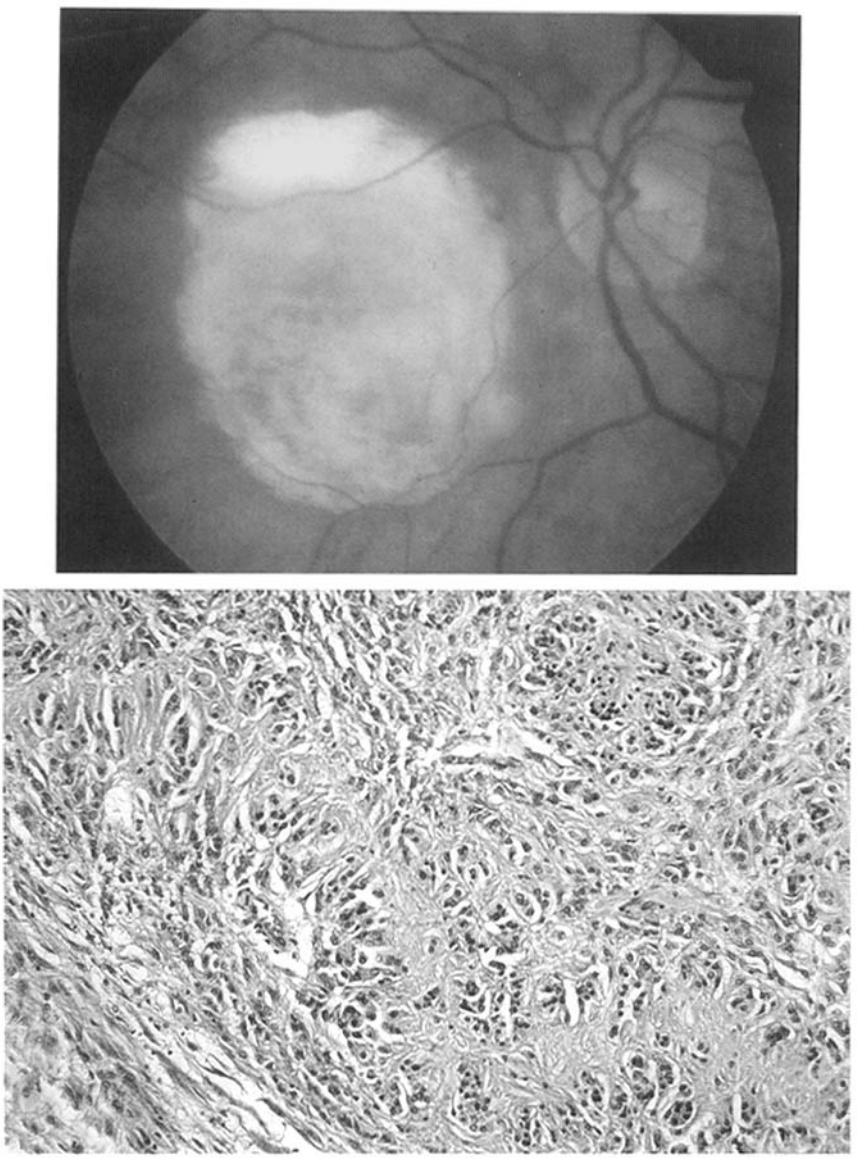


FIGURE 9.18. Astrocytic hamartoma of the retina in TSC. Such lesions may be conspicuous on funduscopy (*top*). Histologically (*bottom*), they consist of spindle and epithelioid astrocytelike cells identical to those of SEGAs (see Figs. 9.15, 9.17, 9.19 through 9.29).

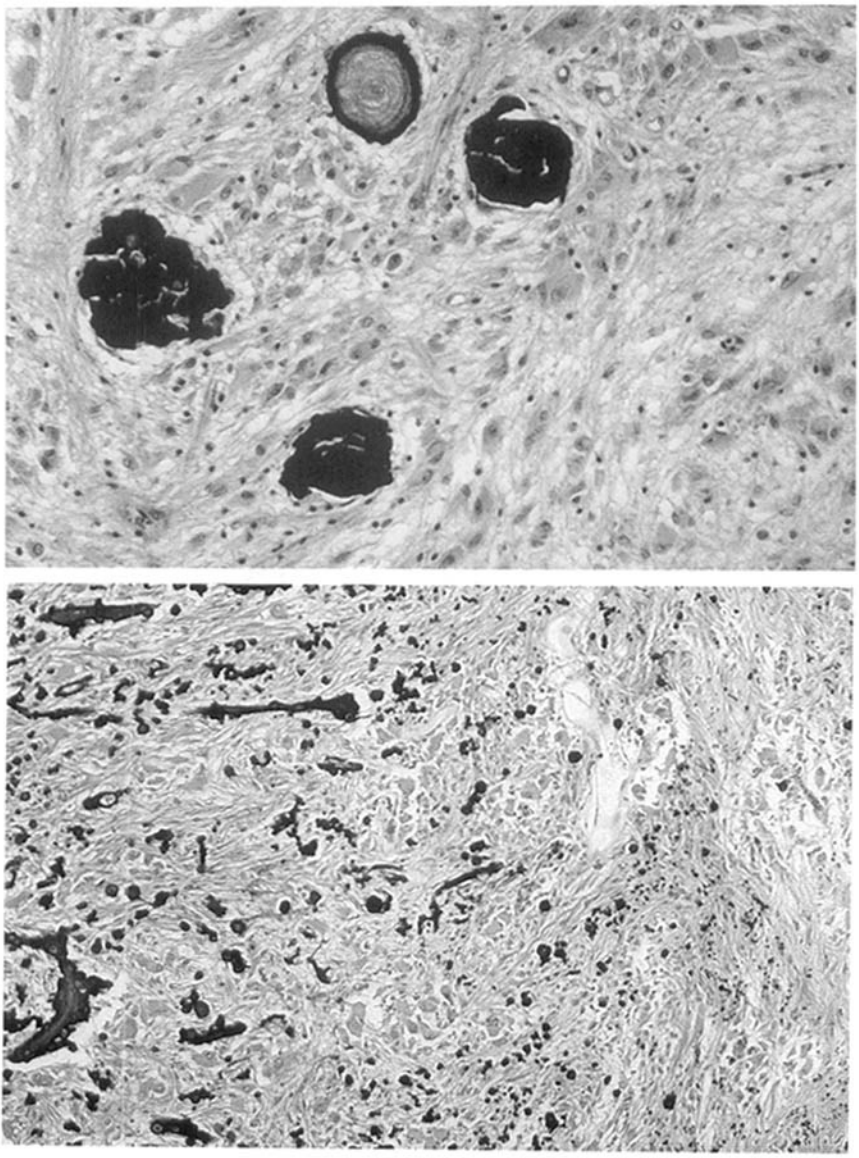


FIGURE 9.19. SEGA. Calcification varies greatly, ranging from spherical and lamellated (*top*) to perivascular "pipestem" or droplet deposits (*bottom*).

the Mayo Clinic series of TSC patients was approximately 6%[19]; it is of note that no such lesions were encountered outside the setting of TSC. Ostensibly non–TSC-associated SEGA have been reported, however, some being said to show subtle morphologic differences from syndrome-related SEGAs.[13,30,31] The question always remains whether these often pre–magnetic resonance imaging era cases were sufficiently studied to exclude

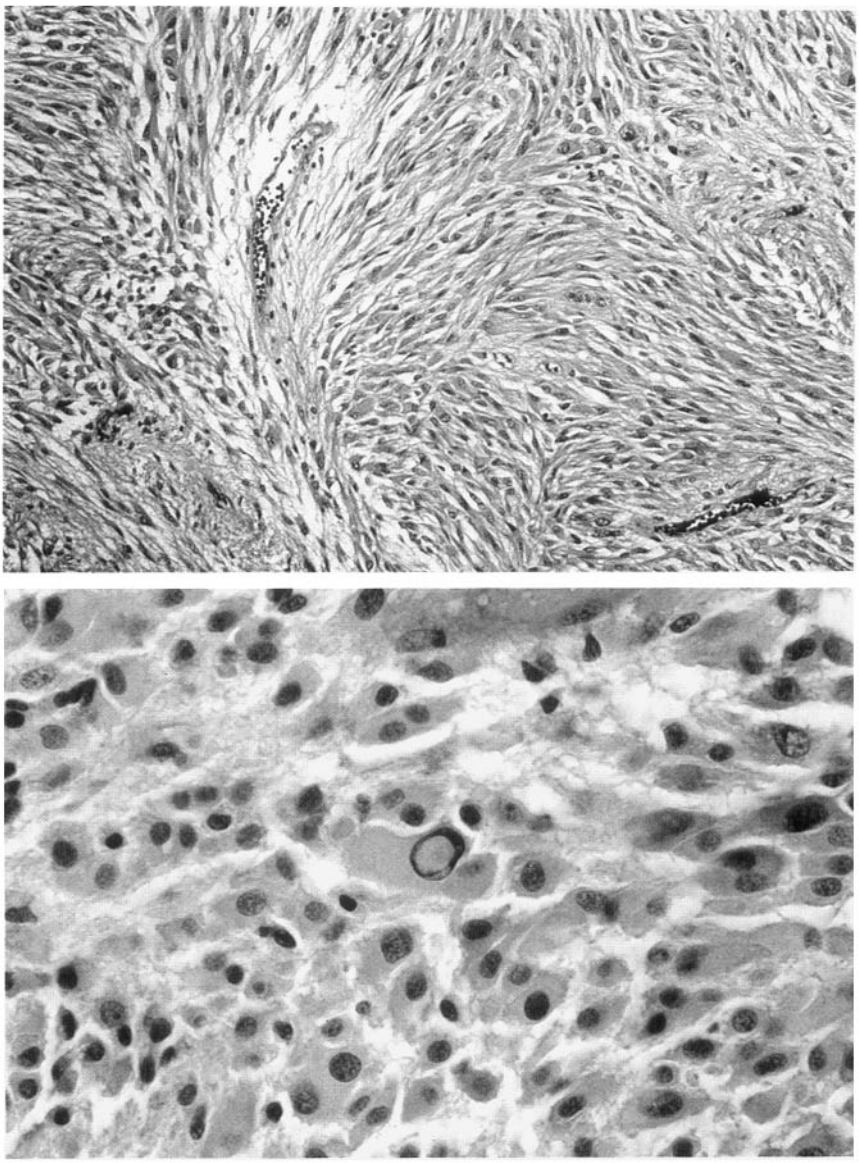


FIGURE 9.20. SEGA. The essential features of the tumor are its composition of spindle cells, often appearing to sweep from vessels in a vague pseudorosette manner (*top*), as well as epithelioid cells (*bottom*). Nuclear pseudoinclusion formation is a common finding (*bottom*).

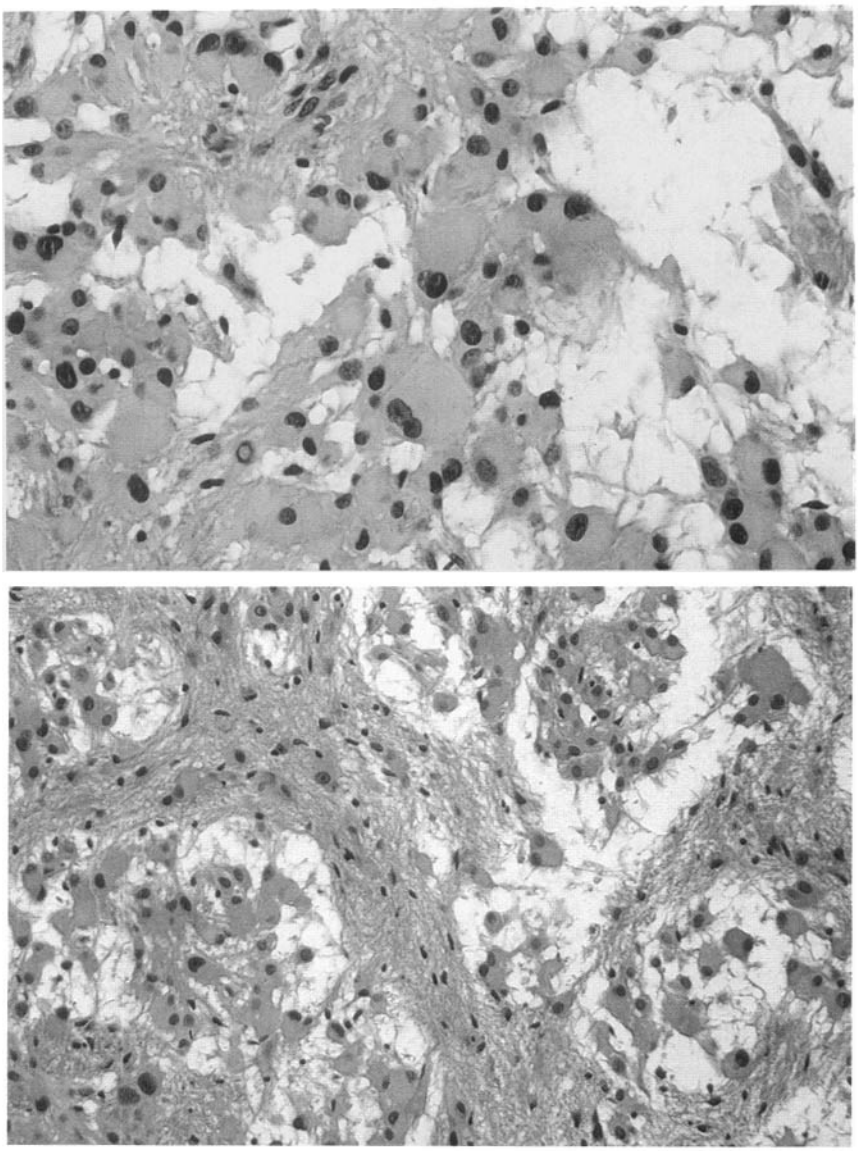


FIGURE 9.21. SEGA. Epithelioid cells, whether patternless (*top*), perivascular (*top upper left*), or nested between bands of cells with aligned processes (*bottom*), generally do not assume giant proportions.

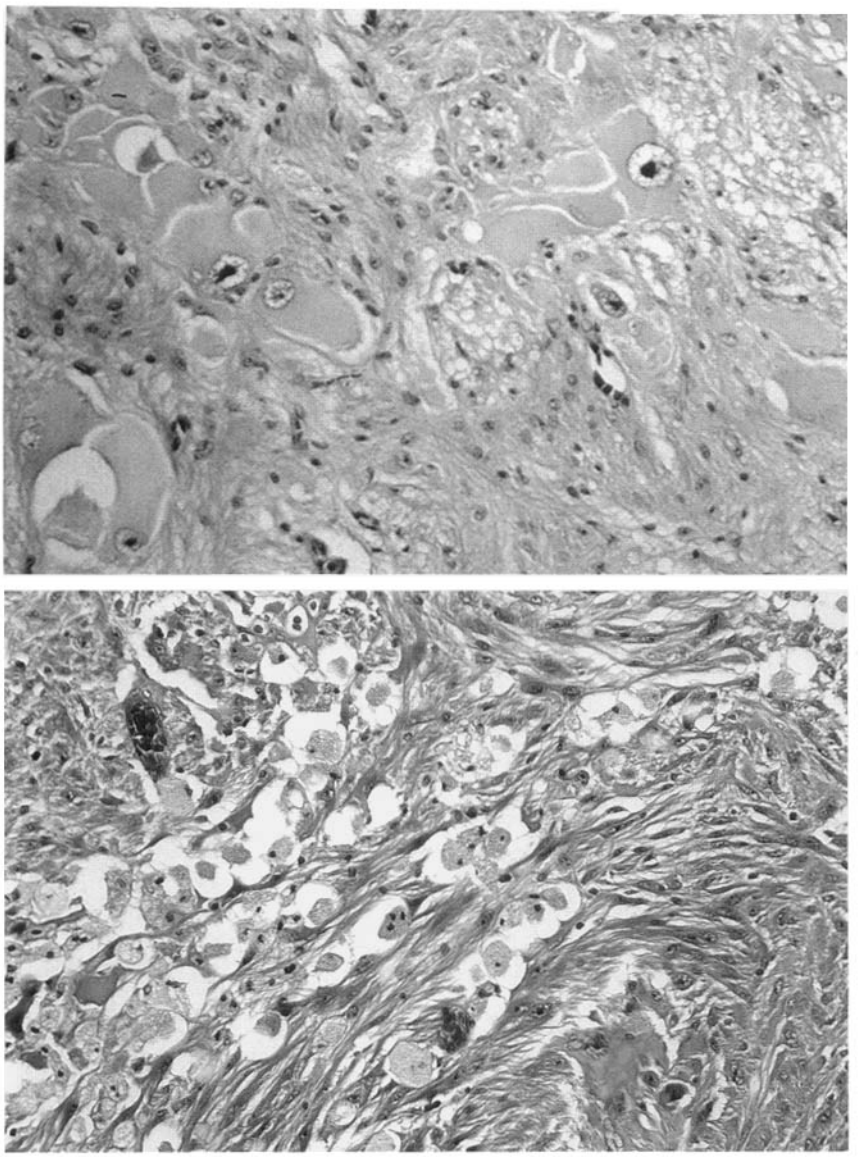


FIGURE 9.22. SEGA. Large SEGA cells occasionally show a remarkable resemblance to neurons (*top*, *bottom*). As a rule, however, they lack Nissl substance and show transition to small- and intermediate-sized epithelioid cells.

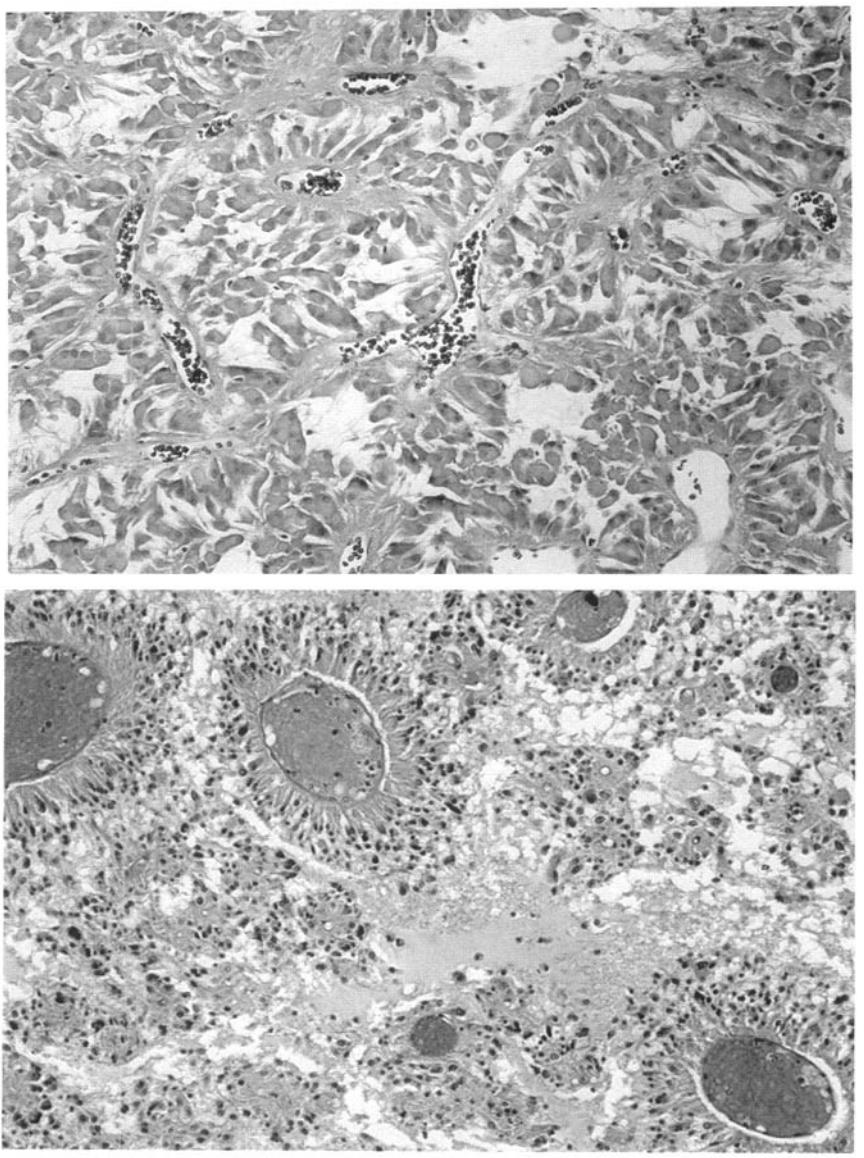


FIGURE 9.23. SEGA. Perivascular cell arrangement may range from subtle (*top*) to occasionally striking (*bottom*). Careful scrutiny of tissue between such ependymal-like rosettes reveals cells with the more typical orientation and cytology of SEGA.

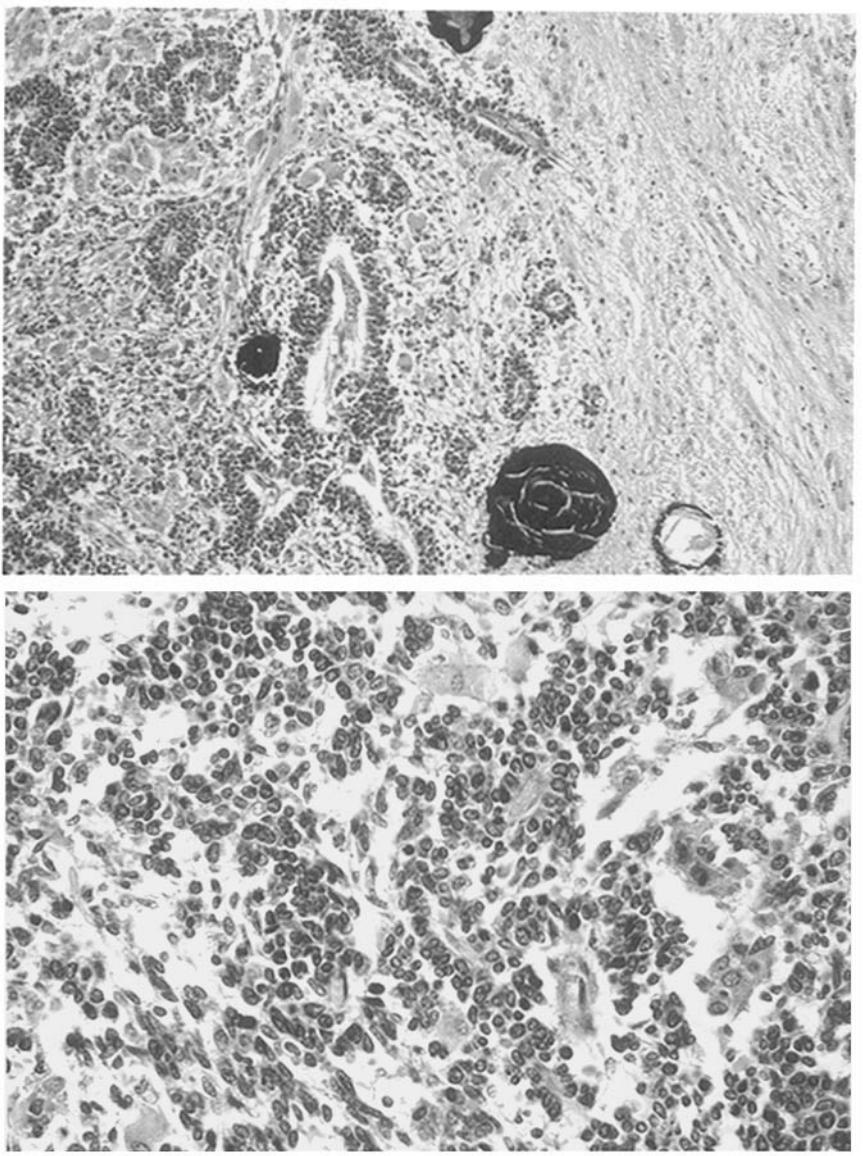


FIGURE 9.24. SEGA. Tumors with unusual histologic patterns, such as are illustrated in Figure 9.23 and this biphasic lesion (*top*) with a small-cell, perivascular component (*bottom*), engender occasional misdiagnoses.

subtle signs of TSC, and whether a thorough clinical workup was undertaken by an experienced clinician.

Short of the more frequent finding of calcification in subependymal nodules, the latter and SEGAs are histologically identical (Figs. 9.14 and 9.19). Their cells, although variable in size and often large, are rarely giant in proportion. Rather, the cells are polygonal, spindle, or epithelioid in appearance (Figs. 9.20 through 9.22). Because of their pink cytoplasm, the majority resemble large, plump astrocytes. Cells resembling neurons are less frequent. As previously noted, SEGAs exhibit a variety of histologic patterns. In the Mayo Clinic series of 20 histologically verified, TSC-associated SEGA, cell arrangements include aligned spindle cells sweeping from vessels (Fig. 9.20), clusters of epithelioid or ganglionlike cells, and giant cells with prominent nucleoli[19] (Fig. 9.22). An unusual finding was that of cells arranged in pseudorosettes resembling those of ependymoma (Fig. 9.23 *top*). Indeed, SEGAs featuring well-formed pseudorosettes are occasionally misdiagnosed as ependymoma.[29] Small cell components are rarely seen (Fig. 9.24). On occasion, the vascular stroma of SEGAs is sufficiently prominent as to lend an angiomatous appearance. Vascular hyalinization and calcification (Figs. 9.19 and 9.24) no doubt contribute to the rare occurrence of spontaneous intratumoral hemorrhage.[20] Despite morphologic diversity, when taking clinical and radiographic data into consideration, the histologic diagnosis of SEGA generally poses no problem.

The cytology of SEGAs has also been the subject of study[32] (Fig. 9.25). Their usually single nuclei vary from round to spindle shaped and from somewhat vesicular to hyperchromatic (Figs. 9.20 and 9.21). Nucleoli may be prominent (Fig. 9.22). Few are convoluted or frankly bizarre. Eosinophilic nuclear inclusions representing invaginations of cytoplasm are a common feature[33] (Fig. 9.20). Various other nuclear inclusions of uncertain composition and significance have also been described.[34–36] Mast cells are often present in varying number.

The issue of malignancy in SEGA has been largely settled. Occasional mitotic figures may be seen, but endothelial proliferation or necrosis is uncommonly encountered (Fig. 9.26). Unlike in diffuse, fibrillary astrocytomas, wherein these features correlate with rapid growth or malignancy,[37] limited expression of these features in SEGA may not imply a less favorable prognosis.[19,30] Indeed, the large, systematically studied Mayo Clinic series found that cytologic atypia (80%), mitotic activity ranging from one to slightly greater than five mitotic cells per high-power field (47%), endothelial proliferation (13%), and even focal necrosis (6%) did not affect the prognosis (Fig. 9.27). The same lack of correlation appears to be true of proliferation marker labeling indices, which range from low[21,38] to moderate (B.W.S., unpublished data). As a rule, SEGAs exhibit a diploid DNA pattern; no aneuploidy was observed in the 1991 study of Shepherd et al.[19]

Immunohistochemistry The histologic and immunohistochemical similarity of SEGA and of the far more common subependymal nodule is readily

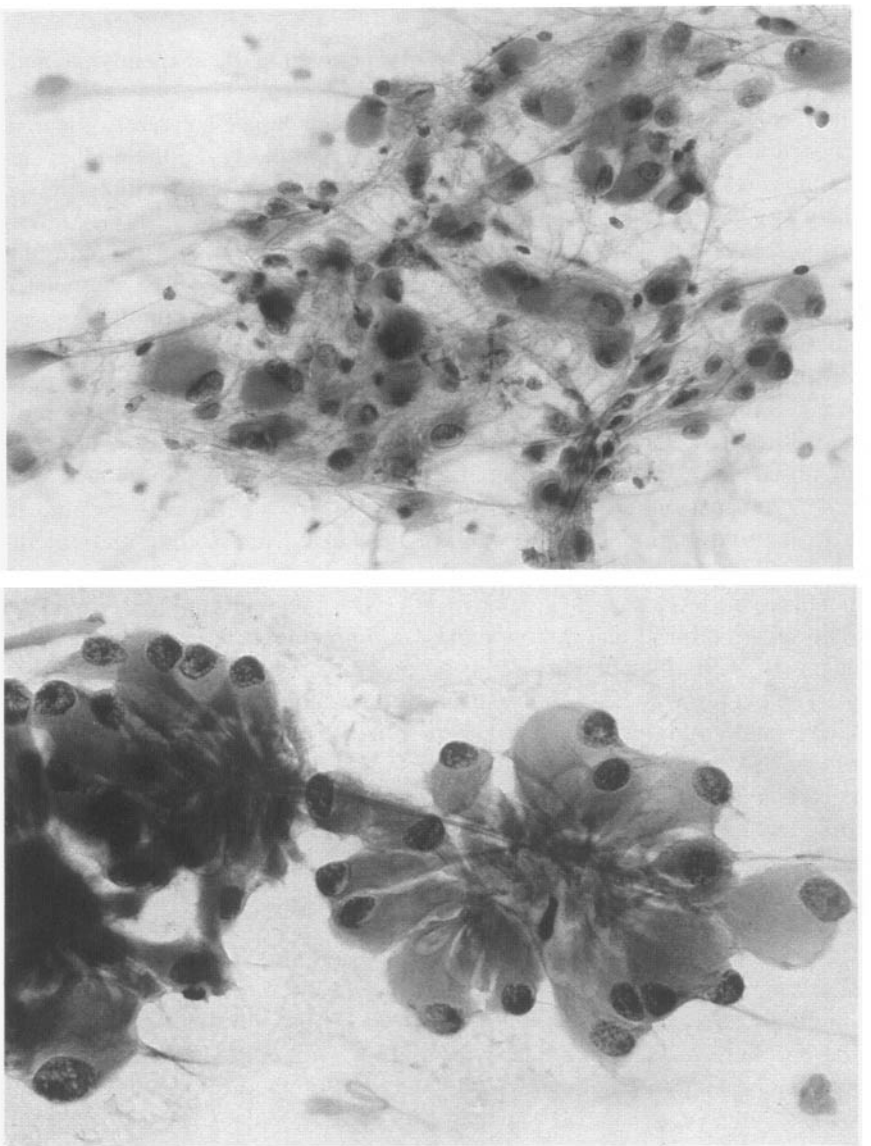
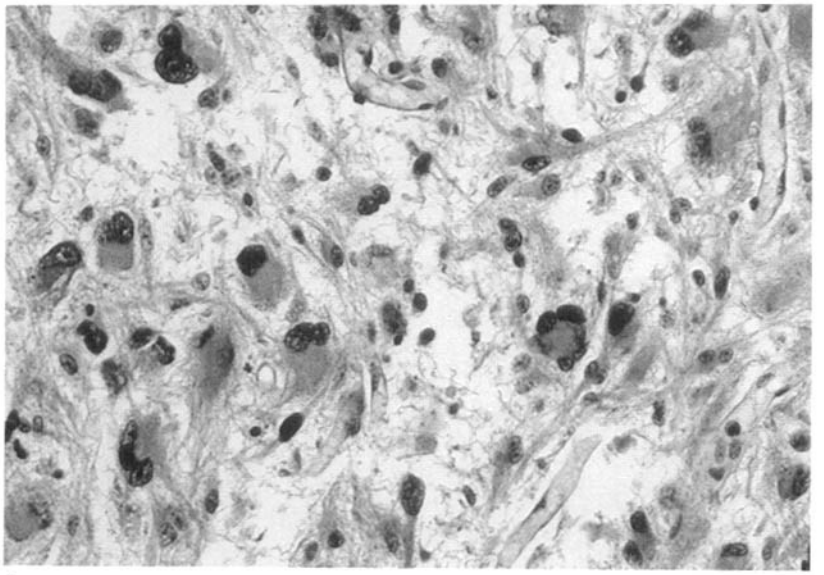
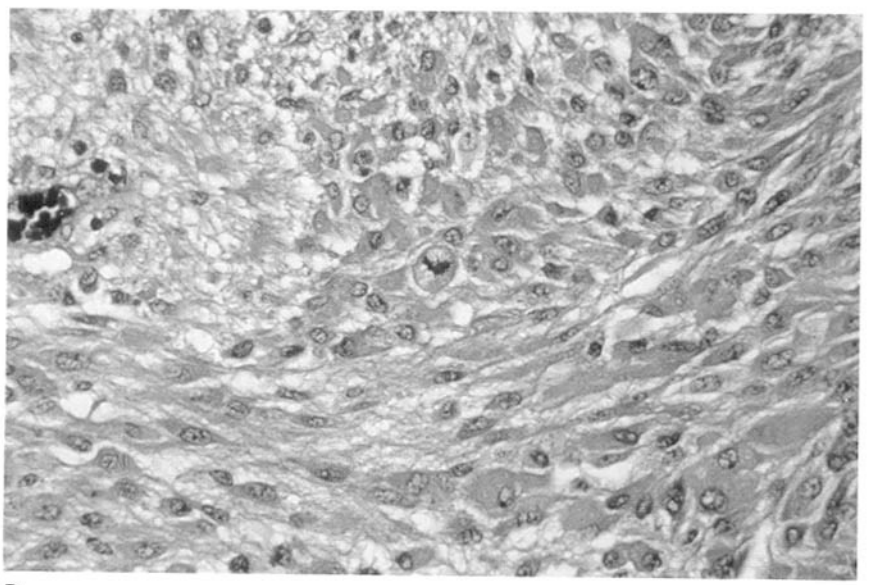


FIGURE 9.25. SEGA cytology. Smear preparations show the epithelioid cells to possess eccentric nuclei, often delicate chromatin, moderate quantities of pink cytoplasm, and limited process formation (*top*). In tumors featuring perivascular pseudorosettes (see Fig. 9.23), somewhat tapered cell bodies are seen to radiate toward a center (*bottom*).

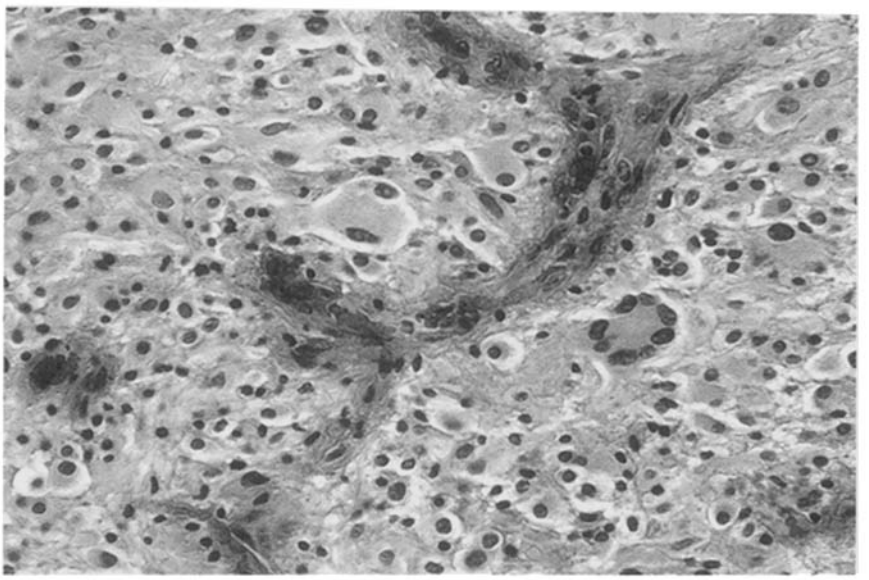


A



B

FIGURE 9.26. SEGA. Features such as nuclear atypia (*A*), limited mitotic activity (*B*), endothelial proliferation (*C*), and necrosis (*D*), which are indicative of aggressive behavior in diffuse or fibrillary astrocytomas, appear to be of no prognostic significance in SEGA. *Illustration continued on following page*



C

D

FIGURE 9.26. (*continued*)

explained by their common histogenesis. Early immunohistochemical studies of SEGAs varied somewhat in their conclusions, reporting glial,[39,40] neuronal,[41] or combined glioneuronal[13,15,42–45] differentiation (Figs. 9.28 and 9.29). Two more recent, exhaustive studies, one of SEGAs[43] and another of both tubers and SEGAs,[13] found not only immunohistochemical and ultra-

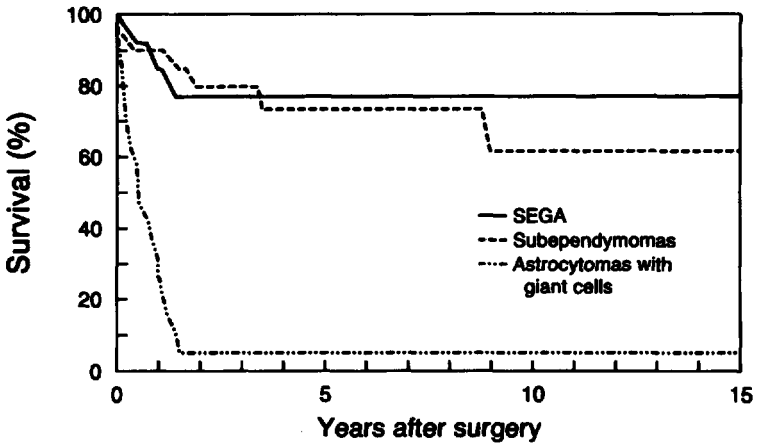


FIGURE 9.27. SEGA. Survival of patients operated for SEGA is comparable to that for subependymoma and differs markedly from that of patients with diffuse astrocytomas containing giant cells. (Reproduced with permission from Shepherd et al.[19])

structural evidence of divergent glioneuronal differentiation in SEGAs, but similarities between the large pink cells of both lesions.[13] In the study of Hirose et al.,[13] the large cells of tubers were positive for vimentin (100%), GFAP (77%), S-100 protein (92%), neurofilament protein (38%), and class III β-tubulin (77%). SEGAs showed a similar immunoprofile, including vimentin (100%), GFAP (50%), S-100 protein (100%), neurofilament protein epitopes (33%), class III β-tubulin (83%), and calbindin (67%). The presence of α-B-crystallin, a component of lens protein and a glial marker, has also been demonstrated.[46] Interestingly, immunoreactivity for neurotransmitter substances (somatostatin, *met*-enkephalin, 5-hydroxytryptamine, β-endorphin, neuropeptide Y) was frequently seen in SEGAs but not in tubers. At the immunohistochemical level, therefore, neuronal differentiation appears to be more fully expressed in the pink cells of SEGAs than in those of tubers.

Ultrastructure Despite early reports of glial differentiation in SEGAs,[47] more recent ultrastructural studies of SEGAs have generally demonstrated both glial and neuronal features.[13,15,45] The study of Hirose et al.,[13] as well as those of Bender and Yunis[15] and of Jay et al.,[14] found the large pink cells of both tubers and SEGAs to contain numerous intermediate filaments, frequent lysosomes, and occasional rectangular or rhomboid, membrane-bound crystalloids exhibiting lamellar, 7-nm periodicity[14] (Figs. 9.30 and 9.31). Some SEGA cells also showed features suggesting neuronal differentiation, including stacks of rough endoplasmic reticulum, well-developed Golgi complexes, secretory vesicles, occasional dense-core granules, and microtubule-containing processes (Figs. 9.32 and 9.33). Immunoelectron-

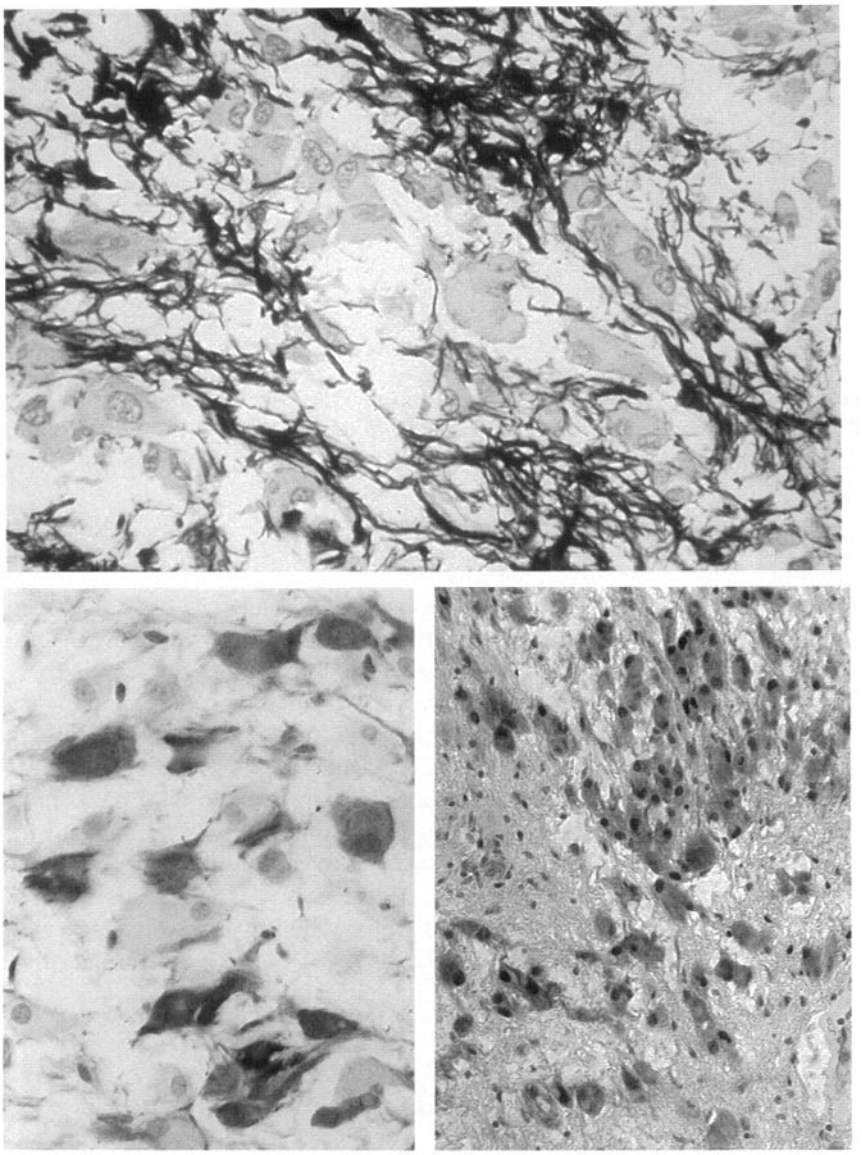


FIGURE 9.28. SEGA glial immunotype. Whereas some tumors show GFAP staining to be limited to processes (*top*), others demonstrate occasional cells with cytoplasmic positivity (*bottom left*). Widespread reactivity is uncommon (*bottom right*).

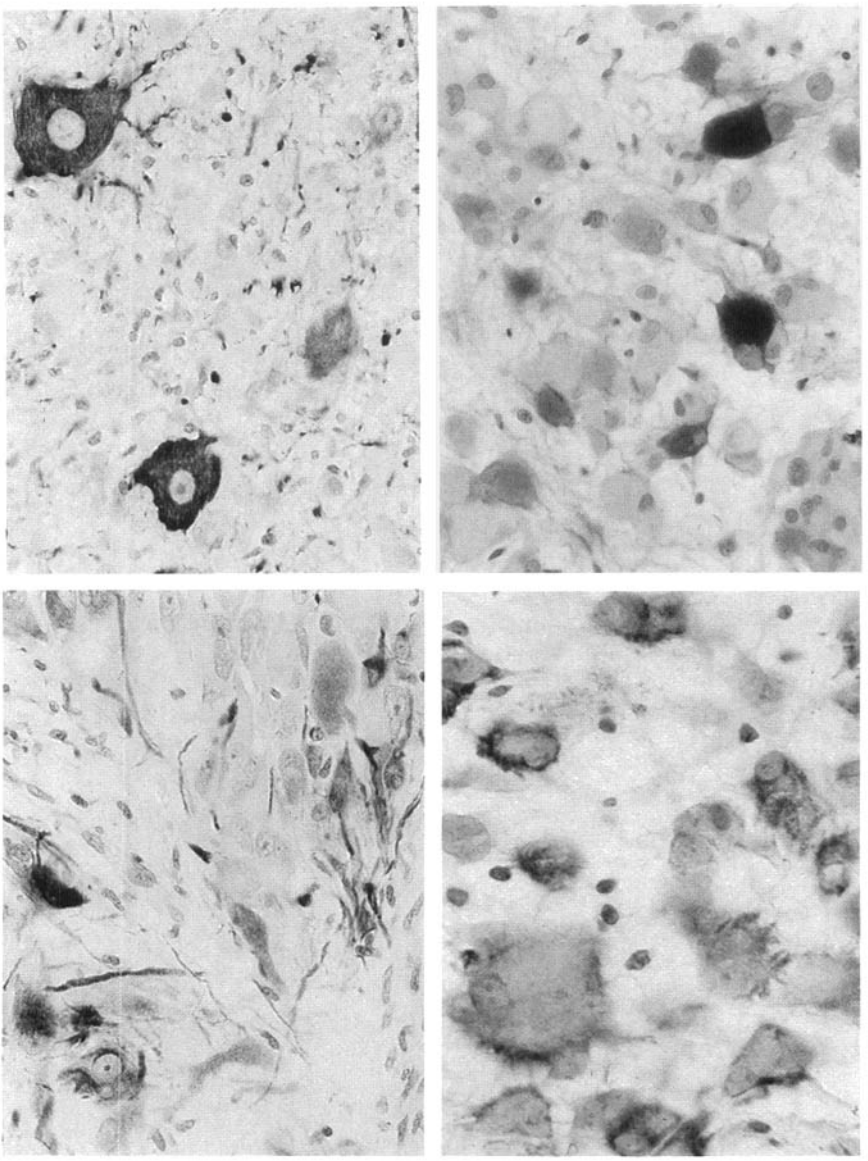


FIGURE 9.29. SEGA. The neuronal phenotype of SEGA cells includes immunostaining for neurofilament protein (*top left*), tubulin (*top right*), calcium-binding protein (*bottom left*), and somatostatin (*bottom right*).

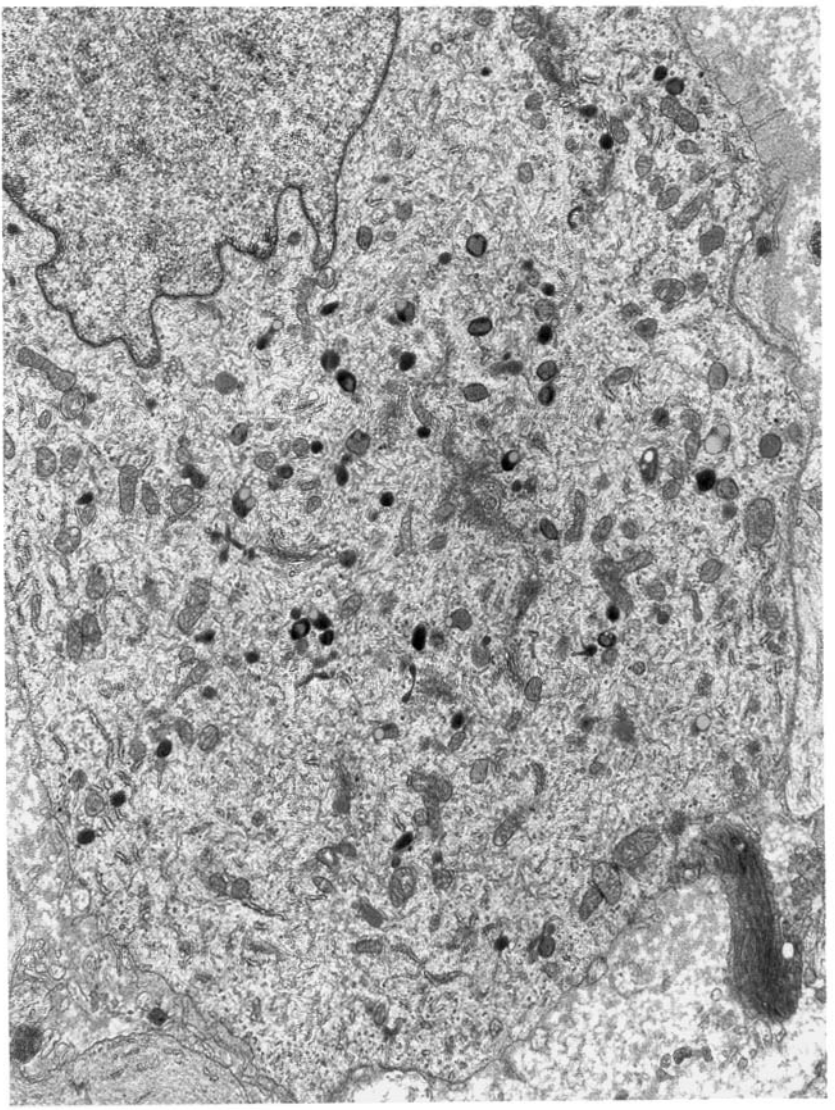


FIGURE 9.30. SEGA. A large tumor cell with primarily astrocytic features shows delicate chromatin, lack of a nucleolus, and simple organelles, including scant, single profiles of rough endoplasmic reticulum, scattered mitochondria, small numbers of despersed intermediate filaments, and occasional dense lysosomes. (×7500)

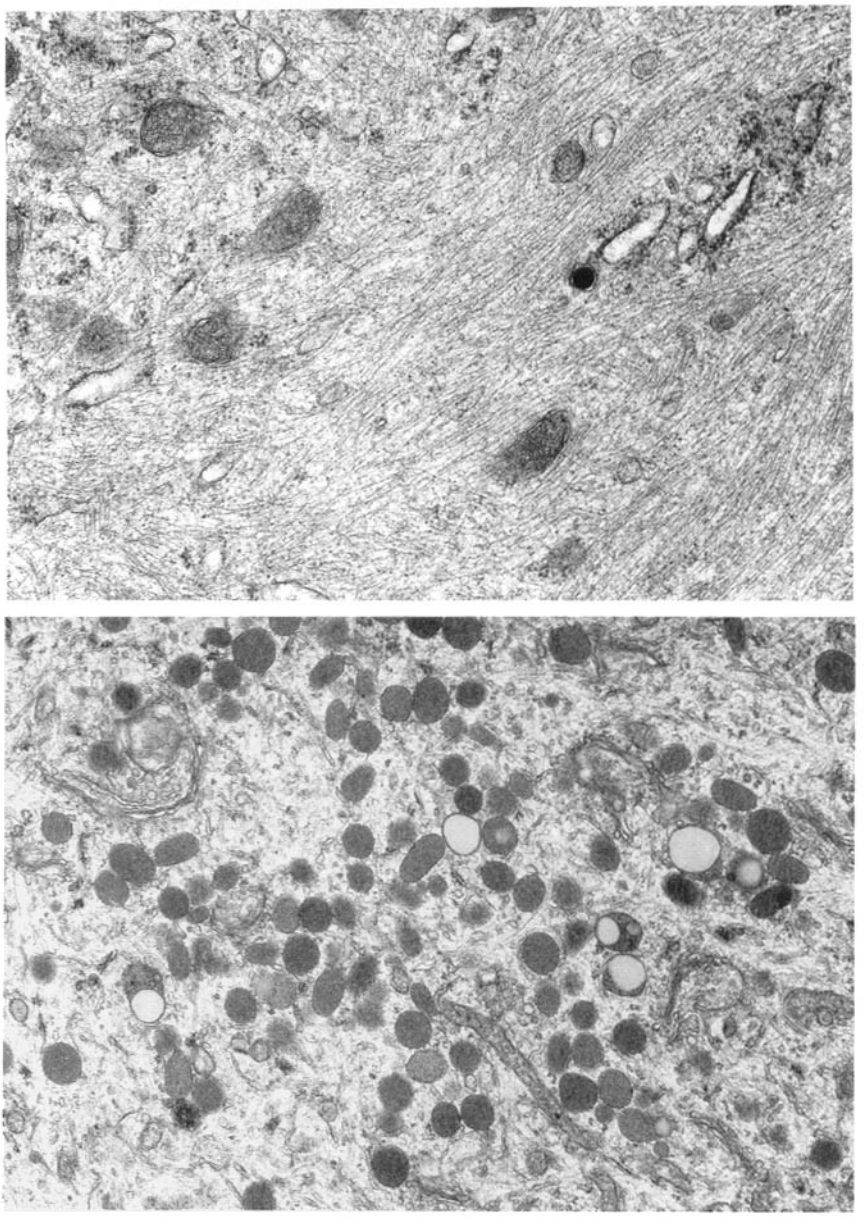


FIGURE 9.31. SEGA. Abundance of intermediate filaments (*top*) and of lysosomes (*bottom*) is occasionally seen. Note dense-core granule in association with glial filaments, an indication of mixed differentiation (*top*). (*Top*, ×30,000; *bottom*, ×12,500)

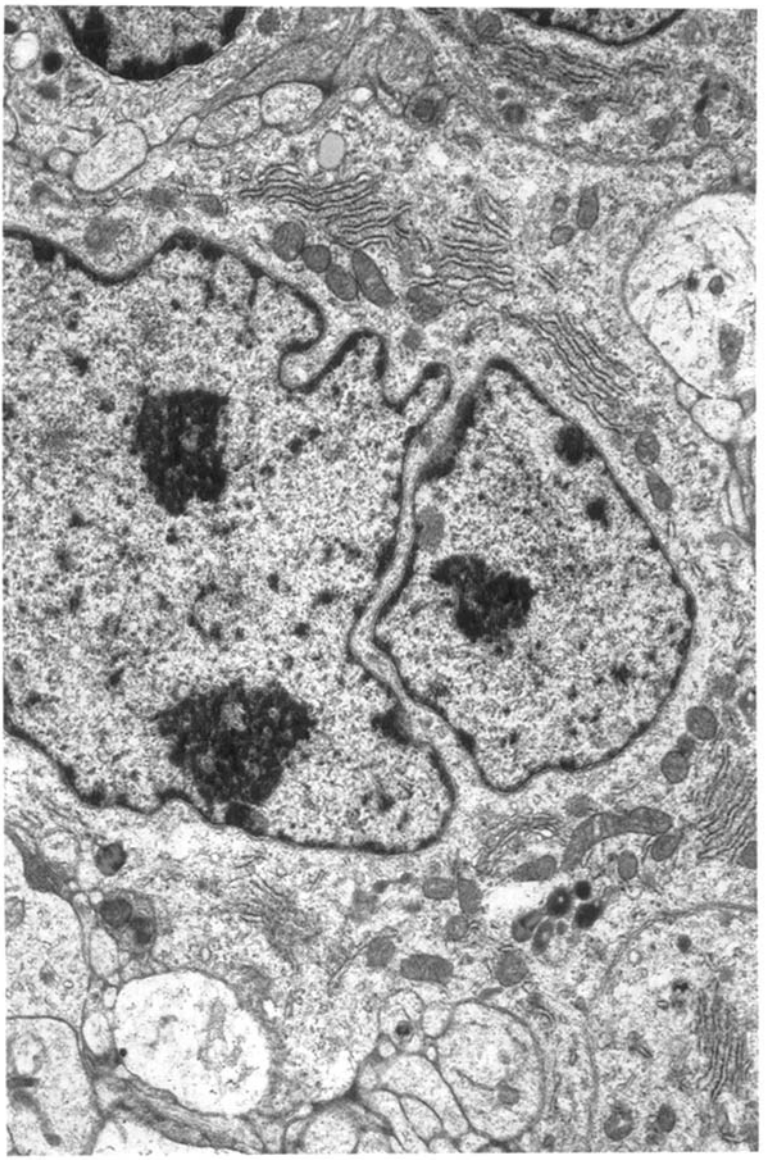


FIGURE 9.32. SEGA. Tumor cells occasionally exhibit stacks of rough endoplasmic reticulum, a feature suggestive of neuronal differentiation. (×25,000)

microscopic studies for GFAP and somatostatin also indicated glioneuronal differentiation in SEGAs[13] (Fig. 9.34).

On the basis of the above-noted studies, it may reasonably be concluded that SEGAs, despite their predominantly astrocytic phenotype, often exhibit characteristics of neurons. Whether these findings should[19] or should not[48] be viewed as bona fide evidence of divergent differentiation, evidence com-

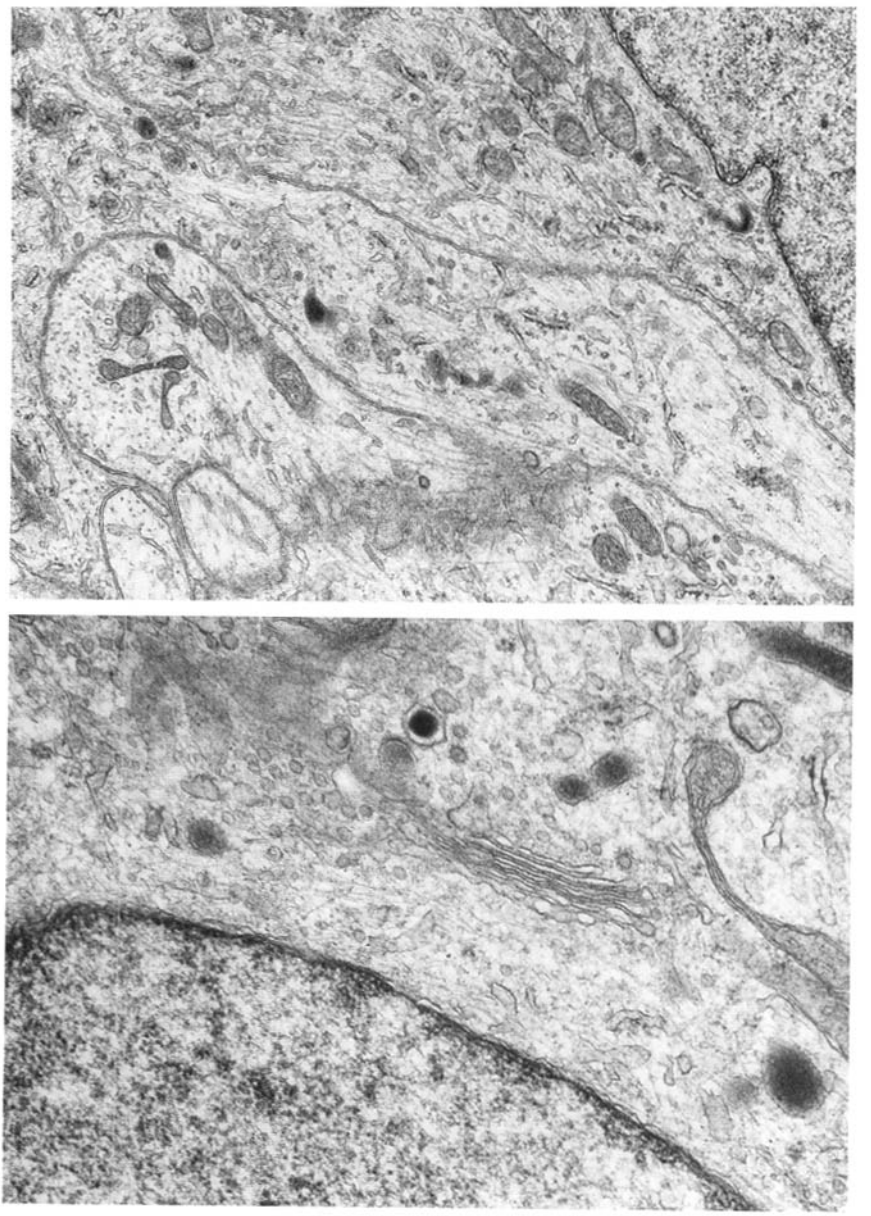


FIGURE 9.33. SEGA. Features suggestive of neuronal differentiation also include long, microtubule-right cell processes (*top*) and occasional dense-core granules, here seen in association with a Golgi complex (*bottom*). (*Top*, ×15,750; *bottom*, ×25,000)

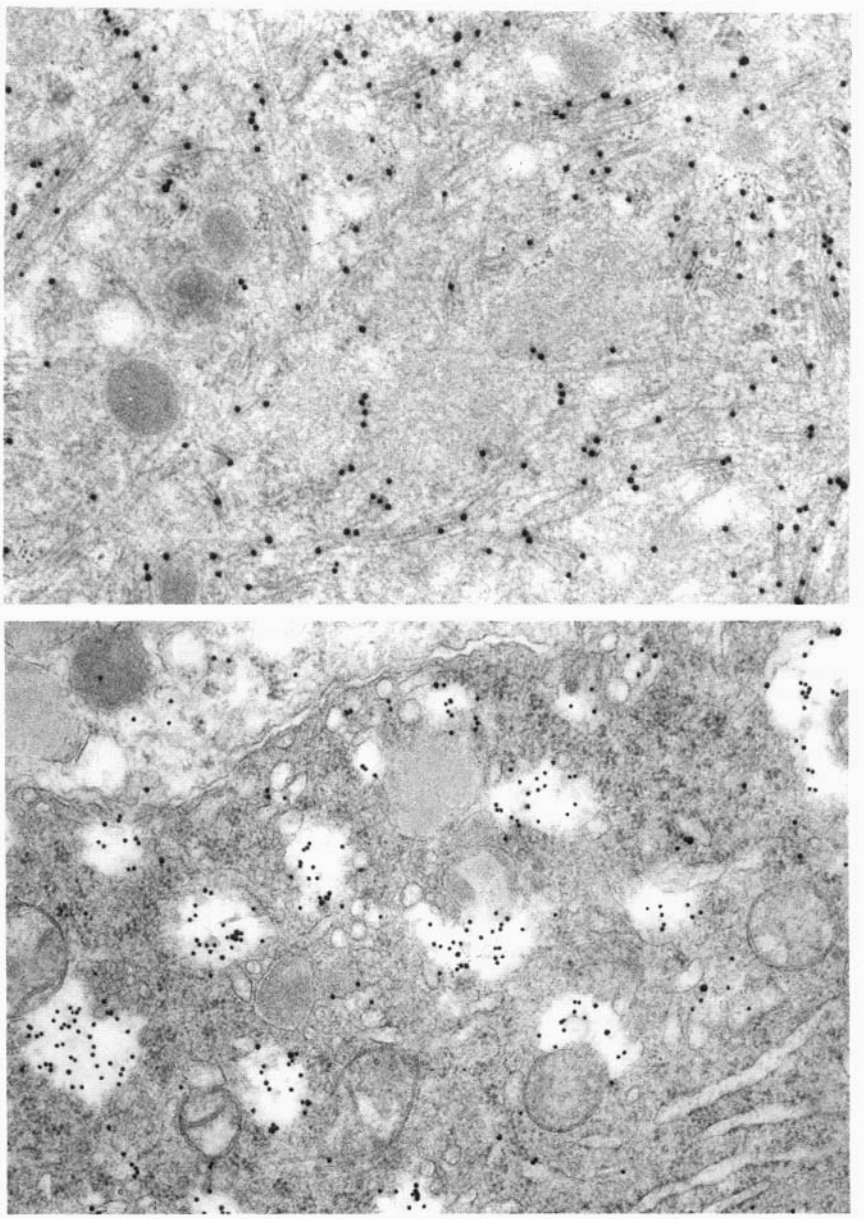


FIGURE 9.34. Cortical tuber and SEGA. Immunoelectron microscopy using the protein A–gold technique shows GFAP labeling of intermediate filaments in a giant cell of a cortical tuber (*top*), as well as somatostatin labeling of irregular, electron-lucent spaces in the cytoplasm of a SEGA cell (*bottom*). (*Top* and *bottom*, ×50,000)

pelling enough to justify use of the alternative designation "subependymal giant-cell tumor" is unsettled.

Prognosis

As previously noted, the occasional finding of histologic features suggestive of malignancy in SEGA may be of little or no prognostic significance. The issue of whether these tumors recur at an appreciable rate is also unclear. We believe that recurrence of SEGAs is both uncommon and linked to their extent of resection. Certainly the reported recurrence rate of 10%[49] greatly exceeds that of the Mayo Clinic experience. Partly explaining this high estimate may be the emergence of separate, new lesions. No doubt this accounts for some "recurrences" reported prior to the availability of modern neuroimaging techniques. For example, we have documented the evolution of three separate, spatially distinct SEGAs arising in one patient over a period of 3 years. In one case, the low rate at which SEGAs are said to recur is not predictable on the basis of their morphology.

Miscellaneous

Convincing examples of other types of CNS neoplasms arising in the setting of TSC are extremely rare. Their occurrence is probably coincidental. Examples include schwannoma,[50] ganglioglioma,[51] oligoastrocytoma,[52] gliomatosis,[35] malignant astrocytoma,[53] glioblastoma,[54] medulloblastoma,[23] (B.W.S., unpublished data), and hemangioma.[49,55] A single case of TSC associated with a paratesticular neuroblastoma has also been reported.[56]

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Ophthalmic Findings

In 1905, 3 years before Vogt[1] defined the well-known triad of tuberous sclerosis complex (TSC), Campbell[2] mentioned its ocular pathology. Berg[3] in 1913 and Schuster[4] in 1914 also described those pathologic findings in the eyes, but it was van der Hoeve,[5–10] in a series of papers written between 1920 and 1937, who gave significance to the retinal findings. In 1921, he described six patients with retinal tumors observed by direct ophthalmoscopy.[6] Despite the relative rarity of TSC, there are many reports in the medical literature with ophthalmologic descriptions that add little to van der Hoeve's original contributions. Retinal and optic nerve involvement are well known today because they constitute the most frequent ophthalmic manifestation of TSC, being present in approximately half of the patients with this disease.[11,12]

Nonretinal Ophthalmic Findings

While so-called adenoma sebaceum may involve the eyelids of patients with TSC,[13] and ciliary poliosis may occur,[14] the external eye is otherwise only rarely affected. Nonretinal findings are uncommonly reported (Table 10.1). Epicanthal folds were reported in two of eight patients examined by Chao.[15] Luo[16] described small, smooth, flat or pedunculated tumors of the conjunctivae that were gray-white in color and approximately 4 mm in diameter in one patient. Both keratoconus[17] and megalocornea[18] have been observed. Thomas et al.[19] reported band keratopathy with subepithelial vascularization and lens opacities. Corneal changes in the form of pannus[20] and band keratopathy have also been described by others.[21,22] Andreani[23] reported a tumor of the ciliary body. The reported disorders of eye motility include nonparalytic strabismus,[9,15,24–26] third and sixth nerve paresis,[26–29] and nystagmus.[26,30] Although primary glaucoma has rarely been observed,[31] secondary

TABLE 10.1. Nonretinal Ophthalmic Lesions Reported with TS[a]

Ocular Tissue	Lesions
Eyelid	Adenoma sebaceum, vitiligo, poliosis, ptosis, epicanthic folds
Conjunctiva	Gray with flat-surfaced pedunculated tumor
Cornea	Megalocornea, keratoconus, band keratopathy, pannus, posterior embryotoxon
Anterior chamber angle	Glaucoma
Iris	Rubeosis, iridocyclitis, coloboma, sector depigmentation
Ciliary body	Neoplasm
Choroid	Coloboma, angioma
Lens	Subluxation, cataract, coloboma
Pupil	IIIrd nerve paresis
Vitreous	Hamartomatous fragments, hemorrhage, pseudoglioma
Globe	Enophthalmos, myopia, phthisis
Extraocular muscles	Nonparalytic strabismus, paralysis of cranial nerves III and VI, nystagmus

[a]Adopted from Archer and Nevin.[37]

glaucoma has been observed from both rubeosis iridis complicating iridocyclitis[32] and vitreous hemorrhage.[33–35] The case reported by Nicholson and Green[36] was later published by Atkinson et al.[33] and again by Archer and Nevin.[37] Sector depigmentation of the iris, reported by several authors,[38,39] may be the ocular counterpart of the ash leaf–shaped cutaneous lesion. Colobomas of the iris, lens, choroid, and retina have been reported,[18,25,40–42] and cataracts[17,33,42–44] have been frequently described. Vitreous seeding,[5,9] clouding,[19,45] and invasion by pseudoglioma[29,32,46] have also been recognized. Phthisis bulbi is rare.[47]

The Mayo Clinic Experience

A review of 139 patients with TSC seen at the Mayo Clinic revealed a number of nonretinal ocular findings that are summarized in Table 10.2. The papilledema observed in five patients was due to chronic intracranial hypertension secondary to ventricular obstruction from an intracranial tumor. Except for one patient who had unilateral optic atrophy and papilledema in the opposite eye (Foster-Kennedy syndrome), the papilledema was always bilateral. Optic nerve pallor subsequent to papilledema was found in three patients.

Fundus Findings

Among the Mayo Clinic patients with TSC in whom an adequate fundus examination was recorded, approximately half (68 of 139) were found to

TABLE 10.2. Nonretinal Ophthalmic Manifestations in Mayo Clinic Series (139 Patients)

Ocular Findings	No. Patients
Coloboma iris, lens, choroid	1
Cataract	1
Choroidal nevus	1
Anomalous discs	
Structurally full	6
Tilted	1
Papilledema	5
Optic nerve strophy	4
Field defects	
Homonymous hemianopsia	4
Arcuate scotoma	1
Central scotoma associated with optic nerve atrophy	1
Inferior temporal defect associated with retinal hamartoma	1
Nonparalytic strabismus	6
Paresis of VIth nerve	1
Nystagmus	1
Vitreous hemorrhage	1

have hamartomas of the retina or optic nerve (Table 10.3). Although there are variations in the appearance of the hamartoma,[12,30,32,37,43,44,46,48–54] two basic morphologic types are generally recognized.

Noncalcified Tumor

The most common type of retinal hamartoma is a lesion that is relatively flat, smooth surfaced, salmon to salmon-gray in appearance, semitransparent, and circular or oval shaped with indistinct boundaries[12] (Plate 10.1). In many instances the color of these lesions is not noticeably different from that of the fundus background. They may be first noticed by the appearance of a circular light reflex surrounding the tumor. Further study will demonstrate the subtle elevation of these tumors and a slight decrease in the visibility of the underlying tissue. They are frequently located superficial to a retinal vessel, usually an artery, and are best found by searching along the vessels as they course from the disc toward the periphery. In this manner they can be identified when a retinal vessel appears to be interrupted or partially obscured. Uncommonly, the tumor may be located primarily in the deeper part of the retina, in which case the superficial retinal vasculature will not be obscured. Either single or multiple, these tumors are usually located in

TABLE 10.3. Fundus Findings in Mayo Clinic Series (139 Patients)

Type of Lesion	No. of Patients
Hamartomas	68[a]
Flat, smooth-surfaced semitransparent lesions	39
Multinodular lesions with calcium	34
Hamartomas having characteristics of both of the above lesions	8
Pigmentary changes	18[b]
Punched-out chorioretinal defects with or without adjacent pigment clumping	
Plaque-like lesions in deep retina	
Pigment clumping	
Isolated black drüsen	
Vascular changes	4[c]
Sheathing; dilated tortuous vessels, double arterial supply	

[a]>49%; [b]13%; [c]3%.

the posterior pole. These lesions were found in more than half of the Mayo Clinic patients who had ocular involvement (39 of 68 patients).

Calcified Mulberrylike Tumor

The second type of retinal or optic nerve tumor is the classic, relatively easily recognized, elevated multinodular lesion resembling grains of tapioca, salmon eggs, or mulberries (Plate 10.2). Von Herrenschwand[55] in 1929 and A. Vogt[56] in 1934 each likened the resemblance of these tumors to a mulberry because of their cluster of small granules, cysts, or refractile, glistening nodules. These lesions are most commonly located near or at the disc margin, although they may be seen in the midperipheral retina as well. When located at the optic disc, they may resemble hyaline bodies or giant drüsen, except that they tend to obscure the underlying retinal vessels. Their sizes range from 1/2 to 4 disc diameters, and they may be several millimeters thick. Occasionally, the retinal vessels course through the nodular structure, (Fig. 10.1), and sometimes the vessels within or adjacent to such tumors may be sheathed. Rarely they may be distributed along the vessels like a string of pearls.[57] The high acoustic density of such lesions may be demonstrated by B-scan ultrasonography, and the calcium may also be demonstrated roentgenographically.[12] This type of lesion was found in half of the patients with ocular involvement (34 of 68 patients).

Mixed Tumor

A third type of retinal lesion contains morphologic features of both of the two types described above (Plate 10.3). The base or border of this lesion

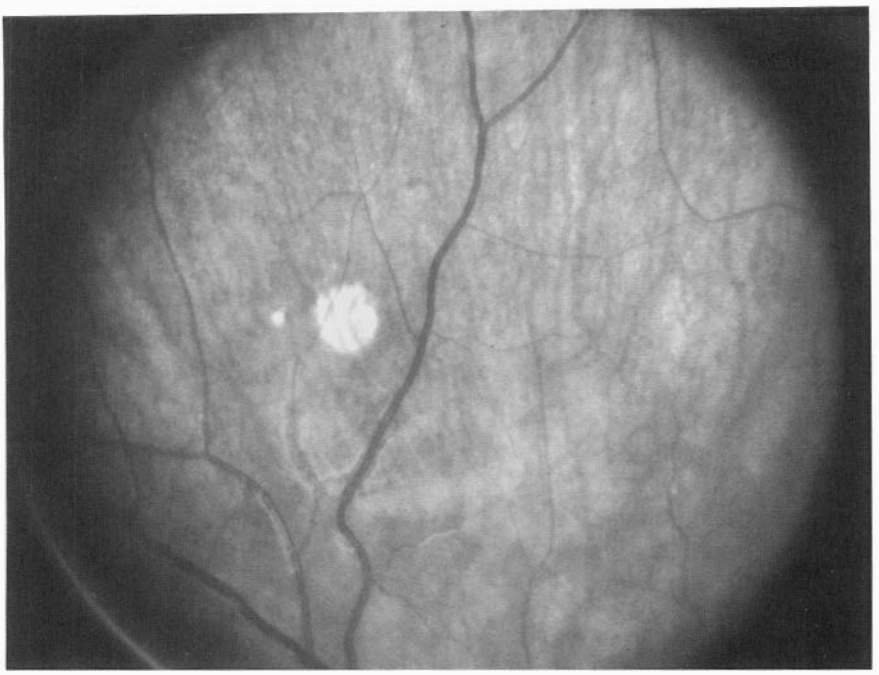


FIGURE 10.1. Punched-out lesion allowing visualization of underlying choroidal vessels.

has the appearance of a relatively flat, soft, and translucent hamartoma, whereas centrally it exhibits an elevated, nodular, and calcified appearance (Fig. 10.2). This mixed type was less commonly observed than the other two types (8 of 68 patients).

Bilateral lesions are seen in slightly less than half of those patients who have retinal involvement, and it is well known that various morphologic types may coexist in the same eye. Almost one third of TSC patients in the Mayo Clinic series have more than one variety of hamartoma. No conclusive correlation between the types of tumors and the age of the patient could be demonstrated. All the retinal tumors in this series have been of endophytic growth. Exophytic growth is rare.

Pigmentary Changes

Although contiguous pigmentary retinal changes, as noted by others,[53,58] were not observed around any of the three basic types of hamartomas in the Mayo Clinic patients, nor was there pigmentation within the tumors as recently reported by Shelton,[59] 18 patients exhibited some other type of retinal pigmentary disturbance. Relative depigmentation may be visible as an annulus around the tumor (Plate 10.3). Some eyes have depigmented lesions with a "punched out" appearance[60] (Fig. 10.1).

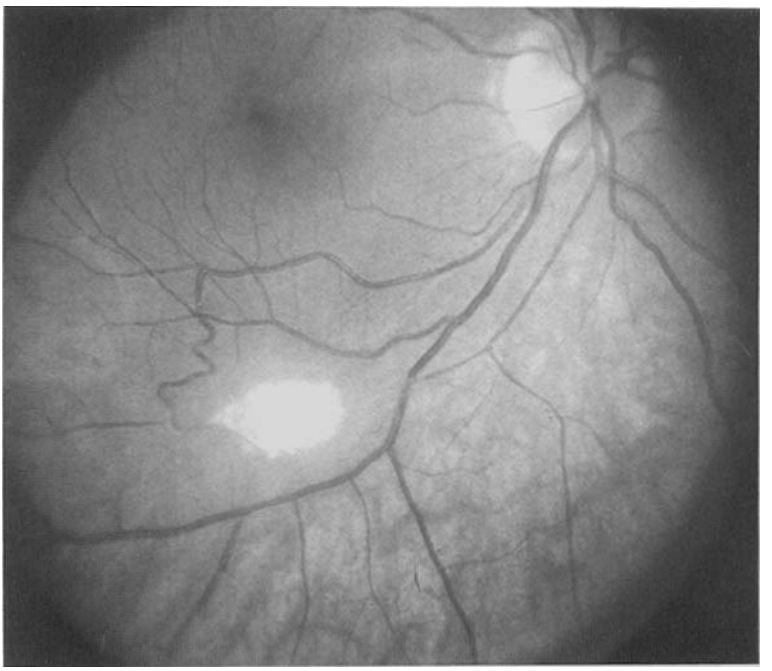


FIGURE 10.2. Mixed-type hamartoma with partial calcification centrally.

Evolution

Generally, the retinal lesions of TSC do not grow. Some authors have maintained that these lesions remain static.[61] Van der Hoeve,[5] however, observed repeated release of the tapiocalike granules into the vitreous in his initial observations on TSC. He also noted hemorrhages appearing and disappearing on and in the tumor mass. Falls[62] speculated that the typical grape or mulberry cluster hamartoma was "actually a late state of the lesion and that the early, or the initial picture, is that of a smooth, superficial, gliotic haze or sheet." On the basis of microscopic information and clinical impressions, other authors also have suggested that some tumors can develop into large multinodular lesions and undergo cystic, hyaline, and calcific changes.[8,63] Some reports indicated that the lesions increase in size and number with time,[7,64] but in some instances lesions that were present may have been overlooked and found only after repeated examinations. Hall[43] pointed out that the difficulty in examining the fundi of an uncooperative or restless child is a valid reason for missing a hamartoma. Also, there is no doubt that certain lesions may be more easily overlooked with direct ophthalmoscopy than with indirect ophthalmoscopy.

The appearance of retinal hamartomas that are in part calcified and nodular and in part flat and transparent (Fig. 10.2) suggests that these lesions are in an evolutionary process, changing from a noncalcified to a more

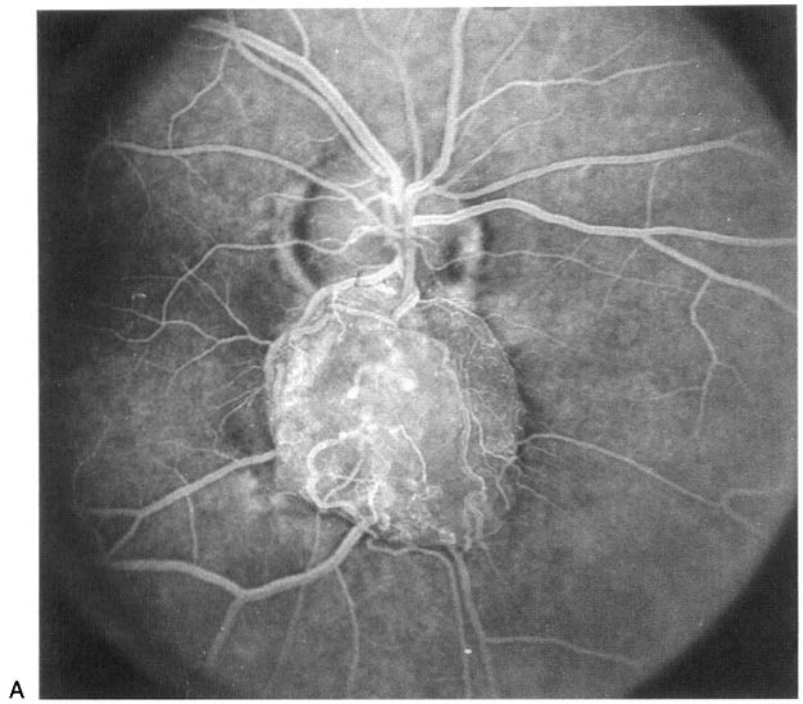
calcified state. However, I have frequently observed flat, semitransparent, noncalcified lesions in adult patients, indicating that evolution to a more calcified state need not be an inevitable result of aging. Furthermore, elevated, nodular, and calcified lesions are not restricted to adults; they were found in infants only a few months old in the Mayo Clinic series. In an effort to learn more about the possible evolution of these hamartomas, Zimmer-Galler and Robertson[65] conducted a long-term follow-up study (6 to 34 years) of 16 Mayo Clinic patients with TSC who had their hamartomas documented photographically (Fig. 10.3). Thirty-seven retinal hamartomas were studied. Most of the retinal lesions remained unchanged even at follow-ups longer than 20 years. However, hamartomas in three patients did show evidence of new calcifications, thus verifying that at least some lesions are capable of change. Another important observation in the study was the documentation of a new retinal hamartoma originating from a site that had been previously photographically documented as normal.[65]

Visual Loss

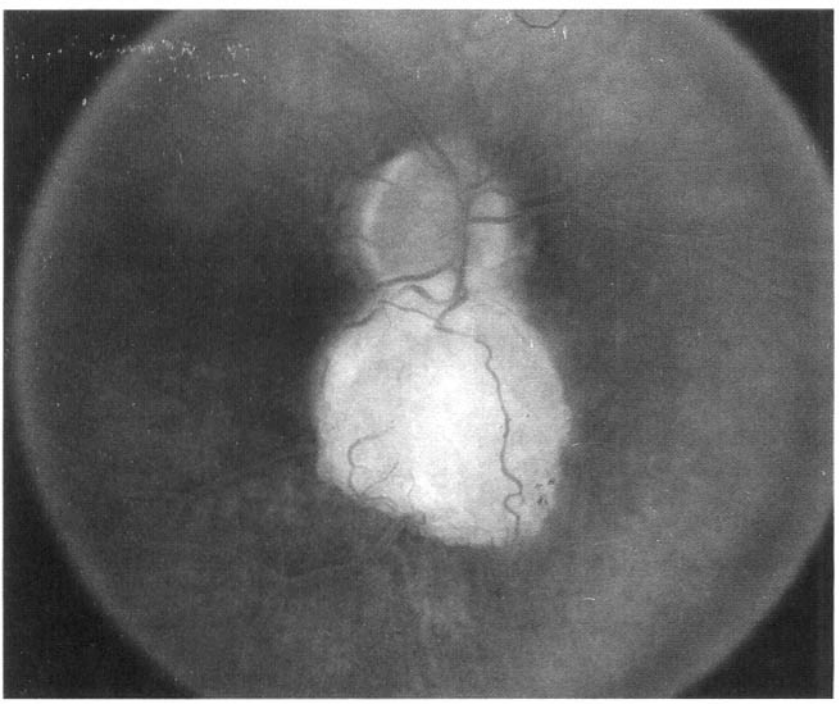
Blindness in association with TSC is rare.[30,33,36,37,64,66,67] Visual loss may be associated with involvement of the fovea by a retinal hamartoma[68] or may develop from retinal or optic nerve lesions, from intracranial tumors causing papilledema and secondary optic nerve atrophy,[17,28,30,43,44,69–72] or from primary interruption of the visual pathways. One Mayo Clinic patient began losing vision in his left eye at the age of 7 years and was found to be practically blind at age 22. A large mulberry lesion extended from the temporal margin of the optic disc into the macular area. Rarely, exudative retinal detachment from incompetence of the tumor vessels may occur.[26,32,51,73,74] Growth of a large astrocytoma of the optic nerve observed over a 7-year period was associated with development of vitreous hemorrhage, blindness, and painful glaucoma in an eye that was later enucleated.[36] Impairment of vision may result from vitreous hemorrhage.[33,37,75] Visual decline from vitreous hemorrhage accompanied by symptoms of vitreous collapse has been observed in a Mayo Clinic patient who had a small lamellar retinal hole on the surface of a retinal hamartoma.

Histopathology

The histopathologic studies of the retinal lesions in TSC have been described by several authors.[6,36,50,51,53,76–83] Generally, they are composed of a feltlike network of glial astrocytes located in the superficial layers of the retina or optic nerve head. The lesions ordinarily are confined to the nerve fiber and ganglion cell layers, where they envelope the superficial retinal blood vessels (Fig. 10.4). Occasionally the tumors involve the full thickness of the retina or the entire papilla.[53] Uncommonly the tumor may be located in the outer



A



B

FIGURE 10.3. *A*, Elevated nodular hamartoma containing numerous tortuous blood vessels that are clearly demonstrated during the arteriovenous phase of fluorescein angiography. *B*, Late phase in angiography showing diffuse tumor staining. Color photograph of this lesion is reproduced in Plate 10.2.

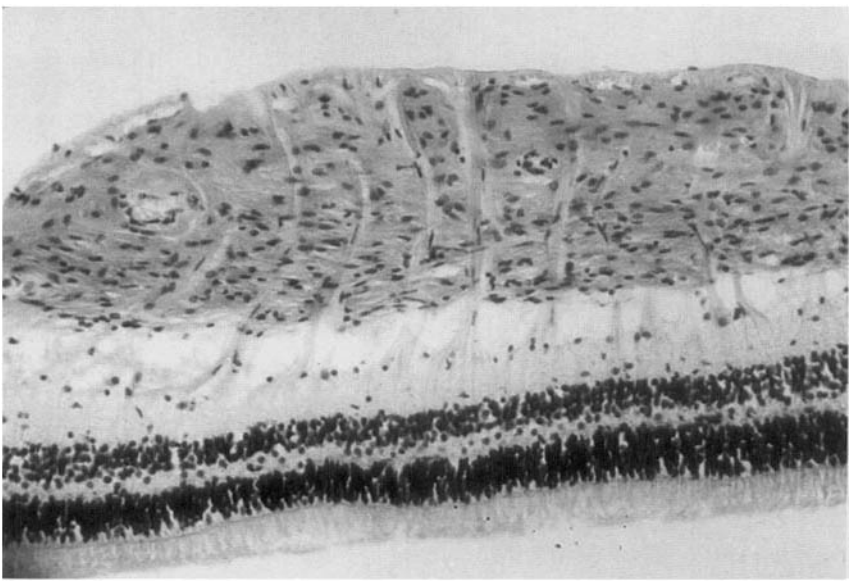


FIGURE 10.4. Astrocytic hamartoma in TSC showing the feltlike interlacing characteristic of the cytoplasmic processes of the astrocytes of the nerve fiber layer. Note the overall thickening of the nerve fiber layer and the blood vessels in the middle portion of the tumor. Müller cells appear to bridge the entire thickness of the tumor. (Reproduced courtesy of Robert Foos, M.D., Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, California.)

retinal layer deep to the retinal vasculature. In such a case, the tumor might be misinterpreted as a choroidal lesion that has invaded the outer retina.[83] The larger lesions tend to undergo cystic degeneration and form hyaline deposits. The cystic areas may contain serous exudates and blood as well as areas of calcific degeneration.[84] The calcific changes are basophilic and, when extensive, tend to form concentric, lamellar configurations (Fig. 10.5).

Reese,[54,80] Hogan and Zimmerman,[77] and Robertson[85] have discussed the similarities and differences between drüsen of the optic nerve and the ocular lesions of TSC. Messinger and Clarke,[53] in 1937, were the first to classify the retinal lesions as hamartomas. Grinker,[86] in 1932, suggested that they probably originated from astrocytes, just as the cerebral tumors. The cells may be large and round, suggesting ganglion cell origin, or elongated and spindle shaped, with large nuclei, prominent nucleoli, and prominent glial fibers. The abundant cytoplasm has poorly defined cell boundaries and the cells tend to merge, giving the appearance of a syncytium. Some cells appear multinucleated; mitoses may also be seen.[83] The cells have been variously considered to be immature[43,63,86] and mature.[64,77,79,85] On the basis of a well-developed vascular network observed in flat preparations, Barsky and Wolter[87] postulated that these lesions may have both an angioblastic and an astroblastic origin. One recent immunopathologic examination demonstrated

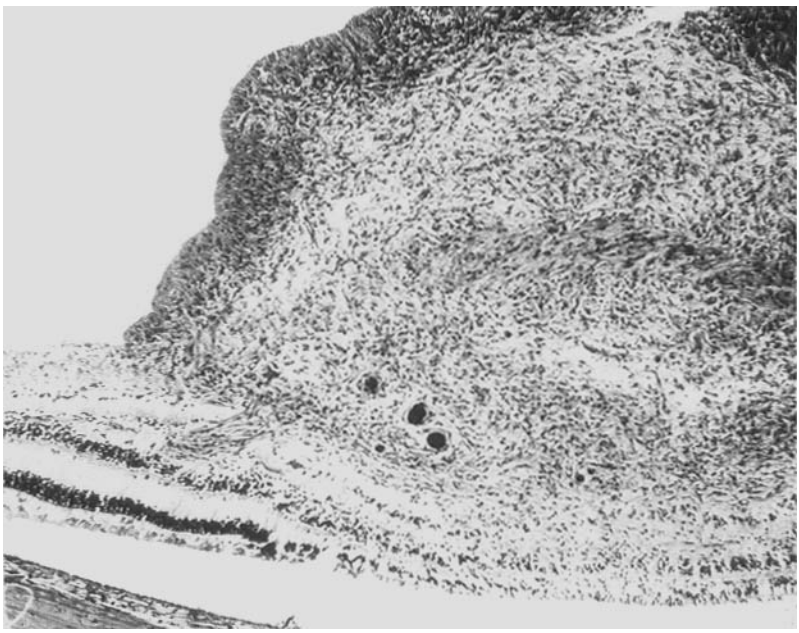


FIGURE 10.5. Elevated high cellular hamartoma arising from the superficial retina and involving deeper structures, including the outer nuclear layer. Note discrete calcareous deposits. (Reproduced with permission from Professor Pierre Danis, Clinique ophthalmologique, Université Libre de Bruxelles, Belgium.)

immunoreactivity for vimentin and glial fibrillary acidic protein.[74] Another showed positive γ-enolase (neuron-specific enolase) staining of the large tumor cells, thus providing support for a neuronal origin of the astrocytic harmartoma.[83]

Differential Diagnosis

Hamartomas seen in the retinas of patients with TSC are indistinguishable from those seen in patients with von Recklinghausen disease.[44,52,88–90] Features of both von Recklinghausen disease and TSC have been noted in one case.[91] Hamartomatouslike lesions of the optic nerve and peripapillary retina have also been observed in retinitis pigmentosa.[85] Although a retinal hamartoma may be the sole recognizable manifestation of a patient suspected of having TSC,[30] in such circumstances it is important to recall that a retinal hamartoma may be mistaken for a retinoblastoma[11,79,92] or other malignant tumor. When the tumor arises from a deep location rather than the usual location in the superficial retina, the tumor may be misinterpreted as a uveal tumor that has invaded the outer retina.[83]

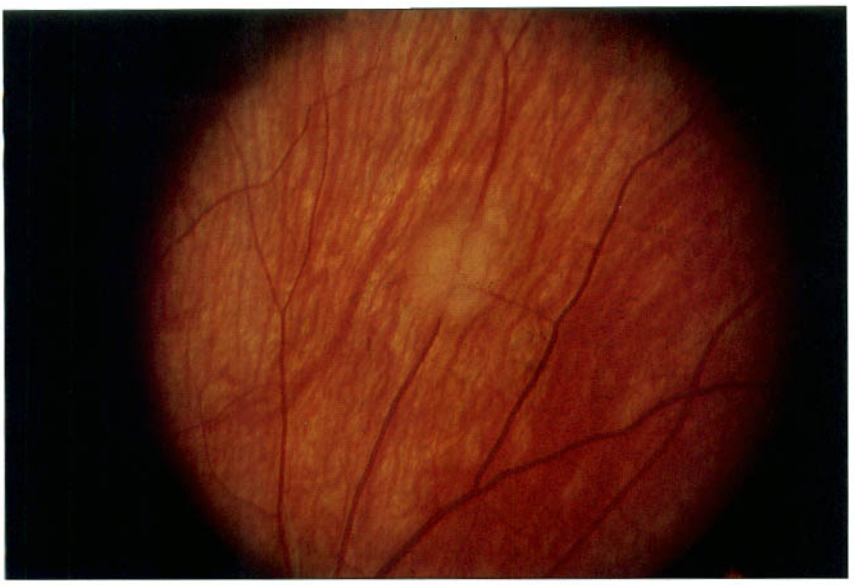


PLATE 10.1. Relatively flat, smooth-surfaced, semitransparent retinal hamartoma overlying retinal vessels.

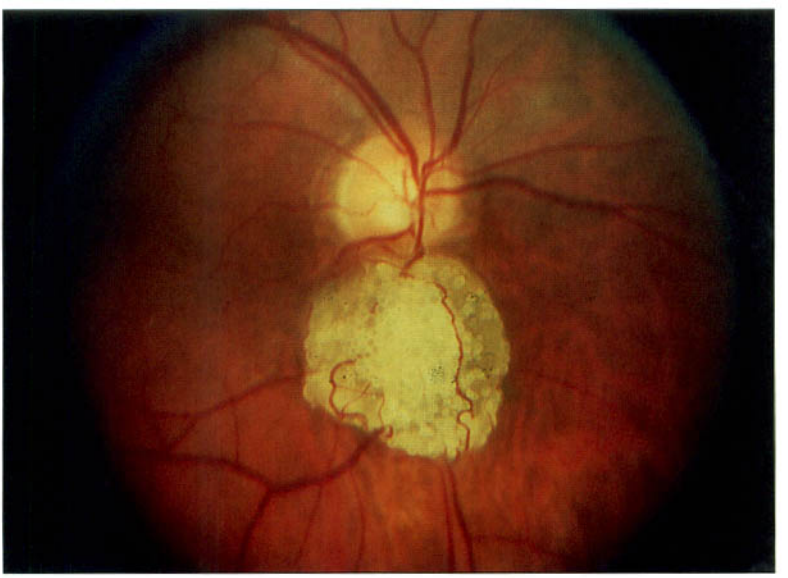


PLATE 10.2. Elevated parapalillary multinodular calcified hamartoma. Note the numerous retinal vessels coursing through the tumor. The fluorescein angiographic apearance is reproduced in Fig. 10.3.

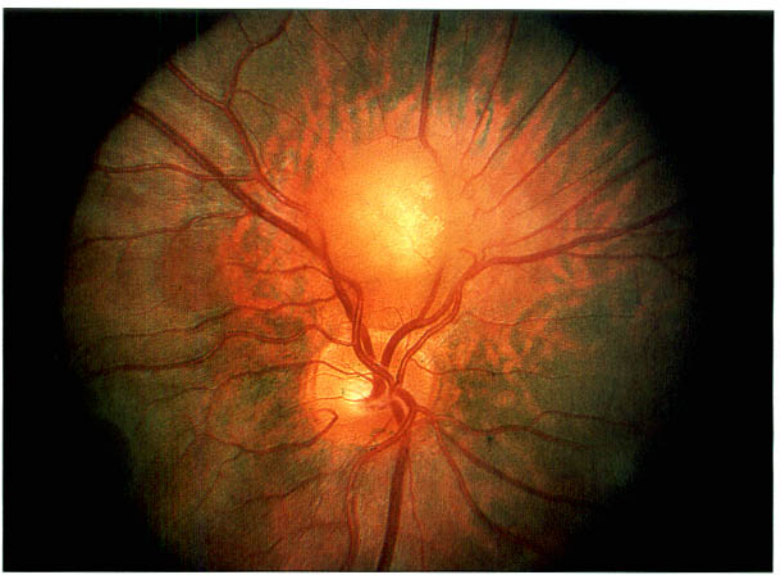


PLATE 10.3. Retinal hamartoma with elevated nodular center and relatively flat, smooth, semitransparent periphery. Note the rarefaction of pigment within the surrounding pigment epithelium.

The presence of adenoma sebaceum, hypomelanotic macules, ungual fibromas, or shagreen patches in the skin, as well as subependymal calcifications in the computed tomography scan of the head, helps confirm that a suspicious lesion is indeed a retinal hamartoma. None of the Mayo Clinic patients had lesions with cheeselike, honeycomb-textured centers surrounded by an annulus of pigmentation, as described in regressing retinoblastoma,[93] nor did any demonstrate evidence of cell clumping in the vitreous or anterior chamber. If no additional stigma of TSC or neurofibromatosis is detected, and if one is in doubt whether a retinal tumor in a child is a hamartoma or a retinoblastoma, the child should be reexamined weekly as suggested by Gass.[51] If it is a retinoblastoma, its growth will soon be noticed, so that appropriate treatment can then be undertaken. Rarely is there a need for tissue to establish the diagnosis of astrocytic hamartoma in presumed TSC.[74] To date I have relied purely on clinical and roentgenologic information to establish the diagnosis of retinal hamartoma in TSC.

Management

Since growth and change of the fundus lesions are rare, treatment is not indicated. Although photocoagulation has been used to treat phakoma, this treatment modality has not been completely effective in promoting ablation. However, those rare tumors causing secondary exudative changes threatening to involve or involving the macula should be studied angiographically to determine the extent of the vascular component prior to deciding whether or not management by photocoagulation might effectively ablate the tumor. Because the nerve fiber layer would likely be destroyed also, the functional loss accompanying the field defect should be anticipated and weighed against the possible advantage of destroying the tumor. Radiation therapy has not been successful.[94] The recognition by the ophthalmologist of a retinal phakoma should prompt inquiries into the family history and arrangements to obtain a neurologic consultation. This is, of course, urgent in the presence of papilledema. Finally, the ophthalmologist must be reminded that mental retardation is an overemphasized symptom of TSC.[95] One half of patients have normal intelligence. Inasmuch as many of these patients may become parents, genetic counseling should be given since they suffer a hereditary disorder of autosomal dominant transmission.

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11

Dermatologic Manifestations

The dermatologic features are an important part of tuberous sclerosis complex (TSC). Adenoma sebaceum was included in the classic clinical triad defined by Vogt[1] in 1908. Even in the neonatal period, lesions that are highly suspicious for TSC may be recognized in some patients by the careful examiner. As the infant or child grows, additional lesions may appear that confirm the suspected diagnosis or provide the key piece to a diagnostic puzzle.

A cross-sectional study of the age-related prevalence of cutaneous features of TSC performed by Webb et al.[2] on 131 affected individuals showed that 126 (96%) exhibited skin signs. There was a trend toward early expression of hypomelanotic macules and forehead fibrous plaques compared with the later development of facial angiofibromas and ungual fibromas. Shagreen patches were usually present by puberty. Ungual fibromas appeared for the first time as late as the fifth decade. Gingival fibromas were present in 36%. Eight percent presented because of the skin manifestations, and 21 received treatment for symptomatic skin lesions.

Hypomelanotic Macules

Critchley and Earl[3] reported "patches of abnormal whiteness" resembling "in size and conformation the café-au-lait stains" in 2 of their 29 patients with TSC.

Fitzpatrick et al.,[4] in 1968, described the "white, leaf-shaped macules," emphasized their significance in TSC, and provided the definitive studies on the incidence, clinical features, pathologic nature, and differential diagnosis of these hypomelanotic macules.

Incidence

Fitzpatrick et al.[4] stated that the majority of patients with TSC have these lesions at the time of birth, although their presentation may be delayed by

months or years.[5] In 80 individuals with hypomelanotic macules who had all lesions counted carefully, 89% had more than one and 40% had more than five. Six individuals had scalp lesions resulting in poliosis.[2]

Clinical Description

The hypomelanotic macules are often present at birth but sometimes can be found only by using a Wood lamp or ultraviolet light. The Wood lamp emits light of 360-nm wavelength, which is selectively absorbed by the melanin, thus exposing the areas deficient in melanin as dull, white patches because they absorb less light. The hypomelanotic macules are asymmetrically distributed over the entire skin surface but are most common over the trunk and buttocks (Fig. 11.1) and are rare on the face. Involvement of the scalp produces poliosis. This, too, may be noted in early infancy.[6]

The number of macules vary from 3 to 4 to more than 100. They are usually 1.0 cm or more in length. The morphology is particularly important; most of them are leaf shaped or lance-ovate (round on one end, tapering on

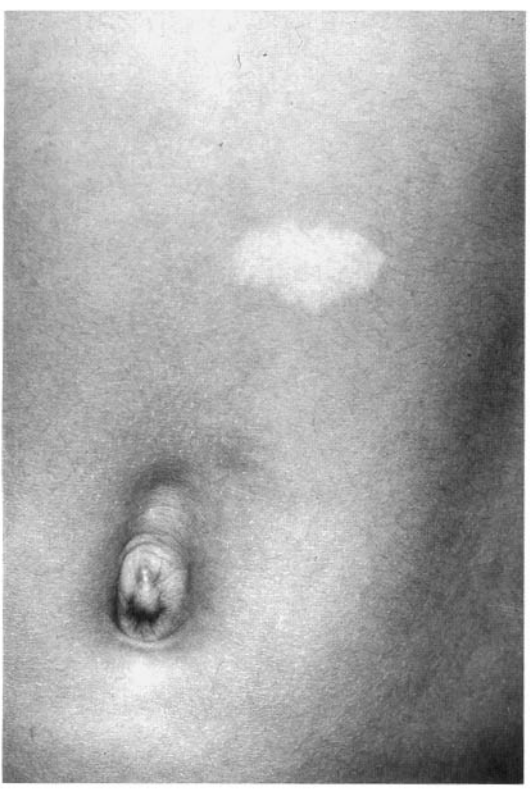

FIGURE 11.1. Hypomelanotic macules. Several macules are visible with ordinary artificial lighting. Note the lance-ovate shape and the typical distribution over the trunk.

the other), resembling the leaf of the European mountain ash tree (Fig. 11.2). Sometimes numerous small macules may be present that resemble confetti. These are most often noted on the distal portions of the limbs.

Course

The hypomelanotic macules are present at birth and usually persist throughout life, although lesions can become less obvious over time and may disappear.[2]

Histopathology

A routinely processed biopsy specimen of a hypomelanotic macule does not serve to establish a precise diagnosis. When histochemically reacted for dihydroxyphenylalanine (DOPA), however, either an epidermal whole mount or a routine biopsy specimen will show a normal number of melanocytes but a reduction in the intensity of the histochemical reaction as compared with an area of normal skin from the same patient.[4] Electron-microscopic examination of a hypomelanotic macule shows reduced number, melanization, and size of the melanosomes within the melanocytes and the keratinocytes. A similar pattern has been found in hypomelanotic hair (poliosis).[4,7]

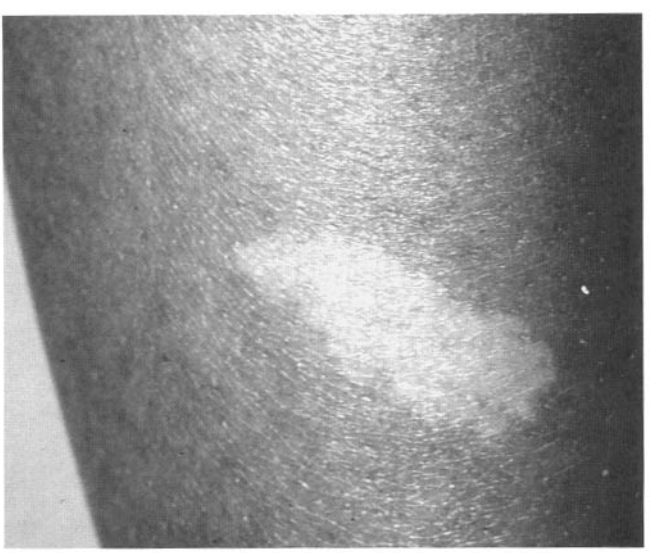

FIGURE 11.2. Hypomelanotic macule. Note the lance-ovate (leaf of European mountain ash tree) shape.

Differential Diagnosis

The presence of hypomelanotic macules at birth, with their dull white color and their characteristic distribution and shape, is highly suggestive of TSC. However, examination of newborns has demonstrated that 0.8% have hypomelanotic macules. These are more common in black (2.4%) than white (0.4%) infants. Some of these lesions may disappear in the first year of life.[8] Moreover, isolated white macules may also occur in vitiligo, nevus depigmentosus, nevus anemicus, piebaldism, and Vogt-Koyanagi-Harada syndrome.

The macules of vitiligo show an absolute loss of melanin with a purer white coloration under natural, ordinary artificial, or Wood light. Their shape is lance-ovate only exceptionally. Vitiliginous macules are distributed symmetrically and have a proclivity to be located near natural orifices and flexures. DOPA-stained sections of the skin with longstanding vitiligo show an absolute absence of melanocytes, but early lesions may contain a few melanocytes. Examination with the electron microscope shows similar findings.

In nevus depigmentosus, the congenital hypomelanosis usually appears in bizarre patterns and linear streaks. In this condition, electron-microscopic examination reveals fewer melanosomes than are present in normal skin. This is similar to the pattern seen in the hypomelanotic macules of TSC. However, the melanosomes in nevus depigmentosus are normal in size and melanization. Transfer of melanin to keratinocytes is markedly decreased in this condition, in contrast to TSC, where it is only mildly decreased.[7]

Nevus anemicus, a circumscribed patch of pale skin, is seen in patients with neurofibromatosis and in healthy individuals. The pale white patch of nevus anemicus does not redden when stroked or warmed, blanch in response to cold, sweat in response to heat, or persist with diascopy as does the hypomelanotic macule of TSC. This lesion is due to a functional vascular abnormality and not to a defect in the pigmentary system.

Piebaldism involves the skin and hair only. Lesions are present at birth and remain unchanged throughout life. Piebaldism is characterized by a white, often triangular forelock of the forehead and scalp, a white macule on the center of the chin, decreased pigmentation of the trunk (with normal pigmentation in a stripe on the back), and white patches on the midarm to wrist and midthigh to calf areas. This skin disease is inherited as an autosomal dominant trait. Examination of the lesions with light and electron microscopy discloses absent melanocytes, as in vitiligo.[7] When associated with lateral displacement of the medial canthi, hypertrophic nasal root, confluent eyebrows, heterochromia irides, or perceptive deafness, the diagnosis of autosomal dominant Waardenburg syndrome is appropriate.

The Vogt-Koyanagi-Harada syndrome may also be considered in the differential diagnosis of the hypomelanotic macules of TSC. The syndrome is composed of chronic bilateral uveitis, poliosis, vitiligo, alopecia, dysacusia, and cerebral symptoms. The hypopigmented macules tend to be permanent and resemble vitiligo.

Diagnostic Value

The hypomelanotic macules of TSC are clinically and pathologically distinct. They are often present at birth, usually persist through life, and constitute the earliest skin sign of TSC. They appear to be highly suggestive of TSC. This must be tempered by the observation that hypopigmented macules may be noted on the skin of healthy neonates. Vanderhooft et al. counted the number of hypomelanotic skin macules in 423 white individuals examined with incandescent, fluorescent, and ultraviolet illumination. Twenty individuals (4.7%) had at least one hypopigmented macule and, of these, four had more than one macule. None had more than three white spots. Indirect ophthalmoscopy performed in 13 of these subjects (65%) did not reveal retinal findings. Apparently none of the subjects had the benefit of imaging studies. These authors concluded that the presence of a few hypopigmented macules on the skin of an otherwise healthy individual without a family history of TSC need not prompt an evaluation to rule out this disorder.[8] The Wood light examination should be carried out for any infant or child with seizures and/or mental retardation and immediate relatives of patients with TSC in need of genetic counseling.

Facial Angiofibroma (Adenoma Sebaceum)

The first description of adenoma sebaceum was made in 1885 by Balzer and Ménétrier.[9] There is, however, an earlier illustration in the atlas of skin diseases compiled by Rayer,[10] published in 1835. Pringle,[11] in 1890, reported "indolent, firm, whitish or yellowish, sago-grain like, solid papules or little tumors imbedded in the skin at different depths, or projecting from it . . . intermingled with these lesions and transgressing their limits in every direction, especially over the cheeks, toward the ears, innumerable capillary dilatations and stellate telangiectasias." Pringle called these lesions "adenoma sebaceum" and a form of "rosacea." In 1908, Vogt[1] associated the lesions of adenoma sebaceum with TSC, and, since then, it has been widely accepted that they are pathognomonic of TSC.

Incidence

The lesions of adenoma sebaceum were found in 83% of the Mayo Clinic series reported in 1967 by Lagos and Gomez.[12] Nevin and Pearce[13] found the lesions in 83.3% of their patients. Webb et al.[2] reported them in more than 80% of patients over 5 years of age. Like hypomelanotic macules, these lesions are likely to be found in almost all patients if the clinician makes careful observations over an extended period of time.

Clinical Description

The hamartomas called adenoma sebaceum are actually angiofibromas because the sebaceous glands are only passively involved. The typical lesions are red to pink papules or nodules with a smooth, glistening surface (Fig. 11.3). They are bilaterally symmetrical, distributed over the centrofacial areas, particularly in the nasolabial folds, onto the cheeks in a butterfly fashion, and on the chin. Rarely are these lesions found on the forehead, on the scalp region, or even laterally on the face. The upper lip is curiously spared, but lesions may be seen on the nasal vestibules and the eyelids.

The forehead and scalp, in contrast, may be affected by large fibrous plaques. These flesh-colored plaques are soft or compressible or doughy to hard lesions. Single large or multiple lesions may be noted (Fig. 11.4). Webb et al.[2] noted these lesions in 36% of their large series. The angiomatous appearance is absent from these lesions. The forehead and scalp plaques may be seen in newborns but usually present at a later age than the facial lesions. These lesions are classified as angiofibromas, although clinically they differ from the papular or nodular adenoma sebaceum because of their cephalad location, larger size, and fibromatous histopathologic appearance.

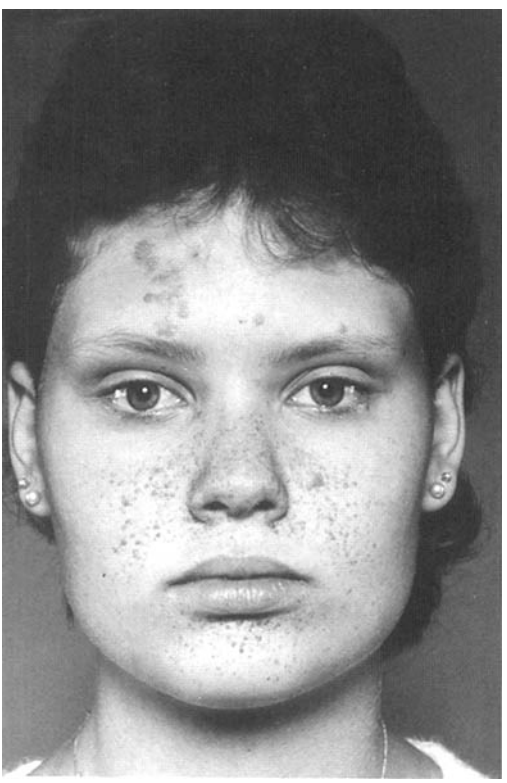

FIGURE 11.3. Facial angiofibroma. Note the small papules distributed symmetrically over the centrofacial area onto the cheeks in a butterfly distribution.

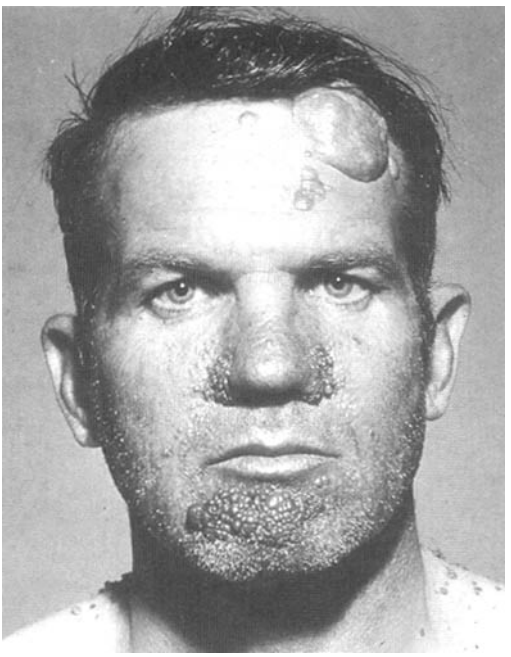

FIGURE 11.4. Facial angiofibroma and forehead plaques. Note the large fibrous plaques on the forehead in this older patient. Also note the more advanced adenoma sebaceum lesions, which are much more numerous, closely set, and larger than the early lesions depicted in Fig. 11.3. Also, "molluscum fibrosum pendulum" tumors may be seen around the patient's neck.

Course

The careful observations of Butterworth and Wilson[14] and Nickel and Reed[15] permit construction of the evolution of these lesions. Sometime in the first year of life, centrofacial flushing is first noted when the child cries. Occasionally, lesions are seen to develop at this time. Early "spider angioma–like" lesions and later fully developed angiofibromas appear by the age of 5 years. As the patient enters puberty, the lesions may become even more numerous and prominent (Fig 11.5). Occasionally, lesions may not present until later in life, even as late as 20 years. However, once it has developed, the angiofibroma persists for life.

Histopathology

Although these characteristic lesions of TSC have been called adenoma sebaceum, as Nickel and Reed[15] have pointed out in a histopathologic study of 74 biopsy specimens from patients of different ages, the sebaceous glands may be atrophic or absent. This is the result of hamartomatous development of the connective tissue and vascular elements of the dermis. The authors emphasized the passive role of the sebaceous glands and noted that the sebaceous glands of prepubescent patients with facial angiofibroma are not pathologically augmented as one would expect if these patients indeed had adenomas of the sebaceous glands.

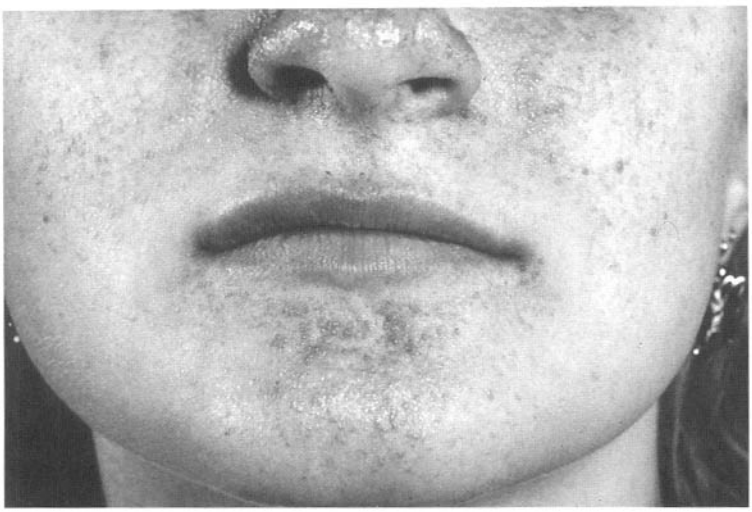

FIGURE 11.5. Facial angiofibroma. Typical papules of adenoma sebaceum in an adolescent boy. Note the relative sparing of the lateral face and upper lip.

The major findings in the facial lesions, dermal fibrosis and vasodilatation, make the term *angiofibroma* preferable to any other one used for them (Fig. 11.6). Elastic tissue is absent from these lesions. As the angiofibromas mature, the collagen becomes sclerotic and layered (Fig. 11.7). Occasionally, large stellate fibroblasts give the tissue a "glial appearance." Histologically, several other entities may resemble facial angiofibromata, including fibrous papule of the nose, perifollicular fibroma, and acquired digital fibrokeratoma. These lesions, and others with a histologic resemblance, are clinically distinct from the characteristic facial angiofibroma lesions.[16]

Histologically, the forehead and scalp plaques are fibromata without the vascular components. Hyalinization and sclerosis of the collagen fibers is a common finding in older lesions.

Differential Diagnosis

In the adolescent patient, angiofibromas may be mistaken for acne vulgaris. Furthermore, acne vulgaris may occur in association with TSC, but the classic open and closed comedones of acne vulgaris are not found in the prepubertal patients with facial angiofibroma. When there is doubt about the diagnosis, a skin biopsy will readily demonstrate the pilosebaceous origin of the acne vulgaris, differentiating it from facial angiofibroma.

Acne rosacea may also be confused because, like the angiofibroma, it has a centrofacial distribution. Also, acne rosacea may be associated with

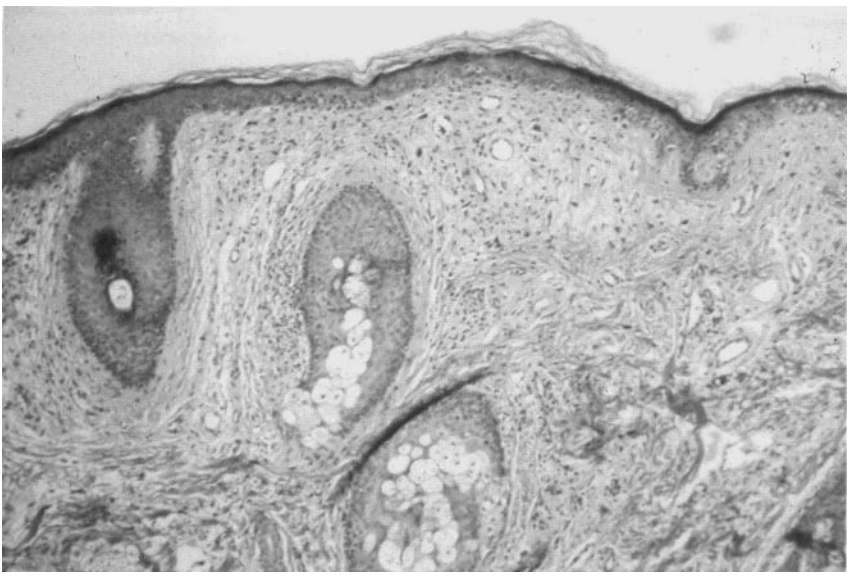

FIGURE 11.6. Facial angiofibroma, cheek. Early facial lesion demonstrating mild dermal fibrosis surrounding the piliosebaceous units and many dilated blood vessels in the upper dermis. The sebaceous glands are normal for a patient of this age and are neither adenomatous nor hamartomatous. (Hematoxylin & eosin, ×100)

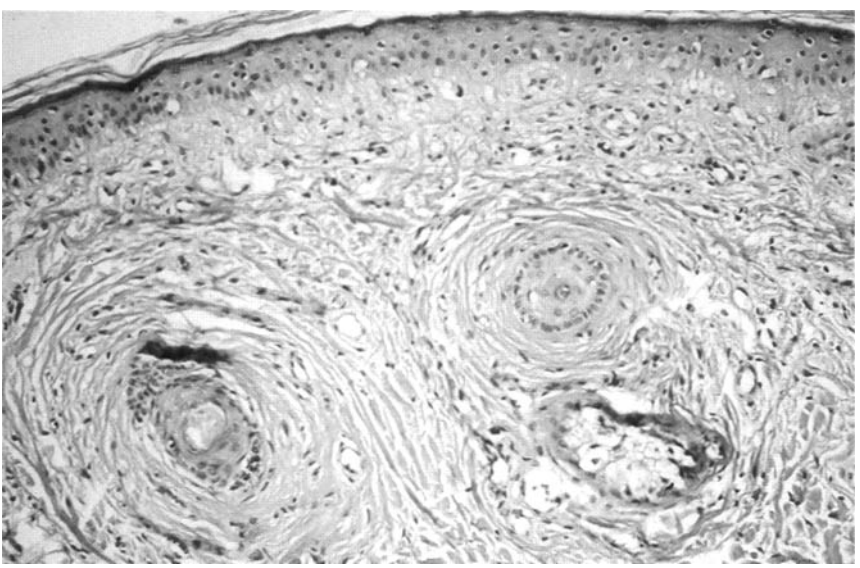

FIGURE 11.7. Facial angiofibroma, cheek. Section demonstrating the sclerotic and layered appearance of a more mature facial lesion. Note the normal sebaceous gland in the lower right corner. (Hematoxylin & eosin, ×100)

facial angiofibroma in the adult patient with TSC. Acne rosacea is a disease of middle life in which papules or pustules develop in an erythematous, telangiectatic background. This type of skin lesion in a patient with facial angiofibroma is probably what prompted Pringle's description.[11] Again, when there is doubt about the proper diagnosis, a skin biopsy will demonstrate the pustules in and around the pilosebaceous unit or the epithelioid tubercles and granulomas of acne rosacea, which differentiate it from the facial angiofibroma.

Clinically, the most difficult lesion to be differentiated is the autosomal dominant genodermatosis multiple trichoepithelioma, also known as epithelioma adenoides cysticum. These lesions characteristically begin at puberty and gradually increase in number and size. They affect the centrofacial area, although the scalp, eyelids, neck, and trunk may also be involved. The lesions are solid, flesh-colored papules and nodules usually without the red or pink vascular component of facial angiofibromas, although some telangiectatic vessels may be noted in the background. Histologically, these multiple trichoepitheliomas are hamartomas of the hair follicle epithelium and are composed of epithelial structures such as horn cysts and islands of basalioma cells.

Multiple angiofibromas have been observed in 28 of 32 patients with multiple endocrine neoplasia type I in numbers ranging from 2 to about 50 and sizes ranging from 1 to 4 mm. The histopathology of these lesions is indistinguishable from those observed in patients with TSC. Two of these patients had confetti-like hypopigmentation on the trunk and neck similar to that in patients with TSC. Several other patients had scattered hypopigmented macules, none lanceolate. Two patients had multiple gingival papules. One patient had a solitary ungual fibroma. Shagreen patches, forehead plaques, and pitting of dental enamel were not observed.[17]

Diagnostic Value

Facial angiofibromas may not be visible until the patient reaches the age of 5 years or older, but, when clinically identified beyond doubt or pathologically confirmed, they are a highly suggestive sign of TSC. Multiple lesions in the characteristic distribution are pathognomonic of TSC. The fibromas of forehead and scalp, which usually appear at a later age than the angiofibromas of nasolabial folds but may be found in newborns with TSC, have identical diagnostic value.

Treatment

The cosmetic deformity of the facial angiofibromas can be improved by several surgical modalities, including laser abrasion, dermabrasion, cryosurgery, and simple excisional or planing techniques. Good cosmetic results are maintained for several years.

Shagreen Patches

The first description of the association of the shagreen patch (from the French *peau chagrinée*, meaning "skin with the appearance of untanned leather") with adenoma sebaceum and seizures was given by Hallopeau and Leredde[18] in 1895. This classic description is worthy of note: "when the patient stands, one sees an elevated mass, divided by longitudinal folds; one can take this mass between the fingers, separate it from the deep layers; actually, it is a part of the skin." The patient had, in addition, adenoma sebaceum and seizures.

Incidence

The shagreen patch was noted in 80% of the Butterworth and Wilson series,[14] in 68% of the Nickel and Reed series,[15] and in 54% of the Webb et al. series.[2] The incidence varies depending upon the age of patients studied, with an incidence of 25% in patients less than 5 years old and 50% or more in patients older than 5 years.[2]

Clinical Description

The patches or plaques are usually found on the dorsal body surfaces, particularly the lumbosacral region (Fig. 11.8), although they may also occur on the upper portion and on the ventral aspect of the trunk (Fig. 11.9). The shagreen patch may vary in size from a few millimeters to 10 cm or more. They are slightly elevated above the surrounding skin surface, are yellowish brown or pink in color, and have the texture of pigskin or an orange peel. They are firm or rubbery in consistency.

Course

The shagreen patch is rarely found in infants and becomes more common after the first decade of life. The lesion persists through life.

Histopathology

The shagreen patch is a connective tissue hamartoma. Two principal types have been described. The first and most common variety is characterized by a band of normal superficial dermis and a haphazard arrangement of collagen fibers in the mid and deep dermis (Fig. 11.10). The second variant is characterized by a uniform hamartomatous proliferation of collagen throughout the dermis (Fig. 11.11). Both types are connective tissue nevi containing various amounts of vascular structures, adipose, collagen, elastic tissue, smooth muscle, and skin appendages. There is no increased vascularity.

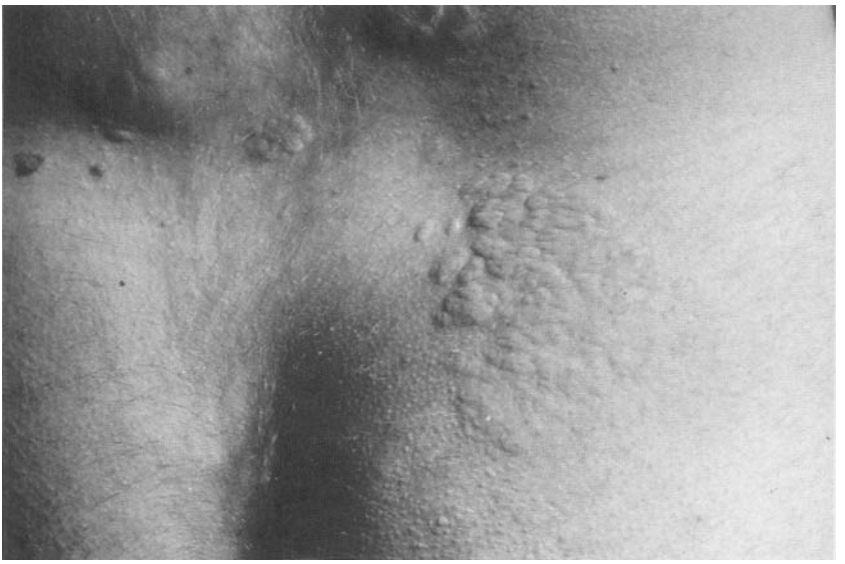

FIGURE 11.8. Shagreen patch. This large plaque is noted in a typical site on the back. The lesion is elevated and has the appearance of pigskin. Note also smaller papular lesions on the upper back as well as several hypomelanotic macules.

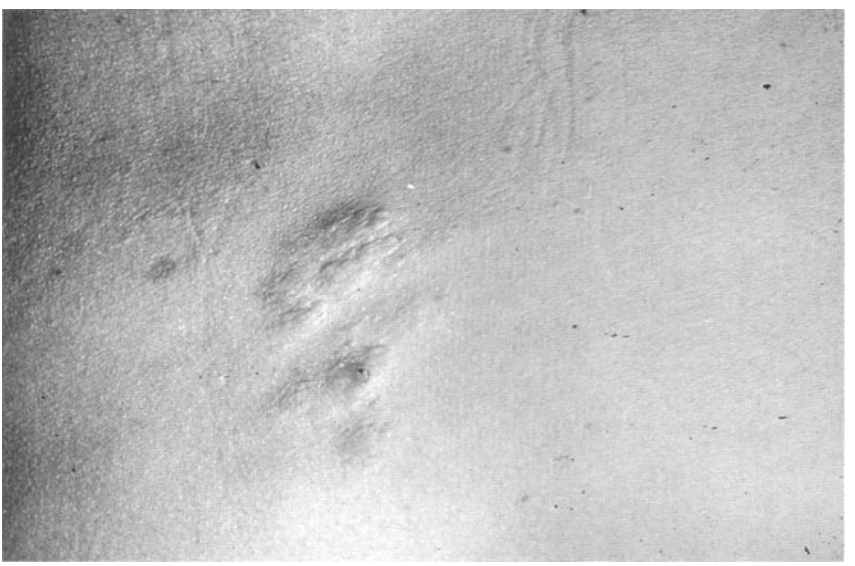

FIGURE 11.9. Shagreen patch. This lesion represents a subtler form of the shagreen patch. It is ventrolateral in location, small, and more infiltrative, and its borders are less well demarcated.

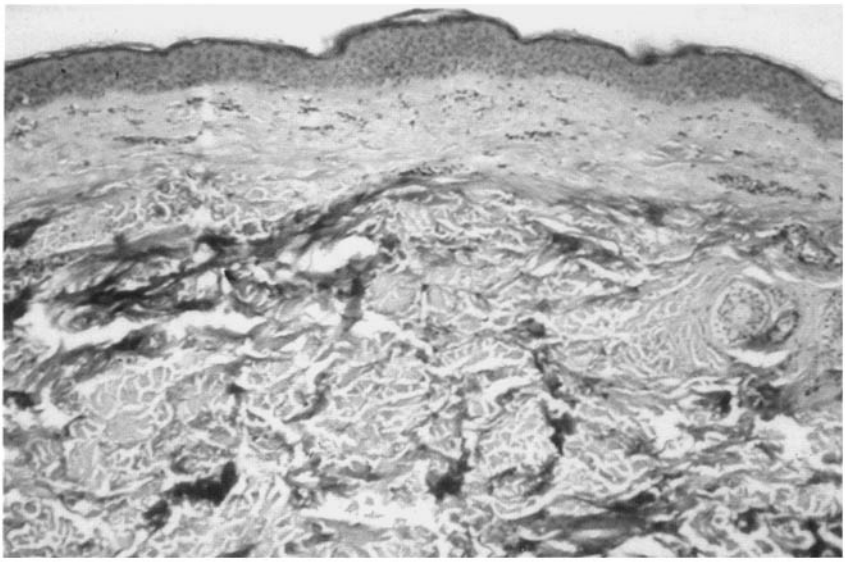

FIGURE 11.6. Facial angiofibroma, cheek. Early facial lesion demonstrating mild dermal fibrosis surrounding the piliosebaceous units and many dilated blood vessels in the upper dermis. The sebaceous glands are normal for a patient of this age and are neither adenomatous nor hamartomatous. (Hematoxylin & eosin, ×100)

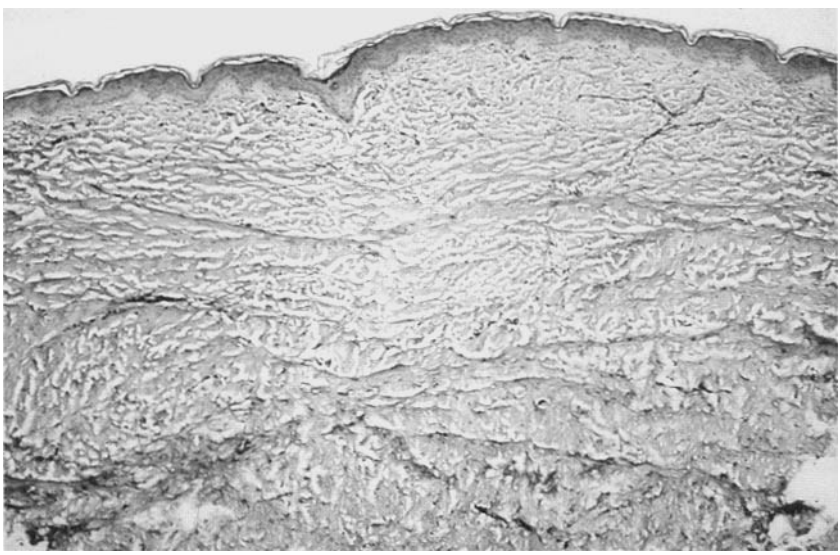

FIGURE 11.7. Facial angiofibroma, cheek. Section demonstrating the sclerotic and layered appearance of a more mature facial lesion. Note the normal sebaceous gland in the lower right corner. (Hematoxylin & eosin, ×100)

Their general appearance is one of excess collagen and elastic tissue in disproportion to the amount of muscle, adipose tissue, appendages, and vascular structures. A biopsy is rarely needed for the confirmation of the diagnosis of a shagreen patch.

Differential Diagnosis

The shagreen patch is a clinically distinctive lesion and a common cutaneous manifestation of TSC. However, it differs neither clinically nor histopathologically in any way from other connective tissue nevi that may occur as isolated developmental defects or in families.[19] The connective tissue nevi associated with osteopoikilosis[20] in the Bushke-Ollendorff syndrome present in childhood, are often bilaterally symmetric and are located ventrally or dorsally. Conversely, the connective tissue nevus or shagreen patch of TSC is invariably asymmetric, usually dorsal in distribution, and rarely observed before puberty.

Diagnostic Value

The shagreen patch is second to hypomelanotic macules among the most common cutaneous lesions found on the trunks of patients with TSC. Although not absolutely a pathognomonic sign, it is a very helpful finding when attempting to establish the diagnosis of TSC.

Ungual Fibromas

In 1903, Kothe[21] described subungual fibromas in association with adenoma sebaceum. Several other authors reported similar findings in the next few years. Koenen,[22] in 1932, reported this lesion in six of nine members in three generations of a Dutch family affected with TSC. Koenen tumor is the name often used in the European medical literature for these characteristic lesions.

Incidence

The frequency of Koenen tumors in TSC patients ranges from 52% in the Nickel and Reed[15] series to 88% in adults over 30 years of age in the Webb et al.[2] series.

Clinical Description

The ungual fibromas are dull, red- or flesh-colored papules or nodules arising from the finger (Fig. 11.12) or toe nail bed to be located in the lateral nail groove, under the nail plate, or along the proximal nail fold. They are more

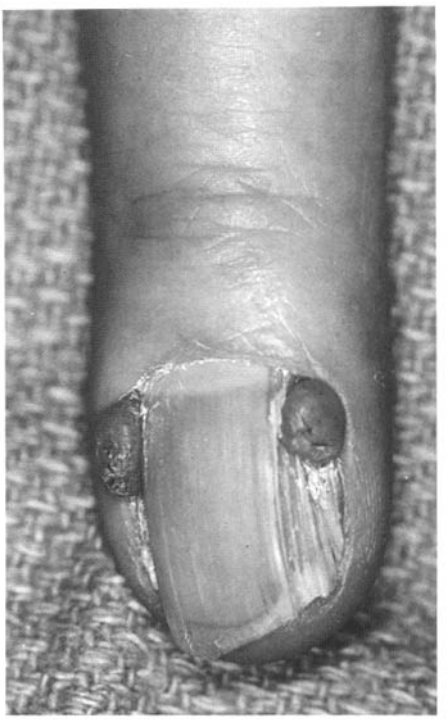

FIGURE 11.12. Ungual fibroma. This fibrous nodule is arising from the lateral nail bed along the lateral nail groove, causing a nail dystrophy.

common on the toes than on the fingers (Fig. 11.13) and range from 1 mm to 1 cm in diameter. It has been said that pressure from shoes stimulates their growth. They are more common in the female patient.

Course

Ungual fibromas characteristically appear at or after puberty, although on occasion they appear earlier in life. These lesions continue to develop with age[2] and tend to regrow after their removal.

Histopathology

Koenen tumors are fibromas or angiofibromas. Histopathologically, they resemble the facial angiofibromas or forehead plaques (Fig. 11.14). The older lesions in particular may contain large stellate fibroblasts with a "glial appearance."

Differential Diagnosis

A patient with a single ungual fibroma and no other sign of TSC may present a problem of proper diagnosis. This problem is emphasized by the report of

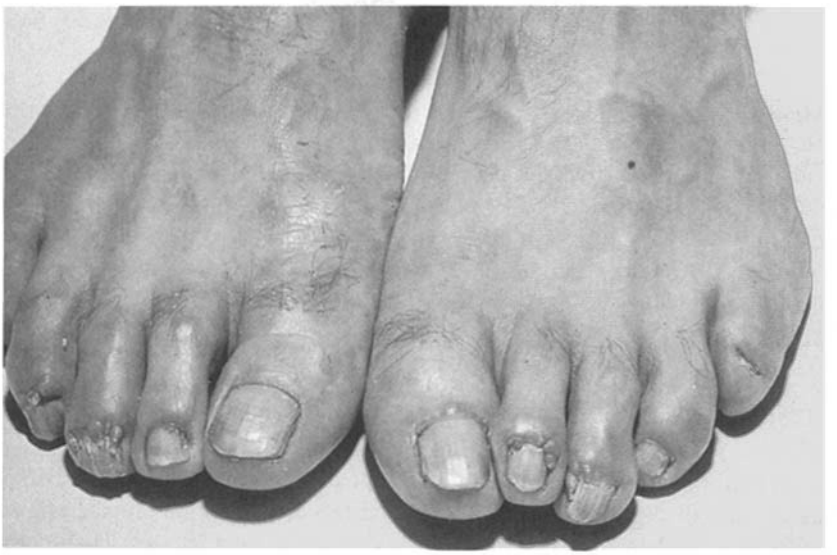

FIGURE 11.13. Ungual fibromata. Numerous grapelike fibrous papules and nodules are visible. Pressure from shoes is thought to stimulate the growth of the fibromata.

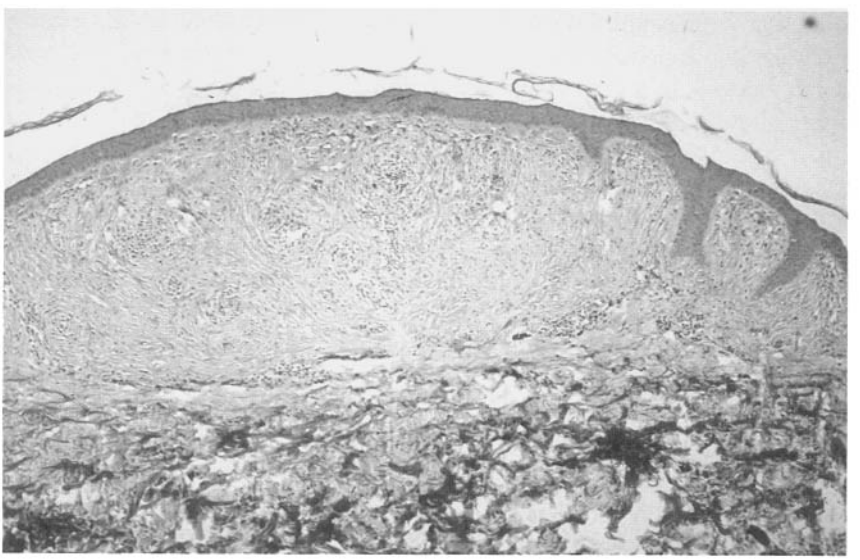

FIGURE 11.14. Ungual fibroma. Nodular angiofibroma from a toe lesion demonstrating hamartomatous changes of both blood vessels and connective tissue. (Hematoxylin & eosin, ×160)

Zeller et al.[23] of seven "normal" patients with periungual fibromata and the accompanying editorial by Schnur[24] illustrating the difficulty of excluding TSC in some carriers. Both acquired digital fibrokeratomas and multiple acral fibrokeratomas may be confused with ungual fibromas, because both tend to be distributed more proximally and do not present in periungual location.

In the differential diagnosis of the skin lesion, one must also consider epithelial inclusion cysts, subungual fibromas, verruca vulgaris, and infantile digital fibromatosis.

Diagnostic Value

The ungual fibroma is another characteristic cutaneous manifestation of TSC that is virtually pathognomonic when multiple, as are the facial angiofibroma, the forehead plaques, and the hypomelanotic macules. Histopathologic confirmation may be required only in unusual circumstances when the ungual fibroma is the sole cutaneous feature in a patient with TSC.

Miscellaneous Nevoid Lesions

Café-au-Lait Macules

These oval or round, flat, hyperpigmented macules of 1 to 5 cm in length may be seen in the skin of patients with TSC. They could create diagnostic confusion with neurofibromatosis, a disease in which café-au-lait macules are an early cutaneous sign. Butterworth and Wilson[14] did not find any café-au-lait macules in their patients, and Nickel and Reed[15] reported 7% in their group of patients. The presence of these lesions in patients with TSC has apparently misled some authors to make the diagnosis of neurofibromatosis in association with TSC. Crowe et al.[25] found that 10% of the general population have one or more café-au-lait macules, but subjects with six or more macules, most of which exceed 1.5 cm in diameter, nearly always have neurofibromatosis. Of their patients with neurofibromatosis, 78% had more than six large macules. It is possible that the presence of one or two café-au-lait macules in patients with TSC is a coincidental finding, and less likely that patients with TSC have café-au-lait macules more often than the general population. Bell and McDonald[26] compared the prevalence of café-au-lait macules in normal subjects (16.0%) and TSC patients (15.4%) and found no statistically significant differences between the two groups.

Soft Fibromas

Multiple or solitary, soft, baglike pedunculated growths on the neck, trunk, or extremities (molluscum fibrosum pendulum) (Figs. 11.4 and 11.15), or

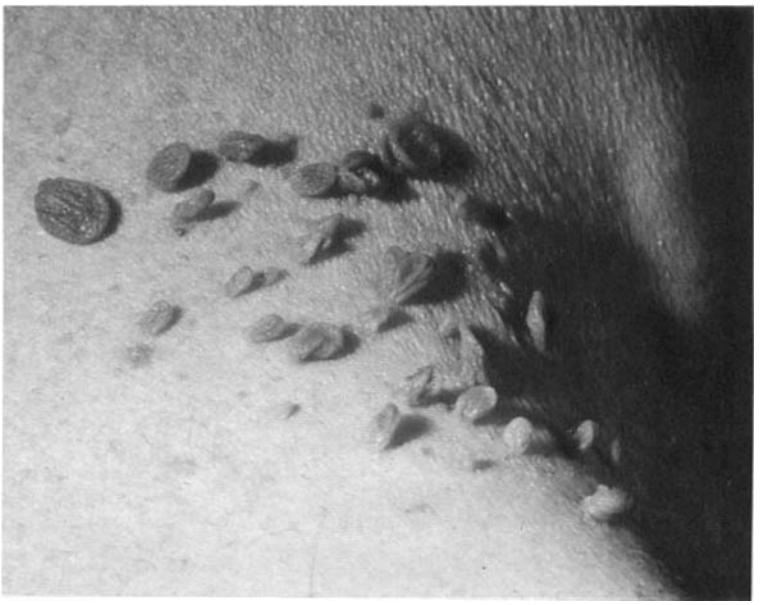

FIGURE 11.15. Molluscum fibrosum pendulum skin tags. Note the saclike pendulous soft fibromata around the neck.

filiform, smooth papules on the neck and axillae and near flexures of limbs, or firm, pedunculated or sessile nodules of the buccal or gingival mucosa may be noted in patients with TSC. Another variant of the fibromatous lesions is one in which large numbers of slightly raised, tiny papules (smaller than a pin's head) are scattered over the trunk or neck (Fig. 11.16). These miliary papules are indistinguishable in color from the surrounding skin, and in size and appearance they resemble coarse "gooseflesh." These lesions are neither clinically nor histologically distinct from the soft fibromas occurring in the general population or in patients with Gardner or the Cowden syndromes, both of which are multiple hamartomatoses. Nevertheless, they are histopathologically distinct from the cutaneous neurofibromas (molluscum fibrosum) of neurofibromatosis.

Dental Pits

Dental abnormalities have been recognized in TSC for many years.[27,28] Dental enamel pitting (Fig. 11.17) is seen in 90% of patients with TSC versus only 9% of the general population.[28] The value of dental examination of families of apparent sporadic TSC patients as a screening test to help identify unsuspected carriers found similar rates of pitting in unaffected first-degree relatives and normal controls. Ten probands with TSC and 20 first-degree relatives (16 parents and four siblings from 11 different families) were examined for evidence of pitted enamel hypoplasia.[28] One hundred percent of

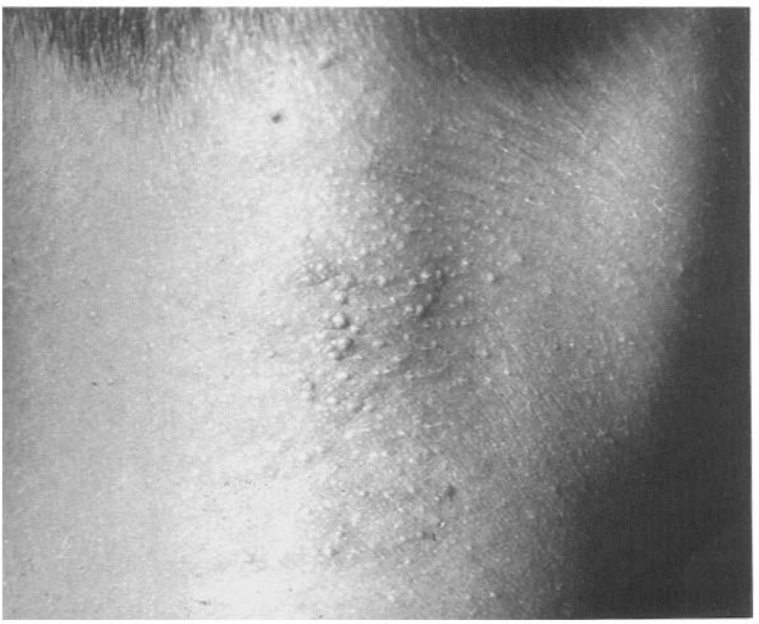

FIGURE 11.16. Miliary soft fibromata. These tiny, slightly raised papules resemble coarse "gooseflesh." These miliary lesions represent another variant of the fibrous tumors of the skin in TSC. Note also the typical ash leaf hypomelanotic macule and the subtle poliosis in the occipital scalp area.

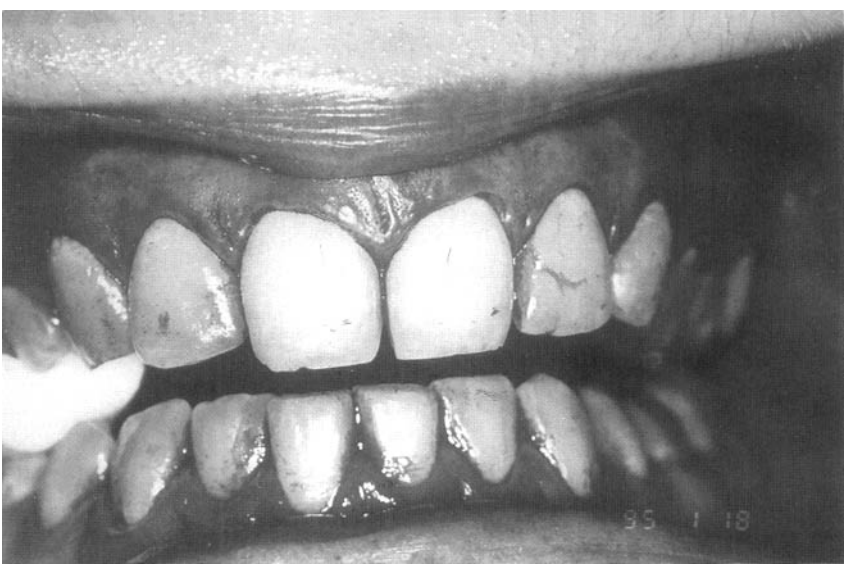

FIGURE 11.17. Dental enamel pitting. This enamel lesion is present in 90% of patients with TSC but only 9% of the general population.

TSC patients demonstrated pitting, compared to 65% of relatives and 73% of 25 controls. Seventy percent of TSC cases had greater than 14 pits per person, compared with only 5% of relatives and 4% of controls. Eighty-five percent of relatives and 84% of controls had less than six pits per person. These results suggest that, although examination for dental pits is a helpful adjunct in establishing a diagnosis of TSC, it is not a useful screening test for first-degree relatives to detect otherwise unsuspected carriers of the disease.

Other Lesions

Many cutaneous tumors, ranging from epithelial-derived appendageal tumors such as trichoepitheliomas and syringocystoadenomas to mesenchymal tumors such as pachydermodactly[29] (localized giantism), lipomata, neurofibromata, and fibromyolipomata, have been reported in association with TSC. These lesions are of no diagnostic significance but may constitute another manifestation of the "dysplastic" defect of patients with TSC, or simply represent a coincidental finding in these patients.

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12

Renal Manifestations

Introduction

Most patients with tuberous sclerosis complex (TSC) develop morphologic abnormalities in their kidneys. These lesions include angiomyolipomas (AMLs), cysts, and renal cell carcinomas (RCCs). Eventually, more than 80% of patients will be found to have renal abnormalities. Only the central nervous system is more frequently afflicted than the kidneys. Two lesions, AMLs and renal cysts, are strongly associated with TSC. Anecdotal evidence also suggests that the association between TSC and RCC is greater than that predicted by chance alone, especially since TSC-associated RCCs tend to manifest differently from sporadic ones. Thus TSC-associated RCCs present at a younger age and have a distinctive immunostaining pattern different from sporadic RCCs. Nevertheless, RCCs occur far less frequently in these patients than do either AMLs or renal cysts. Several other renal lesions have been described in patients with TSC. None, however, has been convincingly demonstrated to be significantly associated with TSC. Lymphangiomatous cysts are usually retroperitoneal in location and only secondarily involve the kidneys. Compression effect may lead to impairment of renal function.

Studies of the Eker rat may advance our understanding of the renal manifestation of TSC. These rats have an automosomal dominant syndrome of renal tumors and cysts.[1] Tumors in the pituitary, spleen, and uterus also occur. The genetic defect in the Eker rat is the insertion of a 5-kb DNA fragment into the rat homologue of the *TSC2* gene.[2,3] Loss of the wild-type *TSC2* allele has been found in both tumors and preneoplastic renal tubular cells[2] from the rats.

This chapter focuses chiefly on the most frequent renal manifestations of TSC: parenchymal and lymphatic cysts, AMLs, and RCCs. Recent developments in the immunohistology and genetics of TSC-associated lesions are addressed.

Cysts

Occurrence

Cysts are the second most common renal manifestation of TSC, following AMLs.[45] Twenty percent of males and 9% of females with TSC will present evidence of cystic disease.[6] Symptomatic renal cystic disease represents the earliest manifestation of TSC, often developing in infancy.[7]

Clinical Findings

Mass effect explains the clinical manifestations of renal cysts, causing impairment of renal function and hypertension. Children commonly present with severe hypertension that precedes the onset of functional impairment.[8] Computed tomography and magnetic resonance imaging are the diagnostic modalities of choice. By themselves, the cysts may be difficult to distinguish from autosomal dominant polycystic kidney disease on imaging studies. Imaging of autosomal dominant polycystic kidney disease seldom shows many large cysts, more often revealing hypoechoic kidneys with a few macrocysts. Severe macrocystic disease in young children most often equals TSC. The coexistence of cysts and AMLs in adults strongly supports the diagnosis of TSC.

Pathology

Cysts in TSC take their origin anywhere within the nephron.[5,9] When small, cysts tend to be clustered within the cortex, later extending into the medulla. The histopathology is characteristic and unique (Fig. 12.1). Micropapillations are common. The lining epithelial cells possess abundant eosinophilic cytoplasm and large hyperchromatic nuclei (Fig. 12.2), and mitotic figures are occasionally seen. Possibly, epithelial hyperplasia represents the initial event, leading eventually to segmental obstruction of a nephron and resulting in cyst formation. Glomerular cysts predominate in infants with unilateral presentation of cystic disease.

Management

Controlling hypertension is critical to the care of patients with severe cystic disease in TSC. Relieving parenchymal compression by surgical decompression does not lead to significant clinical improvement, at least in the short term.

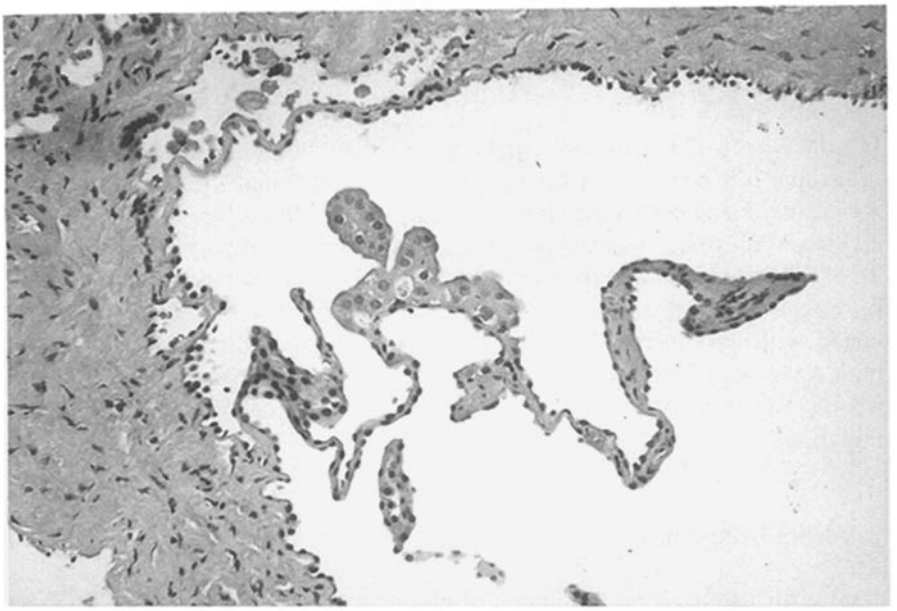

FIGURE 12.1. Renal cyst in TSC. Cystic space with papillary excrescences, lined by hobnail-type cells. (Hematoxylin-eosin, ×200)

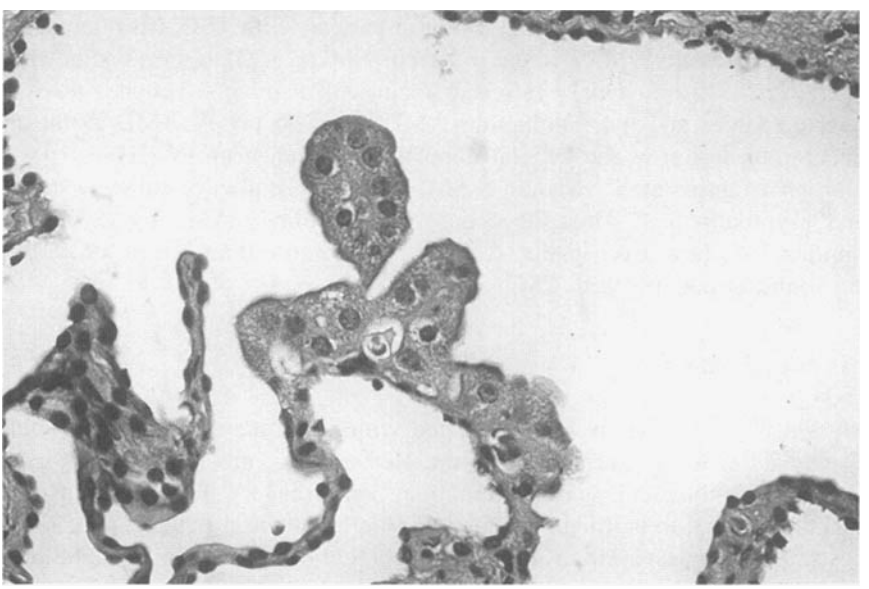

FIGURE 12.2. Renal cyst in TSC. Lining cells with large nuclei and conspicuous nucleoli. (Hematoxylin-eosin, ×400)

Genetics

In infants with TSC and severe polycystic kidney disease, a contiguous gene syndrome has been described in which both *TSC2* and the adjacent autosomal dominant polycystic kidney disease gene, *PKD1*, are deleted.[10] In a recent more extensive analysis of patients with TSC and polycystic kidneys, 23 of 28 patient had deletions of *TSC2* and part or all of *PKD1*.[11] This study raises the possibility that the *PKD1* gene, and not *TSC1* or *TSC2*, is associated with cyst pathogenesis in TSC. In the kidney, therefore, studies of both AMLs and cysts suggest potential clinical differences between tuberous sclerosis caused by *TSC1* gene mutations and that caused by *TSC2* gene mutations.

Angiomyolipomas

AMLs are localized proliferations of blood vessels, smooth muscle, and fat in varying amounts. They may be tumorous malformations rather than "true" neoplasms. By definition, a hamartoma is an abnormal proliferation of tissues indigenous to the organ of origin. Because adipose tissue is not a normal constituent of the mature kidney, however, renal AMLs are appropriately classified as choristomas.

Occurrence

AML is the most common renal lesion in patients with TSC. Eventually, up to 80% of patients will be found to harbor AMLs.[5] AML appears somewhat later in life than do renal cysts and is unusual in infants. Females tend to develop larger and more numerous AMLs than do males. AML is not an uncommon lesion in the general population, found in up to 2.1% of postmortem examinations.[12] Sporadic AML is virtually always solitary, small, and asymptomatic.[13] Thus the occurrence of solitary AML by no means implies TSC in a given patient.[14] Probably no more than 2% of all AMLs are found in patients with TSC.

Clinical Features

Most AMLs, whether or not associated with TSC, are asymptomatic. Dull flank pain is a nonspecific complaint. Hemorrhage into large tumors may lead to life-threatening exsanguinating hemorrhage.[15] Hypertension and functional renal impairment are far less frequent than in patients with renal cysts. Nevertheless, AMLs may be associated with chronic renal failure, often in conjunction with cysts. Of 65 patients with TSC and end-stage renal failure requiring dialysis, AMLs alone were present in 23% of cases, cysts alone in 18%, and both cysts and AMLs in 54%.[16] Ultrasound and computed

tomography are helpful imaging modalities, with ultrasound being more sensitive but less specific than computed tomography. The greater the amount of mature fat within a given lesion, the more reliable the distinction from RCC becomes on imaging studies.

Pathology

The gross appearance of AMLs is contingent upon the relative amounts of vessels, smooth muscle, and fat. Small and early lesions tend to contain relatively greater amounts of smooth muscle than fat. As a consequence, these are pale gray, firm, and elastic. Conversely, kidneys in older patients with TSC may contain innumerable microscopic "lipomas," poorly demarcated from the surrounding parenchyma; microscopic "leiomyomas" are less common. Large and "mature" AMLs possess a gross appearance more typical of mature fat (i.e., a lobulated, yellow, and oily cut surface). All AMLs are well circumscribed, although they may involve structures adjacent to the kidney. Microscopically, the essential feature of AML is thick-walled blood vessels, not dissimilar to those seen in vascular (arteriovenous) malformations (Fig. 12.3). These vessels, which often lack ordered elastic tissue, appear to represent the origin of smooth muscle cells that arise in their outer layers and often form a "collarette" at the periphery of the vessels. The

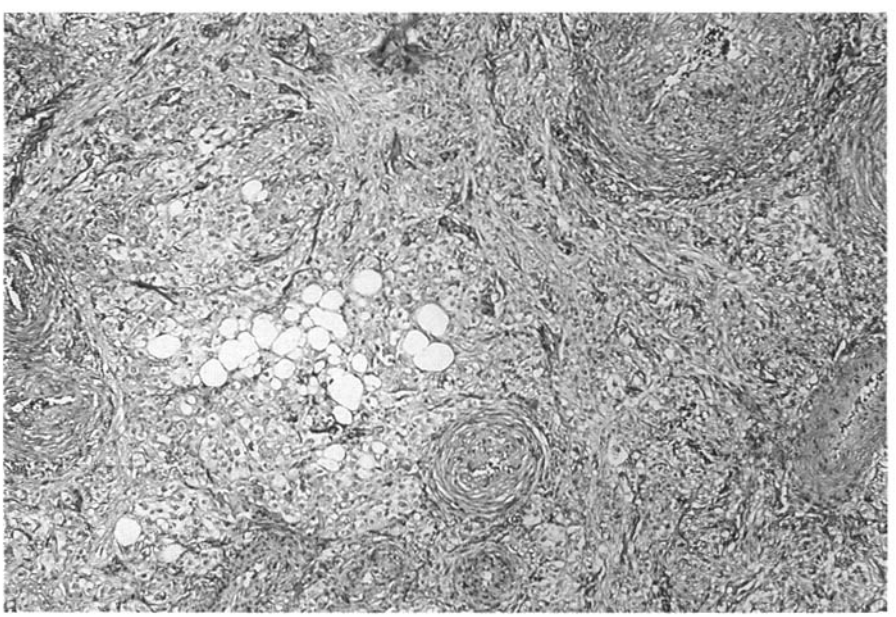

FIGURE 12.3. Angiomyolipoma in TSC. Thick-walled blood vessels. The outer vessel layer of smooth muscle cells blends into a polygonal and spindle cell population away from vessels. Mature fat is left of center. (Hematoxylin-eosin, ×200)

smooth muscle cells, often with large and hyperchromatic nuclei, not infrequently may be mistaken for components of malignant spindle cell tumors. With increasing distance of the spindle cells from the vessels, the smooth muscle nature of these cells becomes more obvious, nuclear density decreases, and, at the junction to the adipose portions, these cells blend or "differentiate" into mature fatty tissue. Immature fat, including lipoblasts, is not a feature of the fatty portion of AMLs.

On immunohistology, the smooth muscle cells are strongly decorated both by antibodies directed against cytoplasmic smooth muscle intermediate filaments (desmin) and by the contractile protein actin (smooth muscle actin). This immunoreactivity becomes progressively fainter as the cells differentiate away from the blood vessels. A marker of cell proliferation, proliferating cell nuclear antigen,[17] is typically seen in the cellular portion of the smooth muscle cells nearest the originating vessel. We and others[18–21] have found a strong reactivity, especially in the smooth muscle portions, against HMB-45 (Fig. 12.4). HMB-45 is an antibody originally described as specific for melanosomes,[22] a cytoplasmic constituent of melanocytes. Melanocytes are known to be derived from neuroectoderm (neural crest). HMB-45 appears to stain immature or abnormal melanocytic cells, such as are encountered in malignant melanoma and in junctional and atypical nevi. Typically, HMB-45 will not stain intradermal nevi and mature adult melanocytes. The spindle cell portion of AMLs is strongly decorated by HMB-45. We have also found Leu-7 positivity in these cells. Leu-7 (CD57) has

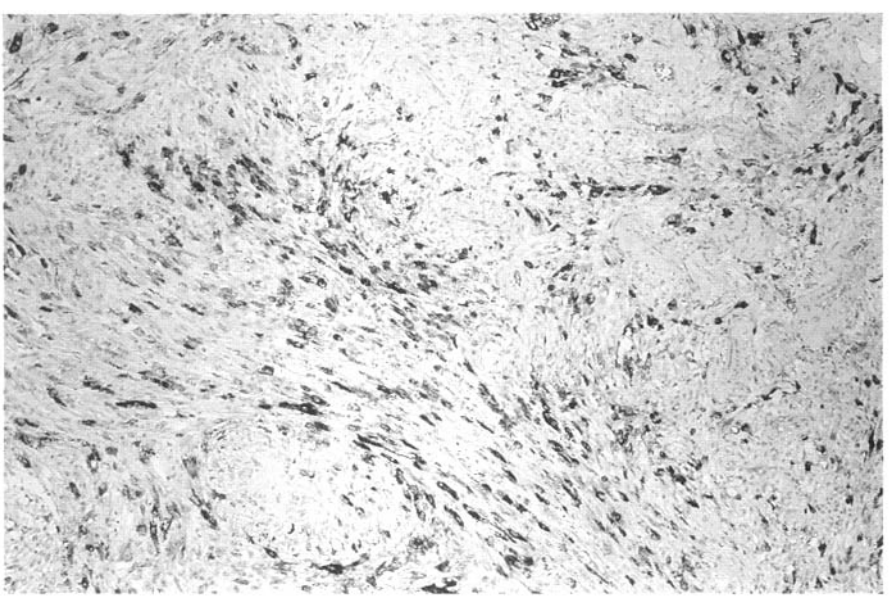

FIGURE 12.4. Angiomyolipoma in TSC. Strong cytoplasmic reaction in spindle cells against melanocyte-associated antigen. (Anti-HMB-45, ×200)

been linked to cells of neural crest derivation.[23] Both sporadic AMLs and those associated with TSC react in this fashion. This reactivity lends support to the notion that AMLs derive in some way from the neural crest.

Genetics

Loss of heterozygosity (LOH) on chromosome 9q34 (*TSC1*) or 16p13 (*TSC2*) occurs in 60% of tuberous sclerosis AMLs.[24] This is consistent with tumor suppressor gene functions for the TSC genes.[25] The germline mutation (the first hit) inactivates one copy of *TSC1* or *TSC2*. Inactivation of the remaining wild-type copy (the second hit) is detected in an LOH analysis. Specific chromosome 16p13 LOH is also found in some AMLs from patients who do not have TSC.[26] These findings indicate that, despite their three different tissue elements (vessels, smooth muscle, and fat), AMLs are clonal tumors derived from a single progenitor cell. Clonality of AMLs has also been demonstrated by nonrandom X-chromosome inactivation.[27]

In AMLs, LOH on chromosome 16p13 (the location of the *TSC2* gene) is more frequently found than LOH on chromosome 9q34 (the location of the *TSC1* gene).[26,28] In a series of 53 AMLs, 28 (57%) had 16p13 LOH and 4 (8%) had 9q34 LOH.[24] No tumors had LOH at both loci. Because most of the tumors in this study were surgical specimens, the higher frequency of 16p13 LOH compared with 9q34 LOH could indicate that *TSC2* AMLs are more often surgically removed than *TSC1* AMLs. Other possible explanations for the finding of more frequent 16p13 LOH compared with 9q34 LOH are (1) that AMLs are more frequent in *TSC2* disease; (2) that TSC in the general population is more frequently caused by *TSC2* gene mutations than *TSC1* gene mutations; or (3) that chromosome 9q34 somatic mutations are often small deletions or point mutations that are missed by an LOH analysis. Further analysis will be required to determine which, if any, of these explanations is correct.

Renal Cell Carcinomas

Occurrence

An association appears to exist between TSC and RCC. The evidence supporting this connection is anecdotal and tenuous and at present not supported by available epidemiologic statistics. Nevertheless, several features of RCC in patients with TSC are unusual enough to warrant a strong suspicion that the association is more than would be expected by chance alone. Five of 403 patients with tuberous sclerosis on file at the Mayo Clinic have had RCC. Oncocytomas also occur with TSC.

Clinical Features

The majority of patients with tuberous sclerosis and RCC have manifested symptoms referable to a renal mass. Most, however, will have had concomitant AMLs, so that the exact origin of symptoms may not be identified with certainty. They are younger by several decades than patients with sporadic RCC, and the tumors are often multicentric and bilateral. The oncocytomas are benign.

Pathology

The gross and microscopic appearances of RCCs in our seven patients[20] (six in the recent report plus one additional patient) are unusual. Six of seven patients have had at least a part of their tumor displaying conventional clear cell histology, indistinguishable from the usual and most frequent variant of RCC. Three of seven patients have had anaplastic carcinomas, with two of three displaying both sarcomatoid and clear cell features within the same tumor (Fig. 12.5). This proportion of sarcomatoid features is far greater than in sporadic carcinomas, in which the incidence of sarcomatoid degeneration is in the range of 1% to 2%.[29] On immunohistology, TSC-associated RCCs differ sharply from sporadic ones. Four of seven tumors have stained positively for HMB-45 (Fig. 12.6), in both the anaplastic and conventional clear

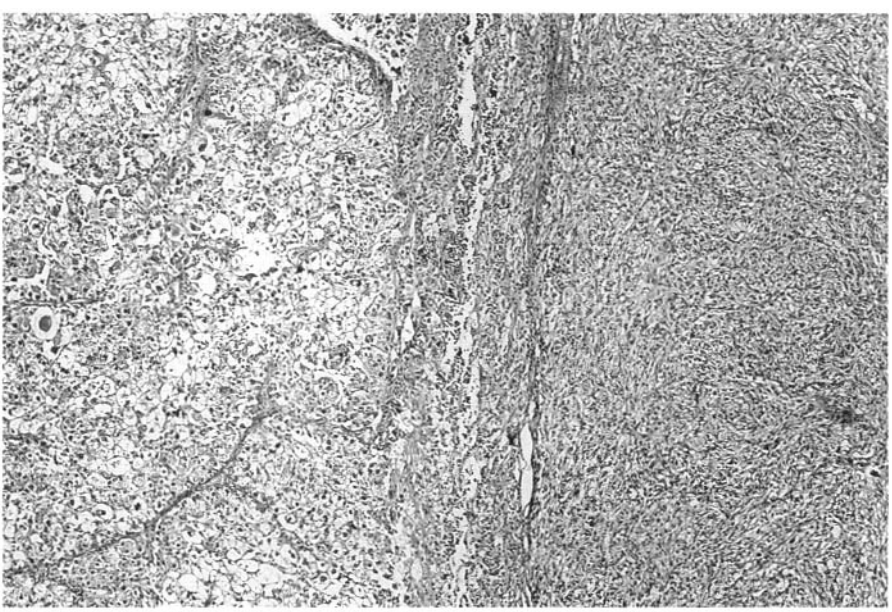

FIGURE 12.5. Renal cell carcinoma in TSC. Sharp transition from clear cell (*left*) to anaplastic/sarcomatoid (*right*) variant. (Hematoxylin-eosin, ×200)

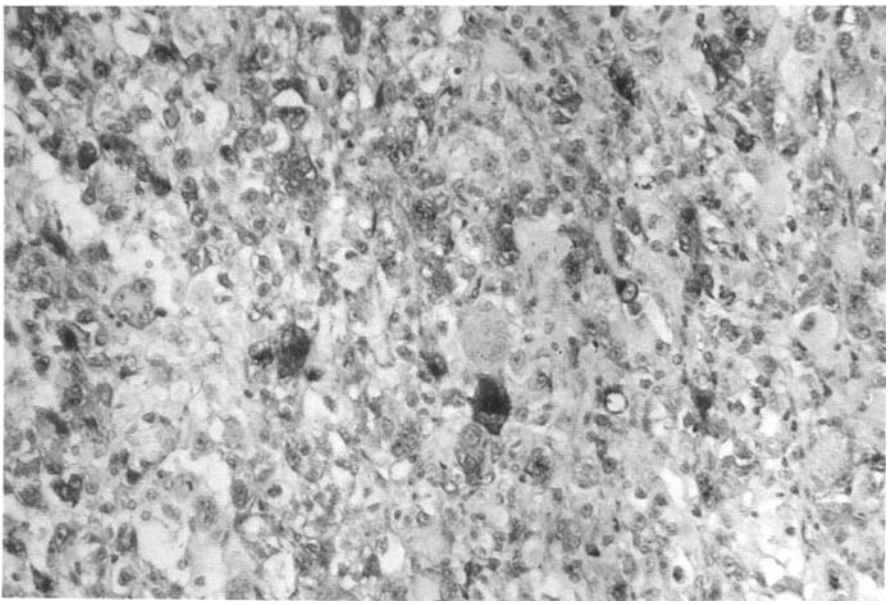

FIGURE 12.6. Renal cell carcinoma in TSC. Focal strong expression of melanocyte-associated antigen in cytoplasm of anaplastic cells. (Anti-HMB-45, ×400)

cell portions. In a control group of 10 sporadic RCCs, none stained with HMB-45 and all stained with cytokeratin markers, which tended to be negative in TSC-associated tumors.[21] In summary, an association between TSC and RCCs appears to exist. These malignant renal cell tumors tend to behave aggressively, afflict younger patients, and display a different immunohistologic profile from conventional sporadic RCCs. The latter parameter suggests an association with tissue derived from the neural crest.

Lymphangiomatous Cysts

Occurrence

Three of 403 patients with tuberous sclerosis on file at the Mayo Clinic have had retroperitoneal lymphangiomatous cysts.[6] Lymphangiomatomatous cysts are an uncommon accompaniment of TSC, yet undoubtedly related to the complex, as is extrapulmonary lymphangioleiomyomatosis.

Clinical Features

Computed tomography is the imaging method of choice and typically will reveal a combination of peri- and intrarenal cysts. Renal failure and hyper-

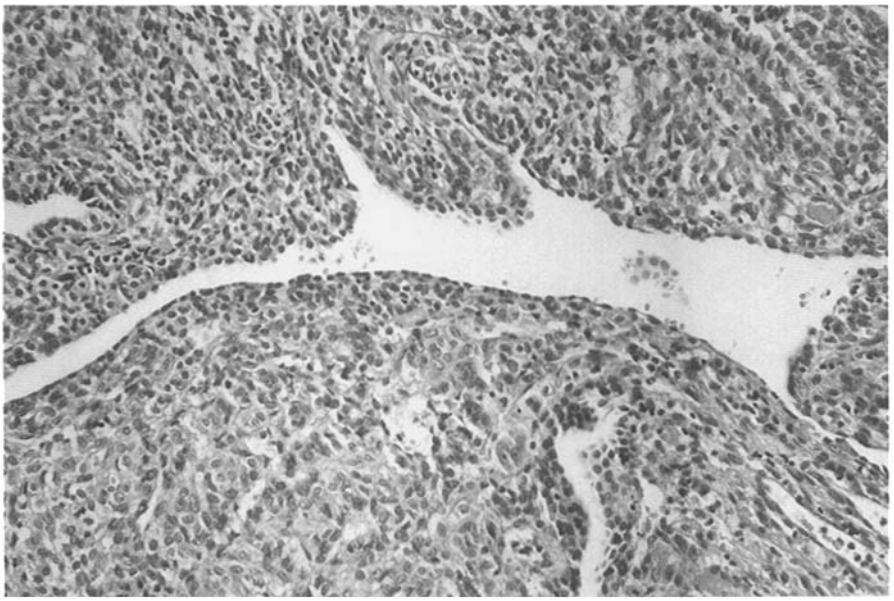

FIGURE 12.7. Lymphangiomatous cyst in TSC. Cystic space lined by flat endothelium. Solid proliferation of spindle cells between cysts. (Hematoxylin-eosin, ×200)

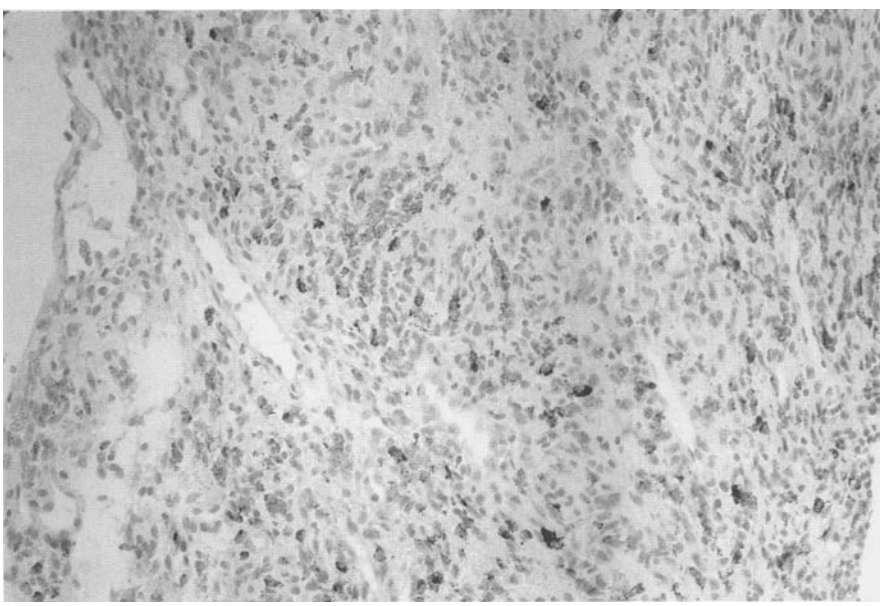

FIGURE 12.8. Lymphangiomatous cyst in TSC. Cytoplasmic reaction in spindle cells against melanocyte-associated antibody. (Anti-HMB-45, ×200)

tension are common clinical presentations. As is the case with lymphangioleiomyomatosis, renal lymphangiomatosis has been known to exacerbate during pregnancy.[30]

Pathology

The cystic and slitlike spaces in lymphangiomatous cysts are lined by flat endothelial cells (Fig. 12.7). The vascular endothelial nature of the lining cells places them apart from the epithelial cells lining TSC-associated cysts. The solid portions consist of compact proliferations of spindle cells. This latter cell population represents the proliferating portion of the lesion as evidenced by conspicuous proliferating cell nuclear antigen expression. The melanoma-associated marker HMB-45 is consistently expressed in the cytoplasm of the spindle cells (Fig. 12.8), along with intermediate filaments (desmin) and contractile proteins demonstrating the muscular derivation of this cell population. This immunoprofile is similarly present in AMLs and lymphangioleimyomatosis, both pulmonary and extrapulmonary.[31]

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13

Cardiac Manifestations

The frequent association of tuberous sclerosis (TSC) and cardiac rhabdomyoma has been recognized for many years. In one series of 40 autopsied cases of TSC there were 12 (30%) with rhabdomyoma of the heart.[1] Conversely, pathologic examination of the brain demonstrated that 11 of 30 patients (37%) with cardiac rhabdomyoma had TSC.[1] Approximately 50% of infants with TSC have been reported to have rhabdomyoma.[2–4] The understanding of the natural history of rhabdomyoma associated with TSC has improved greatly in recent years as a result of the ability to visualize the tumors noninvasively utilizing echocardiography. Older studies based on autopsy data had suggested a poor prognosis.[1] However, echocardiographic studies[3–6] in recent years have suggested a much brighter outlook for afflicted patients, with frequent reports of tumor regression and disappearance, especially in younger patients.

Cardiac rhabdomyoma is the most common cardiac tumor found in infants and children[7]; myxoma is the most common cardiac tumor found in adults. If four reports of large series of primary cardiac tumors in children are combined, 62% (89/144) of the patients had rhabdomyoma.[8] Rhabdomyoma is more frequently found in males than in females; the ratio is nearly 2:1.[1] On occasion, a family history of this cardiac tumor is elicited; at least twice it has been reported to occur in two siblings,[1,9] all of whom had the characteristic findings of TSC.

Clinical Presentation

In the Fenoglio et al. autopsy series of 36 patients with cardiac rhabdomyoma,[1] the largest published series from one institution, 78% of the patients died during the first year of life and only three patients lived beyond age 5 years. Thirty-three of the 36 patients (92%) had multiple cardiac rhabdo-

myomata. Thirteen of these patients either were stillborn or died during the first 24 hours of life, and in nine of them death was clearly due to one or more large intracavitary tumors that obstructed blood flow through the heart. In this series, however, 9 of the 11 patients with pathologic evidence of cerebral tuberous sclerosis had solely intramural tumors, and only 2 (18%) had intracavitary tumors. In contrast, 16 of 25 patients (64%) with rhabdomyoma but without evidence of TSC had one or more intracavitary tumors. These data suggest that a significant proportion of the patients who have TSC with cardiac rhabdomyoma may have tumors confined to the myocardium. These patients, therefore, may have a better outlook than the general population who have cardiac rhabdomyoma, a large percentage of whom have obstructing intracavitary tumors producing cardiac symptoms and causing early death.

Rhabdomyoma in the patient who has TSC may originate in any of the four cardiac chambers. However, tumors are more common in the ventricles than in the atria and are slightly more frequent on the left side of the heart than on the right.[1,2,4]

The TSC patient with cardiac rhabdomyoma may exhibit no cardiac symptoms; echocardiographic evidence suggests that this is true in a substantial majority of afflicted patients. If cardiac signs and symptoms in the patient with cardiac rhabdomyoma are present, however, they are explained by one or more of the three following mechanisms: obstruction of blood flow through the right or left heart, or both, secondary to an obstructing intracavitary tumor; myocardial involvement with secondary deterioration of ventricular function; and disturbances of cardiac rhythm.

Both Kuehl et al.[10] and Shaher et al.[9] reported severe subvalvular aortic stenosis secondary to rhabdomyomata of the left ventricular outflow tract. Kuehl et al. reported a patient who died on the fourth day of life and Shaher et al. reported a 9-month-old infant who underwent successful surgical removal of the obstructing tumors. Foster and colleagues[11] reported an infant who had resection of an intracavitary left ventricular outflow tract rhabdomyoma at 4 days of age and who continues to do well postoperatively, despite the finding at surgery of additional left ventricular intramural tumors that were not resected. Recent echocardiographic evidence suggests that intramural tumors will frequently become smaller with increasing age and may disappear completely.

Case reports also document intracavitary tumors obstructing the tricuspid valve,[9] the right ventricular outflow tract,[9,12] and the mitral valve.[13] Patients with severely obstructing tumors may die during early infancy, but successful surgical removal of the tumors has been accomplished with increasing frequency.[9,11,12] Lababidi et al.[14] described successful surgical removal of two right ventricular outflow tract rhabdomyomata in a 6-day-old infant, and Foster et al.[11] reported a successful surgical result in a 2-day-old infant in whom a tumor obstructing the tricuspid valve was removed.

Figure 13.1 shows photographs of the heart from an infant who died at 8 days of age.[13] This infant had tuberous sclerosis of the brain that was

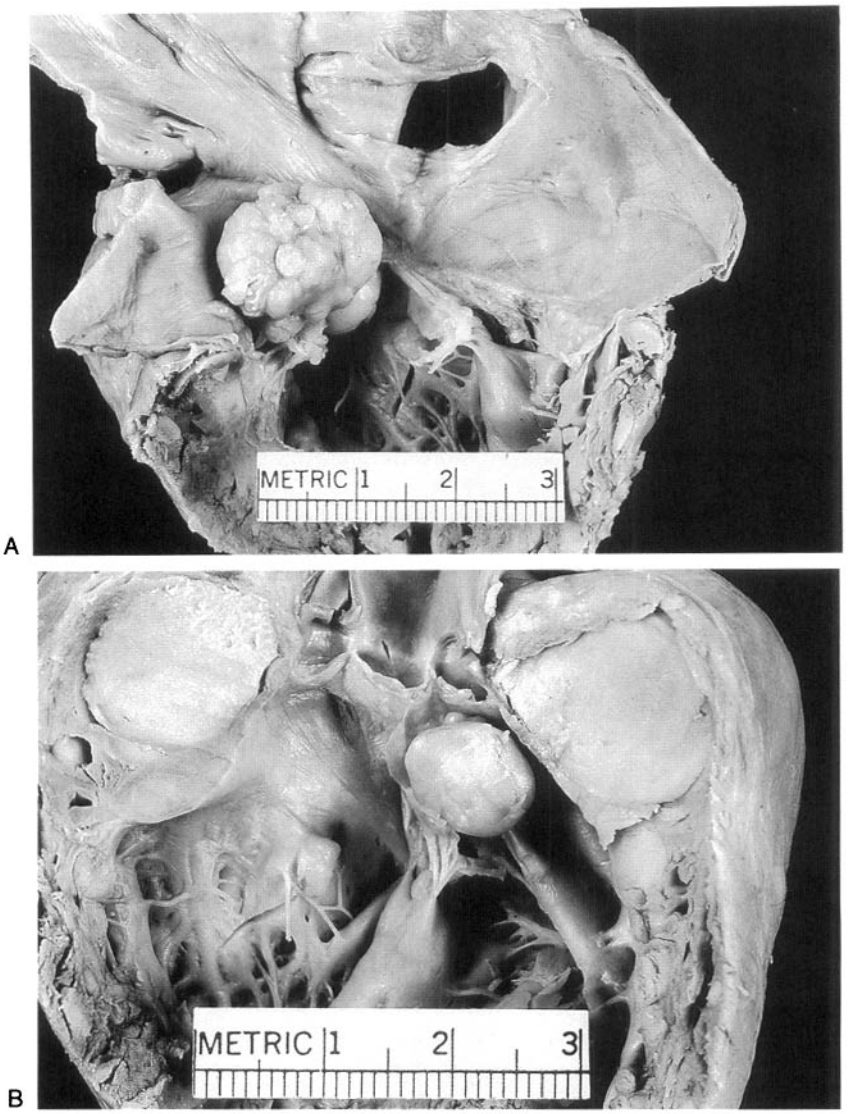
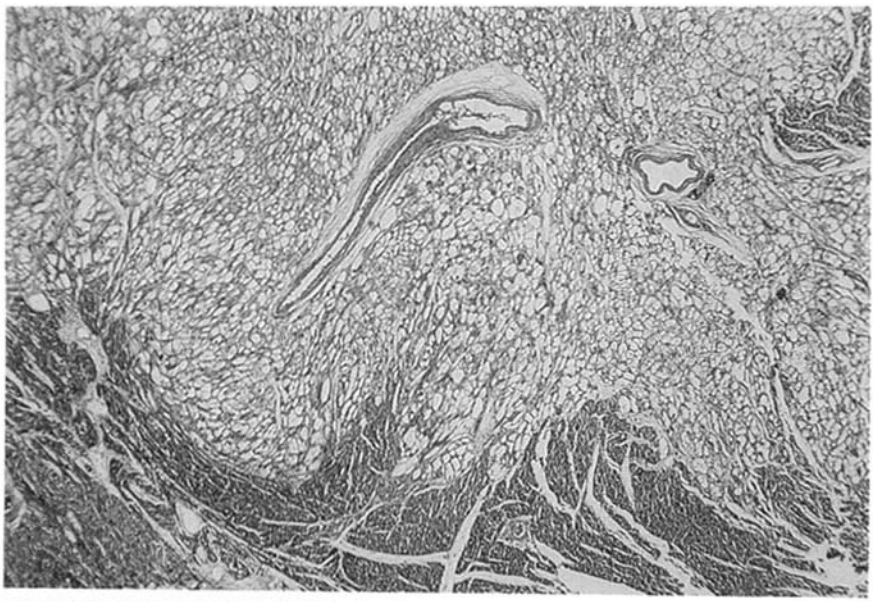
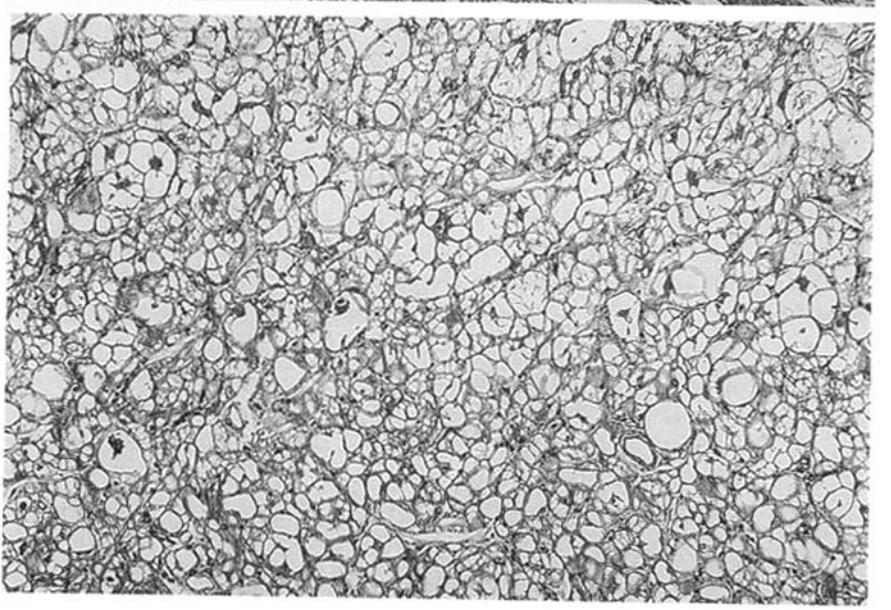


FIGURE 13.1. Heart of an 8-day-old infant with TSC. *A*, Opened left atrium and left ventricle demonstrate a large intracavitary pedunculated tumor that completely obstructed the mitral orifice, simulating mitral valve atresia. *B*, Opened left ventricle and aorta show a globular tumor that was contiguous with the pedunculated tumor shown in *A*. A large intramyocardial and intracavitary tumor located just below the aortic valve, which produced severe subartic stenosis, has been bisected. *Illustration continued on opposite page*

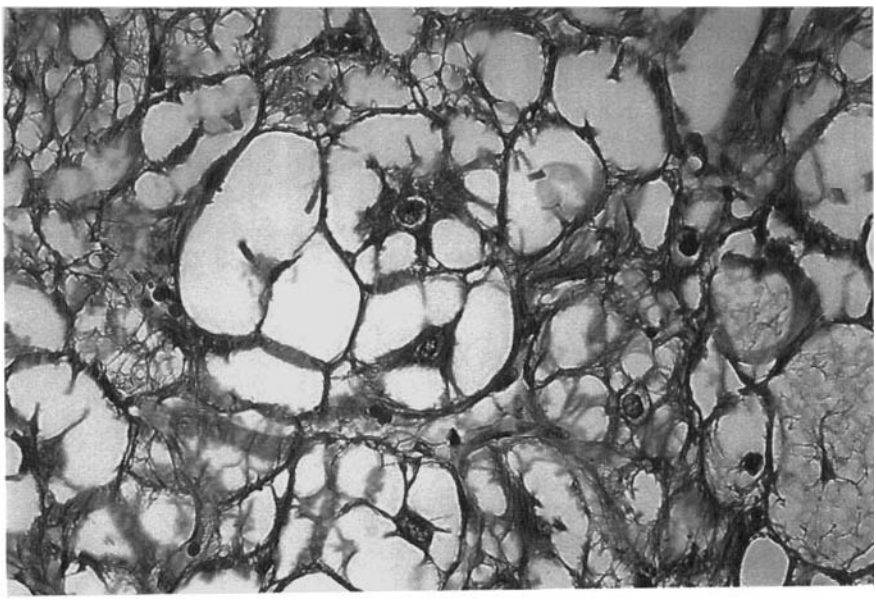


C



D

FIGURE 13.1. (*continued*) *C*, Low-power photomicrograph shows sharp demarcation of normal myocytes (*below*) from tumor cells (*above*). *D*, Medium-power photomicrograph demonstrated classic "chicken wire" appearance of the tumor. *Illustration continued on following page*



E

FIGURE 13.1. (*continued*) *E*, High-power photomicrograph shows so-called spider cells of rhabdomyoma with centrally placed nucleus and adjacent sarcoplasm forming the "spider's" body and peripheral streamers of sarcoplasm forming its legs.

confirmed by postmortem examination. The primary cause of death was a pedunculated rhabdomyoma (1.2 cm in diameter) in the left atrium, which completely occluded the mitral valve orifice and simulated mitral valve atresia. The infant also had multiple intracavitary and intramural tumors. Even if the left atrial tumor had been diagnosed and removed during life, the patient would not have survived because there were multiple tumors in the left ventricular outflow tract that produced severe subaortic obstruction and that did not seem surgically resectable.

Congestive heart failure resulting from myocardial involvement is uncommon in patients with intramural tumors. Shaher and coworkers[9] reported an infant who died at 1 day of age whose left ventricular myocardium had been almost entirely replaced by a large tumor. Van der Hauwaert[15] reported three patients with cardiac decompensation secondary to myocardial dysfunction. In an occasional patient without obstructing intracavitary tumors who survives for some time, the initial cardiac symptoms may mimic a cardiomyopathy because the ventricular muscle has been replaced by noncontractile tumor tissue. However, this clinical presentation is unusual; more commonly such intramural tumors become smaller and less clinically significant with the passage of time.

Cardiac dysrhythmias reported in patients with rhabdomyomata include atrial tachycardia,[9] ventricular tachycardia,[7] complete heart block,[7] junctional ectopic beats,[16] and ventricular fibrillation.[17] These disturbances of rhythm

are usually seen in patients with intramural rhabdomyomata in whom the tumor tissue interrupts the conduction pathways, giving rise to ectopic electrical foci, or leads to an accessory electrical pathway producing preexcitation (Wolff-Parkinson-White syndrome). Case and colleagues[18] reported a 9-month-old infant with multiple rhabdomyomata who presented with a complex supraventricular and ventricular arrhythmia and who, despite aggressive medical therapy, surgical ablation, and pacemaker placement, died of congestive heart failure. Thus, although uncommon, dysrhythmic deaths can occur in these patients. One TSC patient from our institution's series had a normal echocardiogram at 11 months of age and died suddenly 18 months later. Autopsy revealed multiple very small cardiac rhabdomyomata that conceivably could have triggered a fatal dysrhythmia. Thus, despite ever-improving technology, echocardiographic resolution limitations may still occasionally result in some underdiagnosis of tumors, in that very small tumors may not be visualized.

An interesting phenomenon reported in recent years is the association of Wolff-Parkinson-White syndrome with TSC. This has been seen in TSC[19,20] patients with and without cardiac rhabdomyoma.[15] Some of the cells in the cardiac rhabdomyomata found in patients with TSC are structurally similar to normal Purkinje cells. These Purkinje-like tumor cells apparently may function as an anomalous conducting bundle and produce the preexcitation pattern characteristic in the surface electrocardiogram of patients with Wolff-Parkinson-White syndrome. The anomalous bundle comprised by these cells may serve as a reentry circuit and lead to the paroxysmal supraventricular reentry tachycardia that often produces troublesome symptoms in patients with the Wolff-Parkinson-White syndrome.

It has been suggested that rhabdomyoma may be a source of emboli that can cause stroke in young patients.[21] However, in a large group of patients followed at our institution, there has been no history of stroke or other embolic events.

Pathology

Cardiac rhabdomyomas occur almost exclusively in the pediatric age group. They are thought to originate from embryonic cardiac myocytes and to represent fetal hamartomas rather than true neoplasms. Moreover, ultrastructural studies indicate that they are not Purkinje cell tumors and do not represent a localized form of glycogen storage disease. Cardiac rhabdomyomas do not undergo malignant sarcomatous transformation.

Grossly, the tumors form gray-white to yellow-tan lesions that vary from several millimeters to several centimeters. Although they may be irregularly shaped, they appear well demarcated from the surrounding myocardium. Ventricular involvement is more common than atrial, and the tumors may be entirely intramural or may protrude from the epicardial or endocardial surfaces (Fig. 13.1*A*,*B*). In the autopsy series of 36 cases reported by Fen-

oglio et al.,[1] multiple tumors are observed in 92%, and in 50% at least one cardiac tumor protruded into a cardiac chamber. Such intracavitary tumors may commonly cause chamber obliteration or valvular obstruction. Intramural tumors may impinge on elements of the cardiac conduction system. Consequently, the anatomic substrate is provided for the development of heart failure, arrhythmias, cardiomegaly, and valvular obstruction.

By light microscopy,[22,23] cardiac rhabdomyomas are circumscribed but nonencapsulated clusters of glycogen-filled myocytes. The tumor cells are larger than normal myocardial cells and, by virtue of the dissolution of glycogen during routine tissue processing, typically are characterized by a "chicken wire" appearance (Fig. 13.1*C*,*D*) and by so-called spider cells (Fig. 13.1*E*).

By transmission electron microscopy,[1] numerous intercellular junctions are observed that resemble intercalated discs. However, they are found not only at the two poles of the tumor cells but also along the sides. In fact, the cells tend to be polygonal or ovoid and not to attain the cylindrical shape of normal myocardial cells. Thus they resemble cardiac myoblasts. Contractile elements are displaced by abundant sarcoplasmic glycogen.

Diagnosis

In the infant presenting with cardiac symptoms caused by obstructing intracavitary tumors or poor ventricular function secondary to myocardial replacement with tumor tissue, the clinical picture is one of severe congestive heart failure. These infants may exhibit, often in the first few hours after birth, tachypnea, tachycardia, cyanosis, hepatomegaly, and decreased peripheral pulses. Auscultation may reveal a systolic murmur if there is obstruction to right or left ventricular outflow, or a diastolic murmur if one of the atrioventricular valves is obstructed. At times, no murmur is audible but a prominent third or fourth heart sound may be heard. Crepitant rales are often heard over the lung fields. The chest radiograph usually shows cardiomegaly. The pulmonary arterial vascularity may be reduced if there is a right-to-left shunt through a patent foramen ovale, secondary to an obstructing right heart tumor. Obstructing left-sided tumors may produce the picture of pulmonary venous engorgement. Tumor calcification, sometimes observed with cardiac fibromas, has not been described with rhabdomyoma. Occasionally, a bulging tumor mass on the cardiac border produces a clearly abnormal cardiac configuration.

The electrocardiographic findings are extremely variable. Ziegler described diminutive left ventricular potentials.[24] This finding was also exhibited by a patient seen at our institution (Fig. 13.1) who had functional mitral valve atresia. Other cases have demonstrated signs of left ventricular hypertrophy on the electrocardiogram.[9] Abnormalities of cardiac conduction, such as a long P-R interval or prolonged QRS complex duration (right or

left bundle-branch block), are sometimes seen. Complete heart block has been reported, as has the Wolff-Parkinson-White syndrome.[19, 25]

In recent years, two-dimensional echocardiography has become the diagnostic procedure of choice for cardiac tumors.[2–6] This modality allows visualization of intramural and intracavitary tumors and may be used to establish the diagnosis of cardiac rhabdomyomata in tuberous sclerosis patients who have no cardiac symptoms. In 14 consecutive patients with TSC who were examined by two-dimensional echocardiography at the Mayo Clinic in one year, 6 had evidence of cardiac rhabdomyomata, although none had cardiac symptoms.[4] The tumors were circumferential, of variable diameter (0.3 to 2.5 cm), and with a hyperrefractile appearance. They were multiple (two to six masses) in five of the six patients. Cardiac rhabdomyomata were found in the right atrium (one patient), right ventricle (four patients), main pulmonary artery (one patient), and left ventricle (five patients, including one with encroachment into the left ventricular outflow tract). Representative two-dimensional echocardiograms from two of these patients are shown in Figures 13.2 and 13.3.

Bass et al.[2] reported that 8 of 16 consecutive patients with TSC showed echocardiographic evidence of cardiac rhabdomyomata. Multiple tumors were found in three of the eight. The left ventricle was involved in five

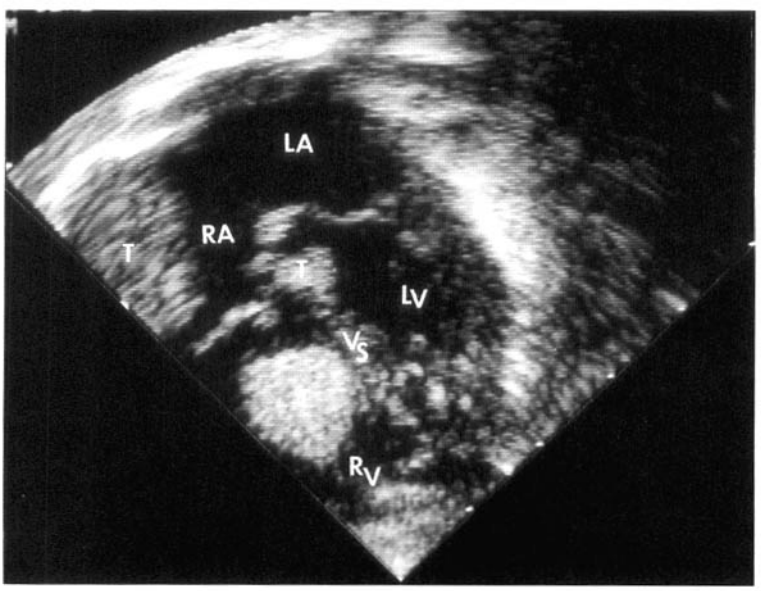


FIGURE 13.2. One-day-old infant with multiple intramyocardial and intracavitary tumor (T) masses. These tumors were first identified on a fetal echocardiogram, obtained because the mother had TSC. The tumor masses, largest in the right heart, have decreased in size with the passage of time and the child remains asymptomatic. LA = left atrium; RA = right atrium; LV = left ventricle; RV = right ventricle; VS = ventricular septum.

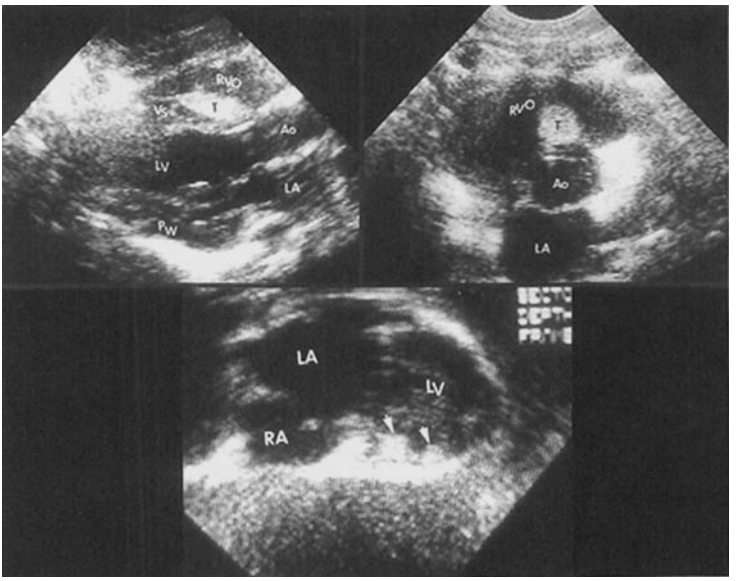


FIGURE 13.3. Two-dimensional echocardiogram in an 11-month-old infant with TSC. *Upper left*, Parasternal long axis view. An intramyocardial and intracavitary tumor (T) is seen in the right ventricular portion of the septum. *Upper right*, Parasternal short axis view. An intramyocardial and intracavitary tumor (T) is seen in the right ventricular outflow. *Lower*, Subcostal four-chamber view demonstrating two intramyocardial tumors (*arrows*) in the free wall of the right ventricle. The patient remains symptom free. RVO = right ventricular outflow; VS = ventricular septum; AO = aorta; LA = left atrium; LV = left ventricle; PW = posterior wall; RA = right atrium.

patients. Of the total of 14 masses seen in the eight patients, 6 were intracavitary and 8 were intramural; no atrial masses were seen.

Because of the high incidence of cardiac rhabdomyomata in patients with TSC, and the ability to diagnose this tumor noninvasively by using echocardiography, it is now our recommendation that all patients with TSC undergo at least one two-dimensional echocardiograpic examination to search for cardiac tumors. In addition, such echocardiographic examinations will allow serial follow-up of identified tumors, many of which will tend to decrease in size or disappear completely with the passage of time.[4-6]

There have been a number of reports of cardiac rhabdomyomata diagnosed in utero utilizing cardiac ultrasound[26-28]; indeed, our patient shown in Figure 13.2 had his tumors identified utilizing fetal ultrasound. Fetal echocardiography is beginning to play a large role in the prenatal diagnosis of cardiac malformations in cases that are considered to be at high risk because of family history of TSC. The ability to make the diagnosis of cardiac tumor at a relatively early age of gestation allows the consideration of therapeutic abortion if this desire has been expressed by the parents, and also allows

early planning of treatment if the fetus is to progress to term. It is our recommendation that, in all cases in which fetuses are considered to be at high risk for TSC and cardiac rhabdomyomata, echocardiographic screening be carried out relatively early in the pregnancy so that, if cardiac tumors are present, the diagnosis can be made prenatally and appropriate plans then established on the basis of this examination.

With the advent and refinement of echocardiography and Doppler ultrasound techniques, cardiac catheterization is no longer necessary to establish a diagnosis of cardiac rhabdomyoma or delineate its hemodynamic manifestations. Indeed, echocardiography is far superior to angiography in imaging these tumors because it allows visualization of intramyocardial in addition to intracavitary tumors and can pick up small tumors previously not seen angiocardiographically.

The echocardiographic findings outlined above are not exclusive for rhabdomyoma, but are shared with other cardiac tumors such as fibroma, myxoma, and sarcoma. However, in the young infant the odds strongly favor the presence of a rhabdomyoma. This diagnosis is almost certain in any young patient with TSC and a cardiac tumor.

Treatment

No treatment is necessary for asymptomatic patients with echocardiographically established tumors; indeed, most patients may exhibit regression in size and number of their tumors or even complete disappearance with the passage of time.[4–6] This is particularly true in infants and young children. Periodic echo-Doppler follow-up of such asymptomatic patients is recommended, however.[4,5]

Only medical treatment is recommended for the patient who has significant symptoms that are caused by intramural cardiac rhabdomyomata. Patients with congestive heart failure resulting from myocardial replacement, a rare occurrence, are treated with digitalis, diuretics, afterload reducers, and salt restriction. The response to this medical program varies depending on the amount of myocardial involvement. Often, satisfactory cardiac compensation can be maintained for some time, and some patients may eventually outgrow the need for medical support if their tumors, as is often the case, decrease in size and number. Intramural tumors producing symptomatic cardiac rhythm disturbances are treated with antidysrhythmic drugs, the choice of medication depending on the type of cardiac dysrhythmia. A cardiac pacemaker may be indicated for the patient with complete heart block or a ventricular dysrhythmia refractory to medication. Patients with paroxysmal tachycardia secondary to the Wolff-Parkinson-White syndrome may require division of the anomalous conduction pathway if the tachycardia is refractory to medical control. It may be possible to accomplish this division in the cardiac laboratory using catheter ablation techniques; if this is not possible, surgical division of the pathway utilizing thoracotomy may be considered.

Patients with critically obstructing intracavitary tumors producing heart failure who do not respond to medical management may need attempt at surgical removal of the tumor. These patients, who are uncommon, are often critically ill newborns with a high operative risk.[10,11,13] A significant proportion of such patients have multiple obstructing tumors, as did our patient (Fig. 13.1). The chance of obtaining a successful surgical result depends upon the size, number, and location of the obstructing tumor masses. However, because successful surgical removal of obstructing rhabdomyomata during infancy has been reported[10,11,29] with increasing frequency in recent years, an aggressive surgical attempt seems indicated in the critically ill patient because the patient's prognosis for survival without operation is poor. In such patients, only removal of the obstructive intracavitary tumor is recommended, with no attempt made to resect associated intramural tumors. A number of infants with severely obstructing intracavitary tumors who have been treated in this way are now alive and doing well some years after operation.

Infant cardiac transplantation has also become an option for the severely afflicted neonate and has recently been successfully carried out.[30]

Summary

Approximately 50% of patients with TSC have cardiac rhabdomyomata. Rhabdomyoma is also the most common cardiac tumor of infancy and early childhood. Most TSC patients with rhabdomyoma will be asymptomatic, and in such patients serial echocardiographic studies will often demonstrate regression in size and number of the tumors or in some instances complete disappearance with the passage of time.

Although signs and symptoms from cardiac rhabdomyomata are found in a minority of cases, if they are present they are produced by one or more of three mechanisms: intracavitary tumors obstruct blood flow through the heart; intramural tumors may cause loss of mechanical contractility by myocardial replacement; and cardiac rhythm disturbances can also be produced by intramural tumors. Symptomatic patients usually display signs of congestive heart failure. Although results of physical examination, chest radiograph, and electrocardiogram are abnormal, they are usually not specific for the diagnosis of cardiac tumor. The two-dimensional echocardiogram, in contrast, is a definitive test for making the diagnosis of both intracavitary and intramural tumors. This procedure has become the gold standard in patients suspected of having cardiac rhabdomyomata, and it is now our recommendation that all patients with TSC have at least one echocardiographic examination to search for these tumors. In addition, the advancement of fetal echocardiography with the ability to diagnose these tumors in utero has led to a recommendation that all fetuses at high risk on the basis of family history have fetal echocardiography carried out so that the diagnosis can be made prenatally. If tumors are present, secondary appropriate planning regarding subsequent management of the afflicted infant can be accomplished.

Treatment is medical for symptomatic patients with only intramural tumors producing heart failure or a cardiac dysrhythmia. The exception is the patient with Wolff-Parkinson-White syndrome, who may require catheter ablation or surgical division of the anomalous conducting bundle, or the patient with complete heart block or a life-threatening ventricular dysrhythmia, who may need a cardiac pacemaker.

For severely symptomatic infants with a massively obstructive intracavitary lesion, surgical removal of the obstructing tumor or cardiac transplantation offers some hope of survival. Although the risk of surgery in such patients is high, because many of them present as critically ill neonates and the tumors may be multiple, an increasing number of cases are being reported in which successful resection has been carried out and good results obtained for several years after surgery. A recent successful heart transplantation in such an infant has also been reported. Pathologic evidence continues to support the hypothesis that cardiac rhabdomyoma represents a fetal harmatoma rather than a true neoplasm. No malignant degeneration of this tumor has ever been reported.

There are several reports of infants whose cardiac rhabdomyomas increased in size or in volume after receiving intramuscular injections of ACTH (corticotropin) for the treatment of infantile spasms.[31,32]

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14

Pulmonary Tuberous Sclerosis

Introduction

Désiré-Magloire Bourneville (1840–1909), a noted French physician, gave the first detailed description of the clinical syndrome of tuberous sclerosis in 1880.[1] Just over 50 years later, virtually all the pathologic lesions currently considered as parts of the tuberous sclerosis complex (TSC) had been observed and were summarized in tabular form by Van der Hoeve in 1933.[2] In either the classic or the *forme fruste* type of TSC, a constellation of hamartomatous and/or neoplastic lesions occurs in almost every organ system of the body, including the brain, eyes, heart, lungs, kidneys, digestive tract, endocrine glands, bones and, more recently, the blood vessels.[3] Now, more than a century after Bourneville's seminal report, we have come to realize that pulmonary tuberous sclerosis, although uncommon, is one of the more important causes of morbidity and mortality of female patients with TSC at the prime of their lives.[3–5]

Pulmonary tuberous sclerosis was first reported by Rene Lutembacher (1884–1969) in 1918.[6] He described cystic and nodular lesions in the lungs of a 36-year-old woman who had a 2-year history of progressive dyspnea before she died from bilateral spontaneous pneumothorax. This patient also had renal tumors (presumably angiomyolipomas); these and the lung lesions were described by Lutembacher in some detail, but he was unaware of their link to TSC and had misinterpreted the pulmonary lesions as metastatic "fibrosarcoma" of the kidney. In 1939–1941, Berg and Vejlens[7–9] were the first to describe the clinical and chest radiographic features of cystic change and an overgrowth of connective tissue in the lungs of patients with TSC.

Pulmonary involvement is among the least common manifestations of TSC and, up to 1971, it had been estimated to occur in about 1% of all cases.[10–18] To date, probably little more than 120 cases of pulmonary tuberous sclerosis have been documented in the literature since 1971.[19–41] The

clinical, chest radiographic, and pathologic features of pulmonary tuberous sclerosis are almost identical to those of the so-called isolated or idiopathic cases of pulmonary lymphangiomyomatosis (LAM). Therefore, most investigators now consider pulmonary LAM to be a *forme fruste* of TSC, with only an occasional dissension[26] or an uncommitted opinion.[25]

Clinical Manifestations

Clinically, patients with pulmonary lesions belong to a distinct subset of all those with TSC. They are almost all women with an average age of between 30 and 35 years at diagnosis; very few have mental retardation, and most were not diagnosed initially as having TSC or another central nervous system disorder.[3–5,11–13,18–20,22–41] There has been some uncertainty as to whether all patients presenting with pulmonary LAM indeed have TSC, but many of those who did show a few signs of TSC, when specifically looked for, had children or other members of their families overtly affected by TSC.[11,17,29,36]

In one of the earlier clinical and genetic studies of TSC, Borberg [10] reported the incidence of 37 patients with pulmonary tuberous sclerosis; among them 26 (70%) had all the classic features of TSC, including mental retardation, and 11 (30%) had only cutaneous and visceral manifestations of TSC. None of the 26 mentally defective patients showed pulmonary involvement, but 4 of the other 11 patients who died demonstrated pulmonary LAM at autopsy. Dawson[11] analyzed the medical records and available pathologic material of 79 TSC patients. Of the 72 patients with mental retardation, 39 were still living and 33 had died; of the latter, 25 (76%) had adequate medical records and complete autopsy, and none showed pulmonary LAM. Only 1 of the 39 living patients exhibited chest radiographic evidence of pulmonary involvement; of the 7 patients without mental retardation, 1 had evidence of pulmonary involvement. Although pulmonary symptoms in TSC are of late onset, as are the chest radiographic findings, Dawson[11] did not believe that death at an earlier age in mentally defective patients accounted for the infrequency of pulmonary involvement in his own or Borberg's[10] series of cases.

TSC patients with pulmonary involvement usually present with dyspnea or pneumothorax. Chylothorax occurs more frequently in patients who have LAM but without the clinical stigmata of TSC at the time of diagnosis.[16,20,27] Respiratory insufficiency is often severe and generally progressive[5,18,29,37]; the average survival from the age of diagnosis to death in older series was 3 to 5 years.[11,18] With effective hormonal therapy and the option of lung transplantation, most pulmonary TSC patients now live longer, with the median time since the onset of LAM among the survivors approaching 10 years.[5,37,39]

The chest roentgenogram in all pulmonary TSC patients typically shows increased markings of the lung parenchyma, consisting of a meshwork of lines either in a close pattern or more open, as a honeycomb, or even with frank cystic appearance (Fig. 14.1). The chest radiographic changes are usu-

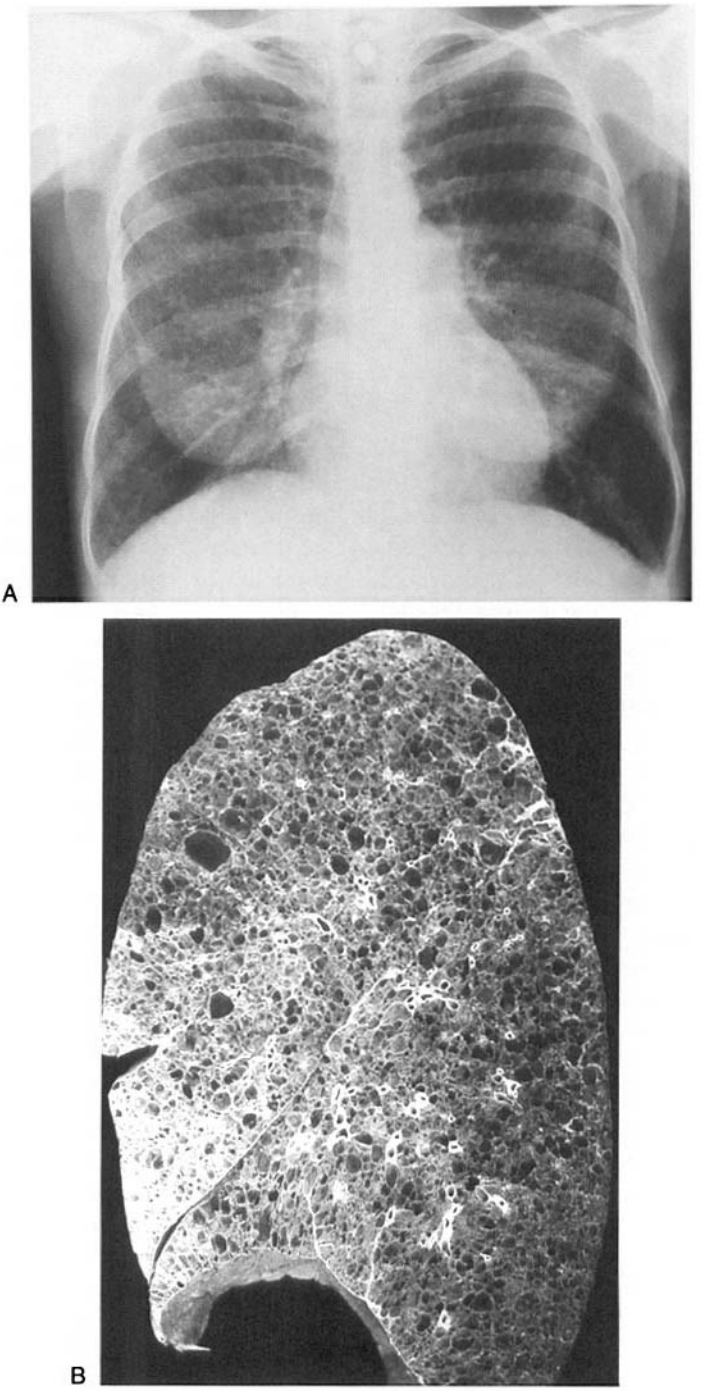

FIGURE 14.1. *A*, Chest roentgenograph of a patient with pulmonary tuberous sclerosis showing bilateral diffuse reticulonodular densities. *B*, Cut surface of right lung from the same patient at autopsy showing honeycomb of large and small cysts and scattered small nodules in the lung.

ally diffuse but may be confined to a localized lung field. Sometimes, in sequential follow-up radiographs, the fine reticular pattern may be observed to progress to a honeycomb pattern over a period of months. The cystic changes are more conspicuous with the presence of a pneumothorax because the normal alveoli would deflate but the air-distended cysts do not. It has long been suggested that the characteristic radiographic findings of TSC lesions in the brain, chest, kidneys, and skeletal system may be diagnostic in and of themselves even when the disease is unsuspected clinically.[14]

Pathologic Features

Among patients with pulmonary tuberous sclerosis, 80% had adenoma sebaceum, 60% had renal angiomyolipomas, and 25% had retinal phakomas.[11,18] Pulmonary lesions were the most common cause of death in TSC patients, particularly those over the age of 40 years,[4] with 59% dying of cor pulmonale and 41% of spontaneous pneumothorax. The prognosis of pulmonary tuberous sclerosis is therefore significantly less favorable than other forms of TSC after the patients reach adulthood.[4]

The gross appearance of affected lungs is most striking (Figs. 14.1 and 14.2). The lungs are voluminous and may be twice as heavy as normal lungs. The normal fine lacy parenchymal pattern is replaced by a multitude of cysts varying from a few millimeters to several centimeters in diameter, resembling a sponge. The cysts are usually empty but may contain either cloudy chylous or clear serous fluid. The cyst walls vary in thickness from being paper thin to 1 to 2 mm in thickness (Fig. 14.2); the origin of these cysts from the bronchial tree has been impossible to demonstrate despite careful morphologic examination of the lungs. The pleura may be focally scarred and thickened as the result of previous and recurrent pneumothoraces and repeated paracenteses. The vasculature and lymphatics are often grossly unremarkable.

Microscopically, the lung cysts may appear unlined, but a cuboidal and rarely ciliated epithelium occasionally can be identified (Fig. 14.3). The interstitium between adjacent cyst walls consists of a proliferation of immature-looking smooth muscle cells with poorly defined, interdigitating, cleftlike lymphatic spaces (Fig. 14.4). These foci of spindled cell proliferation may occur independently of the lung cysts throughout the parenchyma and subpleurally, as well as in extrapulmonary tissues. The vascular smooth muscle and endothelial cell identity of these pulmonary and extrapulmonary proliferations in TSC has been identified ultrastructurally and immunohistochemically.[3,12,21,22,40] The pulmonary nodular lesions in TSC patients are morphologically indistinguishable from pulmonary LAM.[3,19–21,25–32,35–41] However, the extrapulmonary proliferation of smooth muscle and endothelial cells in lymph nodes, lymphatics, and thoracic ducts occurs more commonly in idiopathic LAM than pulmonary tuberous sclerosis (Fig. 14.5), to which the higher incidence of chylothorax in pulmonary LAM of patients without

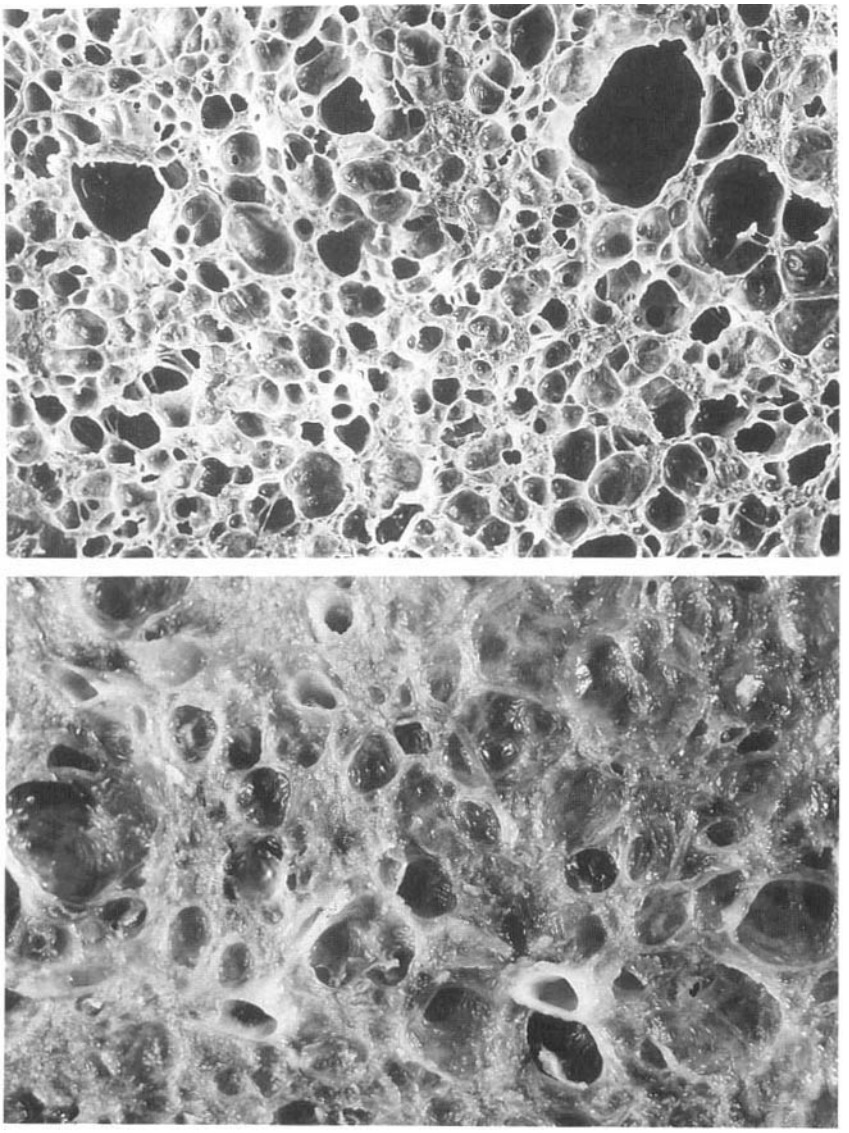

FIGURE 14.2. Close-up views of the gross appearance of two lungs with pulmonary cystic disease: the cyst walls may be paper thin (*top*) or 1 to 2 mm thick (*bottom*).

TSC has been attributed.[16,19,20,27–32] Sex steroid assay of the lung tissue is more often positive in pulmonary LAM and negative in TSC.[32,35]

Pathophysiology and Lung Function Tests

Earlier descriptions of pulmonary tuberous sclerosis have mainly been limited to the clinical, roentgenographic, and anatomic features of the disease,

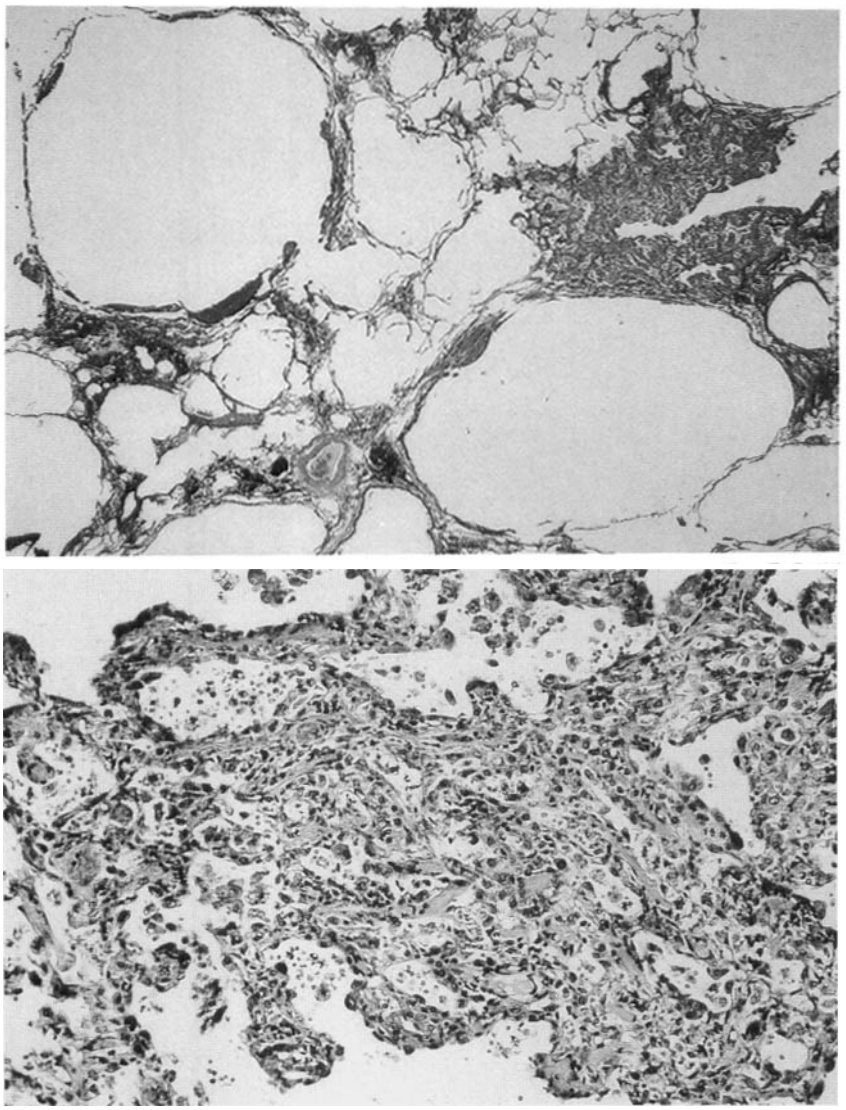

FIGURE 14.3. Low-magnification view of cystic lesions in the lung (*top*) and close-up view of a focal adenomatoid nodule (*bottom*) in pulmonary tuberous sclerosis. (Hematoxylin and eosin; *top* ×16, *bottom* ×160)

but in the past three decades there have been increasing numbers of reports describing pulmonary function tests and blood gas measurements.[15,24,29,32] In these recent reports, obstructive patterns of the lung function impairment have been consistently observed, and the associated hypoxemia may be quite pronounced. These changes resemble those of emphysema rather than of diffuse interstitial fibrosis.[23,29]

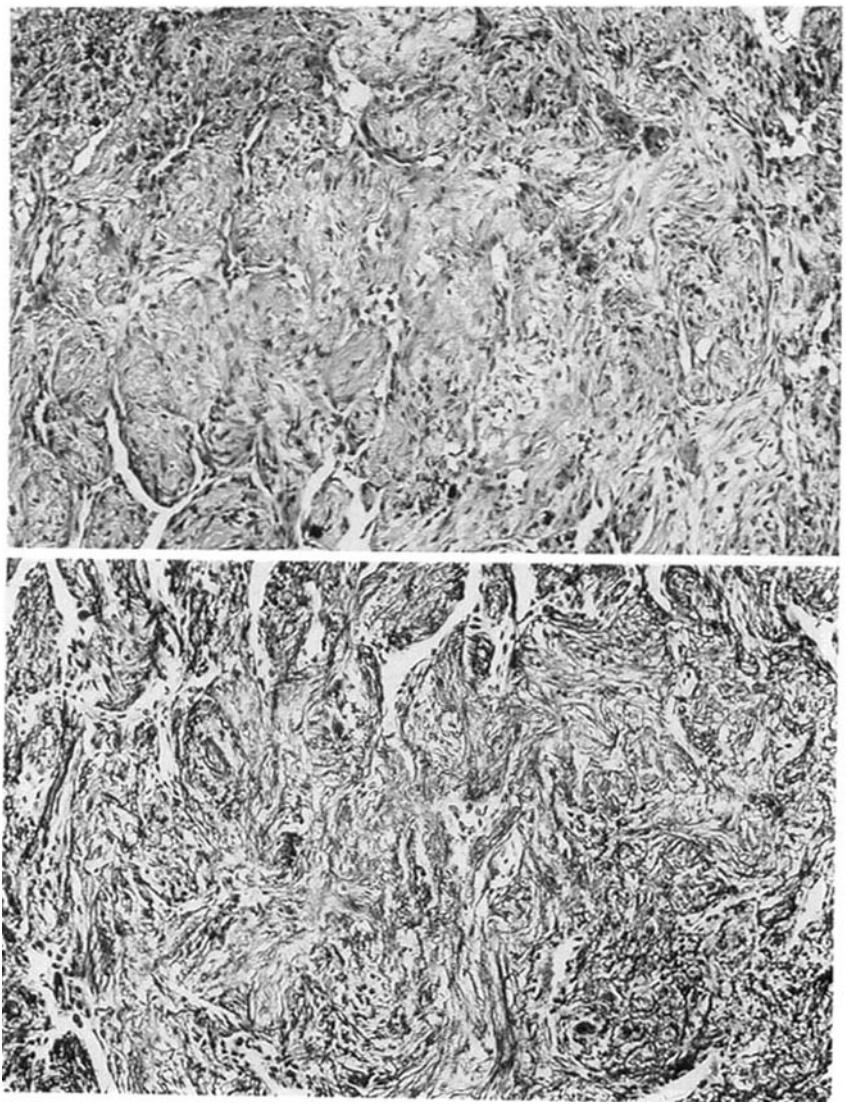

FIGURE 14.4. Pulmonary lymphangiomyomatosis with tightly knotted smooth muscle cell proliferation (*top*) and ill-defined, interdigitating, cleftlike lymphatic spaces (*bottom*). (*Top*, hematoxylin and eosin stain, and *bottom*, reticulin stain; ×160)

Lung function testing in pulmonary LAM is characterized by a combined restrictive and obstructive pattern, and a strikingly low diffusion capacity has often been observed.[25,27–29] Total lung capacity as measured by gas diffusion techniques may be reduced, but it is normal or slightly elevated when measured radiographically or by plethysmography,[26,27,29] suggesting

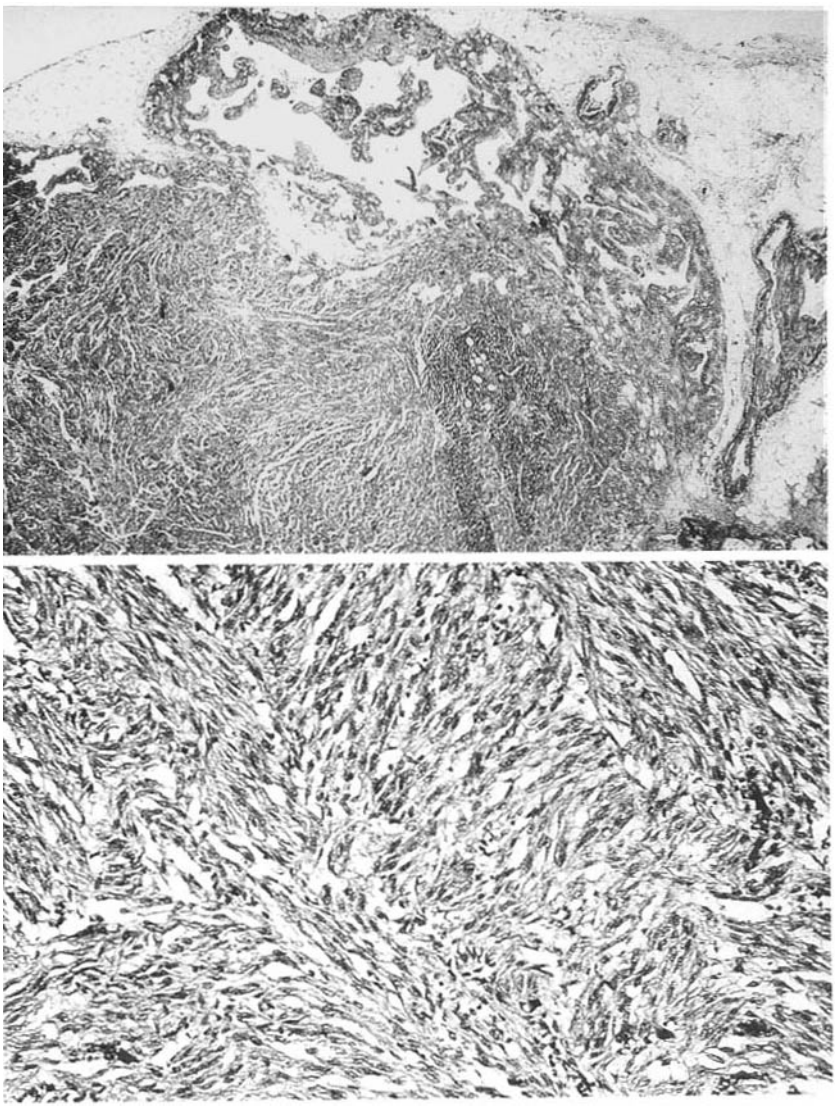

FIGURE 14.5. Low-magnification view of a mediastinal lymph node with lymphangiomyomatosis (*top*) and close-up view of the criss-crossing pattern of smooth muscle cell proliferation (*bottom*). (Hematoxylin and eosin stain; *top* ×16, *bottom* ×160)

that severe air trapping occurs in pulmonary LAM. Quantitative studies have shown that the small airways are narrowed and collapsed because of the surrounding emphysema, and some airways contain excess smooth muscle. These findings suggest that the airspace lesions are more important than muscular proliferation in small airways in producing airflow limitation.

Conclusion

"The major pathologic features of TSC can be viewed as developmental errors, not so much in the structural formation of whole organs as in focal derangement of the shaping, differentiations, and positioning of cells, leaving other parts of an affected organ to acquire a fully normal developmental state."[42] Pulmonary tuberous sclerosis is morphologically indistinguishable from isolated LAM and fits this interpretation of the structural anomalies of TSC. The insidious lung involvement in persons without the usual stigmata of TSC may delay diagnosis until the respiratory insufficiency becomes an irreversible cause of morbidity and mortality in these patients.

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15

The Endocrine System in Tuberous Sclerosis Complex

The endocrine glands are infrequently reported to be affected in patients with tuberous sclerosis complex (TSC). Three patterns of endocrine gland involvement have been seen in TSC: (1) tumor growth in endocrine glands not associated with an increased number of hormone-producing cells or increased secretion from the gland (i.e., angiomyolipoma, a nonfunctioning mesodermal hamartoma); (2) hypothalamic dysfunction without demonstrable hypothalamic tumor; and (3) proliferation of hormone-producing tissue in the adrenal glands, thyroid, gonads, pancreatic islets, parathyroid glands, or pituitary gland. A fourth pattern of endocrine gland involvement that has been recently described is dysplasia of the thyroid gland.

Angiomyolipomas may arise in the adrenal glands or gonads of TSC patients. These mesodermal hamartomas are often found in the kidneys of patients with TS. It is not surprising that these tumors grow in the adrenals and the gonads as well as in the kidneys because the embryogenesis of these endocrine glands occurs in the mesonephros or primordial kidney.

In the vast majority of TSC patients with abnormalities of hormone-secreting cells, only one endocrine organ is involved. However, one TSC patient has been reported with hyperplasia of the parathyroids; adenomas of the pancreatic islets, pituitary, and adrenal glands; and also thyrotoxicosis. The patient's mother had parathyroid hyperplasia in addition to TSC.[1] These two cases, along with the finding of angiofibromas in 88% of patients with multiple endocrine neoplasia type I (MEN I)[2] and another case of tuberous sclerosis with insulinoma,[3] raise the possibility that the fullest expression of TSC may include a form of multiple endocrine neoplasia bearing some resemblance to MEN I (parathyroid hyperplasia associated with adenomas of the pituitary and pancreatic islets).[4]

Adrenal Gland

The endocrine gland most frequently affected in patients with TSC is the adrenal. The most common adrenal abnormality in tuberous sclerosis is angiomyolipoma, which is morphologically similar to tumors found in the kidney or other tissues of patients with TSC.[5–7] In one report,[8] adrenal angiograms of two of eight patients with TSC manifested "a whorled type arterial appearance with a rather well defined circumferential peripheral arterial network, multiple aneurysms, and arteriovenous puddling of contrast material." These abnormal structures were most likely angiomyolipomas. Therefore, such tumors may be present in the adrenal glands of 25% of TSC patients.

Solitary or multiple adenomas are found in the adrenal cortex of some TSC patients; some of them are cystic and contain viscous or stringy fluid.[9] As mentioned above, adrenal cortical adenomas were present in patients with TSC who also had pituitary and pancreatic islet adenomas and parathyroid hyperplasia.[1] In other TSC patients, paragangliomas of the adrenal medulla have been reported.[9] Another group of patients with adrenal involvement includes patients with lymphangioleiomyomatosis. This condition may be a *forme fruste* of TSC.[10]

Patients with TSC and abnormalities of adrenal function have been described. Holtzman et al.[11] reported an 8-year-old girl with TSC and masculinization of the external genitalia as a result of deficiency of 11-β-hydroxylase.[9] Sareen et al.[12] described a patient with TSC and Cushing syndrome associated with high plasma levels of 17-hydroxycorticosteroids. The cause of Cushing syndrome was unknown. Sareen et al.[12] also described a number of TSC patients with abnormally low urinary 17-hydroxycorticosteroid levels in the basal state and in response to administration of metyrapone and adrenocorticotrophic hormone. Unfortunately, the authors did not specify whether the patients were receiving medications such as phenytoin or other anticonvulsants, which might interfere with 17-hydroxycorticosteroid measurement[13,14] or interfere with the absorption and activity of metyrapone.[15] It is also noteworthy that a number of the patients reported by Sareen et al.[12] to have low levels of 17-hydroxycorticosteroids either in the basal state or in response to administration of metyrapone were also said to have hypothyroxinemia. Hypothyroidism may decrease 17-hydroxycorticosteroid excretion by impairing cortisone metabolism[16]; it may also decrease the steroid response to metyrapone administration.[17]

Thyroid Gland

Thyroid adenomas have been described as one of the characteristic lesions of TSC.[18] Papillary adenomas of the thyroid gland, which are extremely uncommon in the general population,[19] are among the most common thyroid lesions found in patients with TSC. One of the patients described by Perou

and Gray[20] had several small "papillary adenoma-like structures which were not encapsulated within the thyroid gland." Verma and Tailor referred to a patient with a similar lesion.[21] A third TSC patient with papillary adenoma of the thyroid was an 8-year-old boy who died from obstructive hydrocephalus produced by large bilateral intraventricular giant cell astrocytomas. The patient was examined pathologically by Dra. Cecilia Ridaura of Mexico City.[22] Figure 15.1 is a photomicrograph of a section of this patient's thyroid gland that includes a portion of the papillary adenoma. Ghidini et al.[23] reported a 13-year-old boy with a fetal adenoma of the thyroid with atypia. I have seen a 36-year-old woman with TSC, fetal adenoma of the thyroid, and euthyroid Hashimoto thyroiditis. These thyroid lesions are shown in Figure 15.2.

There are three reports describing alterations of thyroid function in patients with TSC. One of the patients described by Ilgren and Westmoreland[1] had thyrotoxicosis associated with congestive heart failure and adenomata of the adrenal glands, pituitary gland, and pancreatic islets. This patient had previously undergone parathyroidectomy for chief-cell hyperplasia of the parathyroid glands. The histology of the thyroid gland was not described. Sareen et al.[12] described three TSC patients with clinical and biochemical evidence of hypothyroidism. Two of these patients had palpable goiters. One of them had circulating antithyroid antibodies, indicating the presence of Hashimoto thyroiditis. The second patient had no detectable thyroid antibodies but had a positive thiocyanate washout test, suggesting a defect in

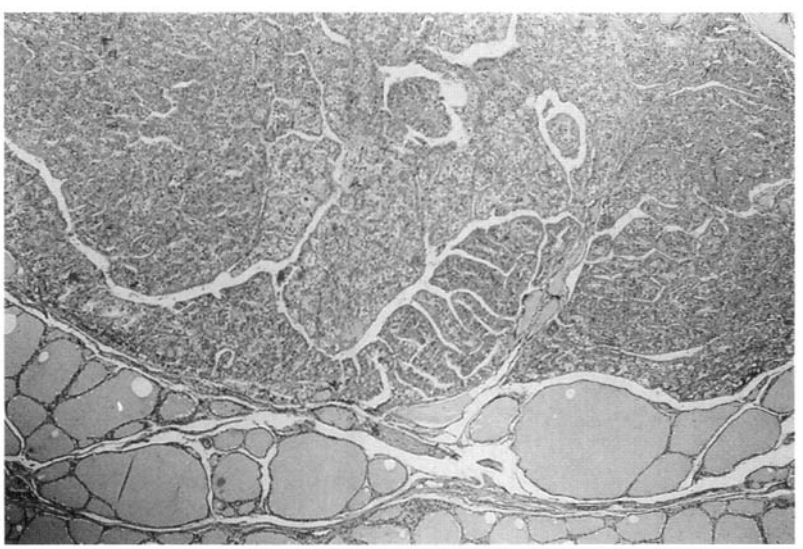

FIGURE 15.1. Histologic section of a papillary adenoma of the thyroid gland found on postmortem examination of a patient with TSC who died with obstructive hydrocephalus. (Hematoxylin and eosin) (Courtesy of Dra. Cecilia Ridaura of Mexico City.)

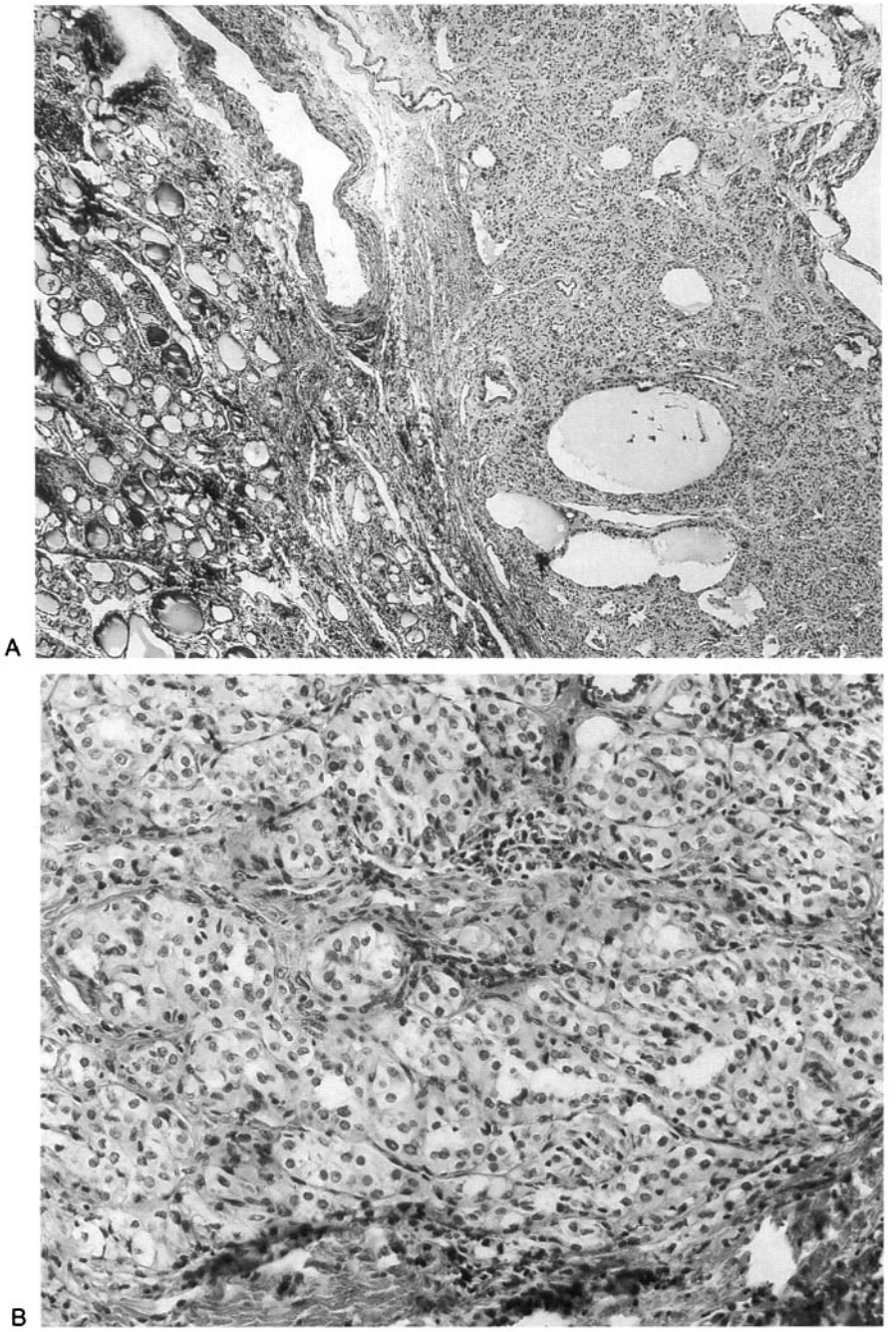

FIGURE 15.2. Histologic section of a fetal adenoma of the thyroid gland of a woman who also had Hashimoto thyroiditis. *A*, Low power (×16). *B*, High power (×36). (Hematoxylin and eosin)

organification of iodine. Bereket and Wilson described a child with TSC and congenital hypothyroidism caused by thyroid dysgenesis.[24]

Pancreatic Islets

Four patients have been reported to have islet cell adenomas of the pancreas in association with TSC. These patients were all young women with symptomatic hypoglycemia.[25-27] One of the three also had hyperthyroidism, adenomas of the pituitary and adrenal glands, and hyperplasia of the parathyroid glands.[1]

Gonads

At least 11 patients with TSC have been reported to have precocious puberty.[28-37] In many of these cases, the etiology of precocious puberty was uncertain. Central precocious puberty is well documented in the TSC patient of Cummings et al.,[35] who also had gynecomastia and hyperprolactinemia. This patient did not have hydrocephalus, nor did he have any lesion of the third ventricle, hypothalamus, or pituitary gland demonstrable by computed tomography (CT) scanning.

While some patients with TSC have had precocious puberty on the basis of activation of the hypothalamic-pituitary-gonadal axis,[35,37] at least one patient had precocious puberty caused by activation of the testes independently of gonadotropin stimulation.[36] His basal gonadotropin levels were undetectable, and his gonadotropin secretory response to administration of gonadotropin-releasing hormone was negligible. This patient's condition is of particular interest in light of the association of gonadotropin-independent sexual precocity with another condition, the McCune-Albright syndrome, which in some ways resembles TSC.[38,39] This syndrome consists of polyostotic fibrous dysplasia, café-au-lait spots of the skin, and sexual precocity caused by a gonadotropin-independent mechanism.[40,41] Indeed, the McCune-Albright syndrome, like TSC, has many features resembling MEN.[42] Unlike TSC, however, the McCune-Albright syndrome is not heritable.[43]

The mechanism of gonadotropin-independent precocious puberty in McCune-Albright syndrome has recently been elucidated. These patients have activating mutations of the α subunit of the guanine nucleotide regulatory protein (G protein).[44] The G protein is a component of the cell membrane that conveys hormonal signals from hormone receptors to the adenylate cyclase enzyme; the latter catalyzes generation of the intracellular "second messengers" such as cyclic AMP. A number of patients with pseudohypoparathyroidism have also been reported to have molecular abnormalities of the G protein.[44] These abnormalities may be responsible for their subnormal response to parathyroid hormone. Two patients with pseudohypoparathyroidism had a G-protein mutation that was inactivating in the kid-

ney (inhibiting parathyroid hormone action) but activating in the gonad (producing gonadotropin-independent precocious puberty).[45] It is conceivable that gonadotropin-independent precocious puberty and perhaps other abnormalities in TSC patients arise through similar mechanisms. The mechanism would have to be somewhat indirect, however, because the gene for the α subunit of the stimulatory G protein is at chromosome 20q13.2, whereas the two established tuberous sclerosis genes are at chromosome 9q33-q34 and at 16p13.3.[46]

Another well-described mechanism for gonadotropin-independent precocious puberty is mutation of the luteinizing hormone receptor producing activation of the function of this receptor. Such activating mutations of the luteinizing hormone receptor have produced autosomal dominant but male-limited precocious puberty in a number of families.[47] If this mechanism is responsible for gonadotropin-independent precocious puberty in children with tuberous sclerosis, the genetic pathogenesis is not immediately obvious because the gene for the luteinizing hormone receptor is at chromosome 2p21 rather than being close to the two most well-accepted tuberous sclerosis genes at 9q33 and 16p13.3.[46]

Another gonadal abnormality that has been described in one patient with TSC is angiomyolipoma of the testis. This tumor has been described as being similar to the lesion occurring in the kidney and in the adrenal gland.[48] Still another testicular tumor, fibroadenoma, was found at autopsy in a TSC patient followed at the Mayo Clinic. A third testicular tumor described in a TSC patient is the Leydig cell tumor, which was associated with eruptive seborrheic keratosis.[49]

Hypothalamus and Pituitary

Abnormal hypothalamic and pituitary function in TSC has been alluded to in at least some of the TSC patients with precocious puberty. The patient described by Cummings et al.[35] had well-documented central precocious puberty in addition to hyperprolactinemia.

Hyperprolactinemia has been described in other patients with TSC. Bloomgarden et al. described a young woman with TSC, amenorrhea, and galactorrhea.[50] The patient's serum prolactin level failed to increase with administration of protirelin, haloperidol, or chlorpromazine. In addition, there was no significant decrease in prolactin levels with administration of levodopa or bromocriptine. Because these challenges usually cause predictable changes in pituitary secretion of prolactin, the investigators thought that prolactin might be ectopically secreted, perhaps by a hamartoma.

Another patient with hyperprolactinemia and TSC also had diabetes insipidus and growth hormone deficiency.[51] Although this patient had a hamartoma of the left lateral ventricle, no abnormality of the sellar or suprasellar regions was demonstrable by CT scanning.

A young male patient with TSC was reported by Ackermann to have diabetes insipidus in addition to pulmonary and skeletal abnormalities,[52] but neither radiographic nor pathologic studies were included.

Hoffman et al.[53] have described acromegalic gigantism associated with a pituitary tumor and TSC in a boy 11 years and 8 months old. Another TSC patient with a pituitary tumor mentioned in other sections of this chapter[1] also had a tumor in the adrenal gland, hyperthyroidism, pancreatic islet cell tumor, and parathyroid hyperplasia.

One of the TSC patients seen at the Mayo Clinic had growth deceleration between 12 and 15 years of age. At 15½ years of age, his height was nearly three standard deviations below the mean. His monozygous twin brother's height (which had been the same as the patient's until the patient's growth deceleration began) was at the mean for his chronologic age. Growth hormone secretion was subnormal in response to arginine infusion but was normal in response to L-dopa administration. CT scan revealed a large ependymoma of the left lateral ventricle associated with hydrocephalus.

Parathyroid Glands

Three patients have been described with hyperparathyroidism in association with TSC. One, mentioned above, had chief-cell hyperplasia of the parathyroid glands and hypercalcemia in addition to adenomas of the pituitary gland, pancreas, and adrenal gland and hyperparathyroidism.[1] This patient's mother had facial angiofibroma in association with hypercalcemia caused by parathyroid hyperplasia.[1] A third patient had only one enlarged parathyroid gland when she underwent surgery for hyperparathyroidism at the age of 14 years. Postoperatively, she experienced transient hypocalcemia.[54]

Summary

TSC has been associated with a number of abnormalities in endocrine tissues. Angiomyolipomas of the adrenal have been reported. Abnormalities of adrenal function have also been described, including at least one patient with Cushing syndrome and another with adrenogenital syndrome caused by 11-β-hydroxylase deficiency. Thyroid adenoma has been described in a very few patients with TSC, and still fewer patients have had either hyperthyroidism or hypothyroidism in association with TSC. Gonadal abnormalities among patients with TSC include precocious puberty, which may be due to gonadal activation by the pituitary gland or to gonadal activation of gonadotropin-independent mechanisms. Angiomyolipomas and fibroadenomas of the testes have also been described. In addition to precocious puberty, patients with TSC may manifest other states of hypothalamic and pituitary dysfunction, including diabetes insipidus, hyperprolactinemia, and growth

hormone excess or deficiency. Finally, hyperparathyroidism has been observed in a few patients with TSC.

The occurrence of many of these endocrine abnormalities in a single patient with TSC raises the possibility that an MEN I like syndrome may be a component of tuberous sclerosis, which is usually incompletely expressed. It is remotely possible that an unidentified gene for TSC may be located on chromosome 11, where the locus for MEN I is located. More likely the genes on chromosomes 9 and 16 which function as tumor suppressers (as does the *MENI* gene) may be expressed in the pituitary and possibly in other MEN I associated sites.

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16

Liver, Digestive Tract, Spleen, Arteries, Thymus, and Lymphatics

Liver

Little is known about hepatic hamartomas; more is known about renal hamartomas. Patients with tuberous sclerosis complex (TSC) may harbor one or several hamartomas, specifically angiomyolipomas (AMLs), in the kidneys, the liver, or both. However, patients without TSC may also develop AMLs in the kidneys or liver. Although not completely studied, it seems that multiple AMLs occur more often and at a younger age in patients with TSC than in those without TSC. This observation may be in part due to the practice of searching for AMLs with imaging methods in all young patients with TSC, whereas imaging studies of individuals without TSC but with an AML of the liver may never be done or may be done only when they are older and have developed abdominal or urinary symptoms. AMLs of the liver do not cause symptoms. These tumors are less commonly found in the liver than in the kidneys. Both renal and hepatic AMLs are more common in female than in male patients, whether or not they have TSC.

The hepatic AML is hamartoma that contains a variable amount of adipose tissue, normal-looking parenchyma, and hyperplastic smooth muscle fibers forming the thick walls of blood vessels (Fig. 16.1). There are aneurysmatic dilatations. The diagnosis of liver AML is most often made by ultrasound; computed tomography (CT) scan is helpful for the diagnosis, especially if it reveals fatty tissue within the tumor. Fat is demonstrable with CT but not with ultrasound, and the former can separate AMLs, among other tumors, from venous lakes of the liver, a common lesion that does not contain fat and is unrelated to TSC. The same can be said of magnetic resonance imaging, which displays a hyperintense signal on T_2-weighted images, indicating that fat is present in the tumor. In two ultrasound studies of subjects with TSC, the proportion who had hepatic AML was 24%[1] and 16%.[2]

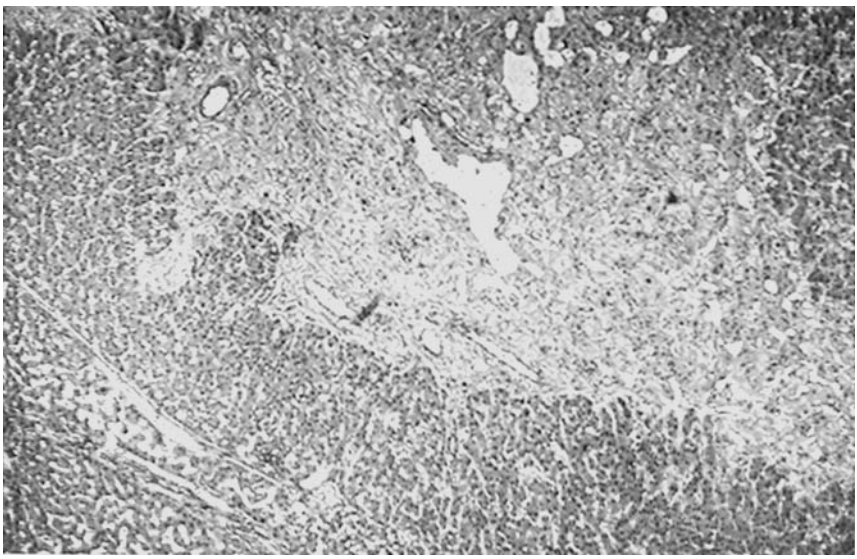

FIGURE 16.1. Histologic section of a hamartoma of the liver found at postmortem examination in a patient with TSC. (Hematoxylin and eosin, ×100)

Feriz reported the association of racemose angioma of the liver with TSC.[3] Others have reported association of TSC with liver adenomas,[4] lipomyomas,[5] or fatty mesenchymatous tumors.[6] Perou and Gray[7] reported multiple hamartomas scattered throughout the liver of an 8-year-old epileptic and mentally handicapped girl with TSC. Reed and colleagues,[8] in 1963, reported hepatic hamartomas found among their 51 autopsied patients, and Viamonte and colleagues,[9] using selective angiography of the celiac artery, demonstrated stretched hepatic arteries supplying an enlarged right hepatic lobe. Microscopic examination of the surgically removed lesion demonstrated a hamartoma containing mature adipose tissue and focal proliferation of blood vessels of capillary size, indeed, a hepatic AML.

Robinson et al.[10] described two patients with TSC and multiple visceral AMLs in the kidneys and liver. There is a description of a histopathologically confirmed hepatic AML in another TSC patient.[2]

Józwiak and colleagues[2] using ultrasound, examined children with TSC between 3 months and 18 years of age and found areas of increased echogenicity, presumed to be hamartomas, in 12 (23.5%) of 51. There were 20 patients under the age of 5 years, 20 between 5 and 10 years, and 1 older than 10 years. Interestingly, there was a discrepancy between genders, with a 5:1 female prevalence. Unfortunately, the total number of patients in this study was too small to be significant. None of the patients with hepatic AML had symptoms or signs of hepatic dysfunction: the serum levels of liver enzymes were normal. In 9 of the 12 children ultrasound also demonstrated multiple bilateral renal AMLs, and, in addition, two children had many renal

cysts. One 9-year-old girl with hepatic and renal AMLs had a clear cell renal carcinoma that caused her death. These authors do not specifically mention histologic confirmation of the diagnosis in this patient or in any of the 12 children presumed to have a hepatic AML. Józwiak et al.[2] apparently did not rule out a cavernous hemangioma with selective angiography of the celiac artery in any of their patients as Viamonte et al. did.[9]

Cheung et al.[11] reported three female patients, 10, 25, and 41 years old, with TSC and hepatic hamartomas diagnosed by ultrasound and CT scan. The hamartomas were multiple in two of the three patients. There was no pathologic confirmation. These authors did not recommend needle biopsy to confirm the diagnosis of liver hamartoma because, if the lesion is indeed an AML, this is a highly vascularized tumor and consequently is at risk of bleeding either spontaneously or following needle penetration. There is one report in the medical literature of a spontaneous hemorrhage in a hepatic tumor[12] that was confirmed histologically as an AML after surgical removal. It is well known that both renal AMLs and intraventricular subependymal giant cell astrocytomas, may also be very vascular and bleed spontaneously. In sum, the hamartomas in brain, kidney, and liver are potentially lethal.

Digestive Tract

Pathologic findings in the digestive systems of patients with TSC are uncommon. The majority of such findings are in the buccal mucosa, dental enamel, and anorectal junction.

Mouth

The oral lesions of patients with TSC are nodular tumors, fibromas, or papillomas, usually located on the anterior gingival border, particularly in the upper jaw (Fig. 16.2) and also the lips, dorsum of the tongue, palate, and other parts of the buccal mucosa.[13] They appear between 4 and 10 years of age or at puberty.[14] There is no sex prevalence. Gingival fibromas may be obscured by gingival hyperplasia caused by taking phenytoin. It is also possible that this antiepileptic drug accelerates the growth of gingival fibromas.

Macroglossia is an uncommon finding in TSC patients.

Teeth

Dental enamel defects are not uncommon in patients with TSC. In the study by Hoff et al.,[15] dental radiographs of six patients demonstrated that all tooth surfaces had pit-shaped enamel defects with no clear pattern of distribution. The number of pits varied from 1 to 11 and averaged 3 per tooth surface. Three types of defects are demonstrable: (1) small pits not visible without magnification and only 4 μm in diameter; (2) indentations up to 60 μm in

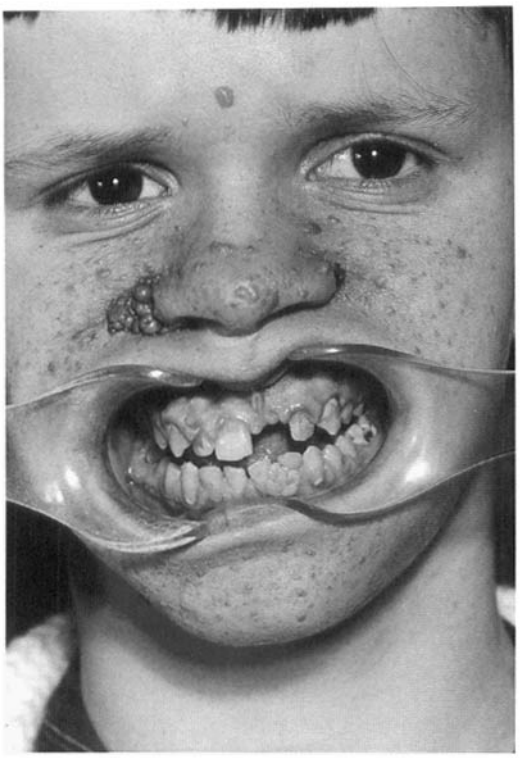

FIGURE 16.2. Large gingival fibroma between the medial and lateral left lower incisors in a patient with TSC who also has facial angiofibromas.

diameter, and (3) craterlike structures corresponding to pits visible with the naked eye, whose diameter is approximately 100 μm. Examination of the enamel structures in longitudinal sections with the aid of a scanning electron microscope shows no abnormality in the enamel of the superficial layers. The enamel near the amelodentinal junction contained amorphous material from which the indentations and craterlike defects extend to the tooth surface. Hoff et al.[15] are of the opinion that the enamel hypoplasia associated with TSC is caused by a malfunction of the ameloblasts, and, because the amorphous material in the enamel is close to the dentin, the first phase of amelogenesis may be defective. When one or more ameloblasts malfunction, small pits appear; when large groups of ameloblasts fail to function normally, indentations or craterlike structures appear.

In another study, Mlynarczyk[16] looked for enamel pits in 50 patients with TSC and 250 controls. He found that 76% of TSC patients 11 years old or younger and 20% of controls have dental enamel pits, while 90% of TSC patients of all ages and 9% of controls have enamel pits. More recently, examination of 87 shed deciduous teeth from 20 patients with TSC and from 142 controls (80 with cerebral palsy, 15 with phenylketonuria, 33 with Down syndrome, and 14 normal controls) revealed enamel pits in all 87 deciduous teeth from the TSC patients. There were no differences in the pits in the

pre- and postnatal enamel (it is known that most of the enamel of deciduous teeth is mineralized in prenatal life). Contrary to the belief that enamel pits may be found in individuals who do not have TSC,[16] none of the 253 deciduous teeth from the 142 controls had enamel pits.[17]

Pharynx and Esophagus

Fibromatous tumors of the pharynx, larynx, and esophagus have been infrequently reported.[18,19]

Stomach and Duodenum

There are few reports of stomach and duodenum lesions in TSC patients. Van Brouwdjik-Bastiaanse reported a large tumor of the stomach, histologically of a mixed type,[20] and Sailer reported small nodular tumors of the duodenal wall, said to be sarcomas.[21]

Pancreas

Pagenstecher[22] suggested involvement of the pancreas in patients with TSC, and Kawamura[23] reported a pancreatic tumor in one patient. Perou and Gray[7] described the postmortem findings of an 8-year-old girl with TSC: the pancreas appeared to be grossly normal but on microscopic examination there were scattered noncapsulated microadenomas composed of acini filled with coagulated acidophilic material. In a few of the lesions, both the acini and the islets of Langerhans seemed to be involved in a proliferative process. The latter were hyperplastic. This patient also had liver and kidney hamartomas, polycystic kidneys, subependymal nodules, and facial angiofibromas. Histologically proven AML of the pancreas has been reported in association with lymphangioleiomyomatosis (LAM), giving support to the concept that LAM and TSC are parts of the same disease process.[24,25]

Rectum

Hamartomatous colorectal polyps of the large intestine are infrequently reported in TSC patients and seem to be uncommon compared with other organ involvement. Apparently they occur only in adults or after the age of 18 years, but, because they are not usually sought in TSC patients, particularly in the young, their true prevalence is unknown. There are only two reports of this lesion in fewer than 20 patients.[26,27] The report by Gould et al.[27] includes 18 patients with TSC, and 14 of them (78%) had polyps in the rectum close to the anorectal junction discovered by sigmoidoscopy or colonoscopy. All but 2 of the 18 patients had seizures. Three of these patients were mildly mentally handicapped and two had facial angiofibroma. The

polyps were small and sessile, and some were filiform. The rectal polyps were too numerous to count in eight patients, approximately 10 in two patients, and 3 or fewer in the remaining four patients. Polyps were palpable by digital rectal examination in 5 of 11 patients. Histologic examination did not reveal adenomatous tissue, neural tissue, or malignant or premalignant changes. In three patients there was splaying of the muscularis mucosa, and six had edema of the lamina propria. The hamartomatous polyps of TSC are nonneoplastic and have no malignant potential.

Spleen

Cares,[6] in reporting the postmortem findings of a patient with TSC, described an angiomatous tumor of the spleen 7 cm in diameter histologically consisting of endothelial sinuses and enlarged and some thrombosed blood channels. Morales[28] reported a patient who died of cardiac failure at the age of 4 days; postmortem examination disclosed cerebral TSC, a cardiac rhabdomyoma, and a nodular spleen. Microscopic examination of the spleen demonstrated accumulation of periodic acid-Schiff–positive material in histiocytes. I know of no similar case in the literature.

Only two cases of splenic tumors have been found in more than 400 patients with TSC seen at the Mayo Clinic. The first one was reported by van Heerden and Longo.[29] The patient was a mentally retarded 12-year-old boy with TSC and seizures who developed an enlarging abdominal mass at the age of 9 years. The large spleen, removed at surgery, weighted 390 g and contained a multinodular bluish red mass 11 cm in diameter that, on sectioning, showed nodules varying between 2 and 6 cm in diameter. The tumor had a normal splenic color and consistency but showed no malpighian corpuscles. Cystic blood-filled spaces of some nodules gave them the appearance of a cavernous angioma. Microscopically the tumor had a distorted splenic architectural pattern with dilated sinusoids lined with a single layer of endothelial cells[29] (Fig. 16.3) and the reticular fibers of the cords arranged in haphazard fashion.

The second case of hamartoma was in a patient seen at the Mayo Clinic when he was 19 years old following a routine physical examination for joining the Armed Forces that had revealed a blood pressure of 200/110 mm Hg. He had no history of seizures and was of average intelligence but had facial angiofibroma, poliosis in the occipital region of the scalp, molluscum fibrosum pendulum of the neck, bilateral intracranial calcifications on plain radiographs, a calcific lesion in the bony pelvis, and a small cyst in a finger middle phalanx. He was treated for hypertension. At the age of 30 years, he was found unconscious in his car. He died 1 hour after admission to a local hospital. Postmortem examination showed a recent massive hemorrhage into a nodular tumor adjacent to the foramina of Monro histologically identified as a giant cell astrocytoma, bilateral renal AMLs, lipomas, concentric hypertrophy of the heart, and a nodular tumor of the spleen 8 cm in diameter.

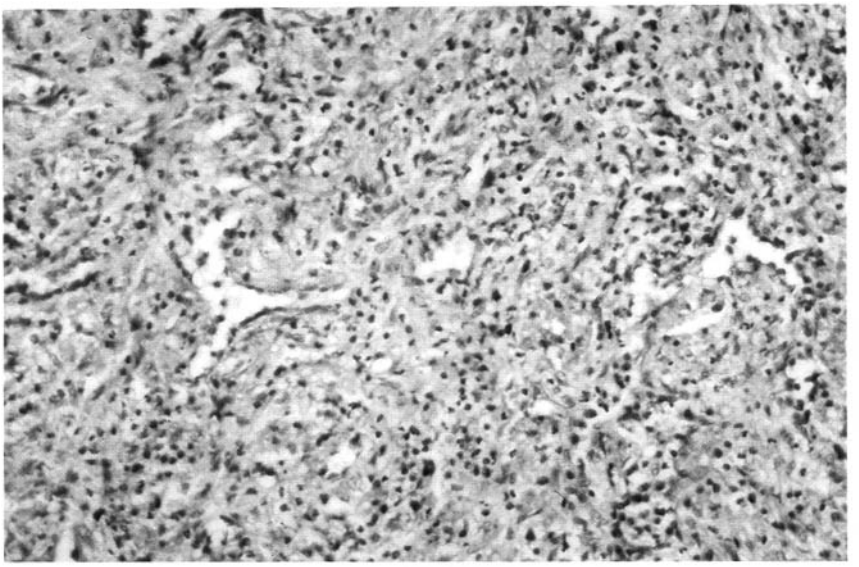

FIGURE 16.3. Histologic section of a multinodular mass in the spleen of a patient with TSC. Note the distorted splenic architecture with absence of malpighian corpuscles, dilatation of sinusoids, and haphazard arrangement of the stroma. (Hematoxylin and eosin, ×160)

The spleen weighed 600 g. Sectioning the spleen showed that the nodular tumor had replaced much of the parenchyma. The nodule was composed of tissue slightly firm and with a variegated appearance where vivid red or red-brown zones intermixed with opaque yellow zones. Microscopically, the spleen architecture was formed by irregular focal zones of pink hyalinized connective tissue supporting thin or very thick-walled blood vessels along with scattered red blood cells, lymphocyte, plasma cells, and numerous large hemosiderin-laden histiocytes. In other areas there were large macrophages having abundant lipid material in their cytoplasm.

Darden and colleagues[30] reported another 14-year-old boy with a hamartomatous spleen, facial angiofibroma, seizures, and mental subnormality. He presented with abdominal pain and left-sided abdominal mass. The spleen, surgically removed, weighted 667 g and contained two well-circumscribed masses measuring 5 and 6 cm in diameter. Microscopic examination showed a "splenic hamartoma with a predominance of vascular elements." It is interesting that this patient also had redundant skin of the left axilla and the left thumb was hypertrophic. Fundus examination disclosed several "white plaques" in the retina. Head radiograph showed calcifications in several regions of the brain, and radiographs of the left humerus disclosed cortical thickening. Abdominal aortogram and selective splenic angiography revealed a large mass supplied entirely by the splenic artery and its branches. There was also arteriovenous shunting of the splenic

hamartoma. Darden et al. commented that only 43 cases of splenic hamartoma had been reported in the literature and in only one previous instance was it associated with TSC. However, there are at least four cases of splenic hamartoma if one adds the cases reported by Cares[6] and van Heerden and Longo,[29] plus the unreported Mayo Clinic case described above in some detail. There is only one patient reported to have TSC and a splenic sarcoma.[31] The 33-year old man presented with ascites, splenomegaly, and uremia, and in addition had typical cutaneous lesions of TSC. Because this is a single case, it is difficult to know whether splenic sarcoma is also a manifestation of TSC or this case was just a coincidental association of two unrelated disorders. If it is a manifestation of TSC, splenic sarcoma would be the second most common hamartoblastoma that may occur, the most common being the renal clear cell carcinoma, at present reported about 10 times (See Chapter 12).

Arteries

Aortic Aneurysms

Fibromuscular dysplasia of the aorta is a cause of aneurysms of the aorta and other large-caliber arteries. Aneurysms of the aorta have been discovered in at least 13 patients with TSC, and 10 of these patients are less than 16 years old. Most of them are children less than 5 years old.[32] Therefore, one can now propose that the association of the two disorders is not coincidental; rather, the aortic lesion is one more clinical expression of a TSC genotype, and perhaps, because it may occur with polycystic kidney disease and TSC, there could be deletion of these contiguous genes.

It has been known since the days of Moolten[33] and Cares[6] that patients with TSC may also have involvement of medium-sized arterioles of the kidneys, lungs, liver, and adrenal glands. The vascular wall has neither arterial nor venous character, but it is similar to that of cirsoid aneurysms of the brain. The wall of a medium-sized renal artery often shows medial layer thickening, the elastic tissue is deficient and undergoes hyalinization, and the lumen is narrow and eccentric. Arteries resembling those of renal hamartomas have also been found in the mesentery of the large bowel.[34] (Intra- and extracranial arterial aneurysms are described in Chapter 4.)

Rabinowicz[35] reported in 1968 a 9-day-old infant examined postmortem who had TSC and coarctation of the aorta. Of the 13 patients reported with TSC and also an aortic aneurysm, four cases involved the descending thoracic aorta[36–39], and nine involved the abdominal aorta.[40–48]

Thoracic Aorta. Larbre et al.[36] reported a mentally handicapped child 2 year and 7 month old with anorexia, refusal to eat all foods and in particular solids, and weight loss of 2 months' duration. He had begun having infantile spasms at age 7 months. He had typical skin lesions of TSC. Radiographs

of the head revealed periventricular calcifications, and chest radiograph demonstrated a mediastinal tumor the size of an orange that, on exploratory thoracotomy, proved to be an aneurysm of the aorta. Autopsy verified he had cerebral TSC and also revealed a cardiac rhabdomyoma, polycystic kidneys, and a saccular aneurysm of the aorta 9 by 6 cm in diameter whose neck was 3 cm below the origin of the left subclavian artery and measured 4.5 cm in diameter. There were additional aortic aneurysms in the process of formation. On microscopic examination, there was practically complete disappearance of all normal structures. The aortic wall intima had thickened areas bulging into the lumen that contained dystrophic, fragmented elastic fibers. In the media there were lacunae containing fragmented elastic fibers and edema. The vasa vasorum had a normal appearance. There was no evidence of atherosclerosis or inflammation of the arterial wall.

Among 430 patients with TSC seen at Mayo Clinic, only 2 had aneurysm of the aorta; in both cases the aneurysm was in the descending thoracic aorta. One of these two patients had had seizures for many years, and the aneurysm was an incidental finding in a routine chest radiograph.[38] He was surgically treated successfully. Microscopic examination demonstrated thin media and extensive transmural disruption of normal musculoelastic lamellae. The other patient, a 3-year-old severely mentally handicapped girl, suffered a rupture of her descending aorta aneurysm that caused her sudden death.[39]

Abdominal Aorta. Freycon et al.[40] reported a 3-year-old patient with West syndrome, retinal hamartomas, hypomelanotic macules, facial angiofibromas, systemic hypertension, and a palpable abdominal mass first noticed after an episode of urinary retention. Surgical exploration disclosed a pulsatile abdominal aortic aneurysm at the level of the aortic bifurcation. The infant died 48 hours after surgery. No autopsy was done.

Hagood et al.[41] described a 22-month-old girl with an abdominal aortic aneurysm, facial angiofibroma, seizures, mental retardation, and essential hypertension. Percutaneous transfemoral angiogram disclosed a large distal abdominal aortic aneurysm and a hypovascular mass in the right kidney. The aneurysm and the proximal 1 cm of the iliac arteries as well as the renal mass were resected. The aneurysmal wall showed fragmentation of the elastic fibers and focal accumulation of mucopolysaccharides. There was bilateral proximal fibromuscular dysplasia of the proximal iliac arteries. The hypertension continued after the nephrectomy.

Thymus

Drut[49] reported an 8-month-old male infant without history of seizures who was unable to sit up without assistance and died after an acute febrile respiratory illness. The postmortem examination disclosed cardiac rhabdomyomas, numerous renal cysts, and "the liver, thymus, lungs, heart, and

appendix which also contained collections of large cells with pale, faintly eosinophilic, homogeneous cytoplasm, a large nucleus, and a prominent nucleolus simulating neurons.'' In addition, there was Langerhans cell histiocytosis infiltrating other viscera, notably, the heart and thymus. Unfortunately the brain was not examined. This patient has been reported as a case of TSC with multiple visceral involvement, including the thymus,[49] but this is not by any means an unquestionable case of TSC.

Lymphatics

A cervical lymph node with leiomyomatous changes was reported by Berg and Vejlens.[50] Wilson and Lo described infiltration of a mediastinal node with smooth muscle tissue in a 42-year-old with TSC and involvement of lungs, kidneys, skin, eyes, and brain.[51] Monteforte and Kohnen noted striking similarities between the cystic pulmonary changes associated with retroperitoneal or mediastinal lymphangiomyoma and the cystic pulmonary lesions of patients with TSC[52] (see Chapter 14). Practically all reported patients with LAM have been females and many of them had an associated chylous pleural and/or peritoneal effusion. These authors[52] presented a patient with LAM and cystic pulmonary disease who, in addition, had bilateral renal AMLs, but the brain, spinal cord, eyes, skin, and other viscera were unremarkable on gross and microscopic examination.

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17

Imaging the Skeleton and the Great Vessels

Radiographic examinations of lesions of tuberous sclerosis complex (TSC) have always been an important ingredient in the assessment of patients afflicted with this disease. In this chapter the classic radiographic features in the skeleton are reviewed. In addition, a few rare manifestations seen in TSC are reviewed: overgrowth of portions of an extremity and abdominal aortic aneurysm.

Skeletal Manifestations

Osseous involvement in patients with TSC may consist of one or several localized areas of sclerosis throughout the skeleton. These radiographic findings are unaccompanied by symptoms, have little significance, and are most often discovered after other features have established the diagnosis. Only occasionally, when the diagnosis is obscure, may these radiographic signs help to decide the diagnosis; rarely are they the first sign to suggest it.

Skeletal involvement is very common in TSC patients; Holt and Dickerson[1] found it in 66% of their patients and Reed and Nickel[2] in 45% of their patients.

Calvarial Sclerosis

On plain radiographs it may be difficult to distinguish intracerebral from vault calcifications, but, with stereoscopic films, multiple projections, or head computed tomography this should not be a problem. Calvarial sclerotic lesions are found in 40% to 50% of TSC patients,[1-3] primarily in the frontal and parietal bones. They are usually round but differ in shape, and their size varies between 0.2 and 2 cm. They are patchy and poorly defined and often have an appearance similar to dense osteomas. Rarely, lesions are wide-

spread in the cranial vault, appearing as generalized osteosclerosis and thickening of the calvarium. Calvarial sclerosis is not seen in infancy or early childhood; it appears within the first decade of life in about half of the affected patients. Microscopic examination shows increased density in the inner table and diploic trabeculae with concentric development of bone around the trabeculae and consequent reduction of the diploic spaces containing fatty tissue.[4] The relationship between cerebral calcification and cranial vault calcification is not established, and they may occur independently.[5]

Spine and Pelvis

Sclerotic lesions similar to those seen in the calvarium are frequently present in the spinal vertebrae and involve the bodies, posterior elements, or both (Fig. 17.1). Pelvic lesions tend to be flame shaped or irregular and in the iliac bones tend to cluster near the sacroiliac joint (Fig. 17.2), but other parts of the pelvis may be involved. Many peripheral sclerotic islands may be present and, being diffuse and poorly marginated, may be mistaken for osteoblastic metastases.

Hands and Feet

Osseous lesions of TSC in the hands and feet differ from those in other parts of the skeleton: they consist of cystlike rarefactions and often are associated with periosteal new bone. The cystlike lesions are seen more frequently in the hands than in the feet. Conversely, periosteal new bone is found more often in the metatarsal than in the metacarpal bones; it is rarely found in the phalanges. The new bone is thick and solid and has a characteristic wavy contour (Fig. 17.3). Cystic changes in the phalanges are present early in childhood, whereas cortical changes in the metatarsals and metacarpals appear at a later age.[6] Microscopic examination of the cystlike lesions shows nonspecific fibrous tissue replacing bone and surrounded by reactive osseous formations.[4,7] Rarely, subungual fibromas cause erosion of distal phalanges.

Other Skeletal Locations

Involvement of ribs and long bones in TSC is uncommon. It follows the pattern of the spine and pelvic lesions. A few cases of involvement under 1 year of age have been manifest as an expanded, relatively dense rib constituting an early sign of TSC.[8] Cystlike lesions or periosteal new bone seldom occur in locations other than the hands and feet.

Little attention has been paid to the association of tuberous sclerosis and scoliosis. In a series of 12 patients, six had scoliosis curvature ranging from 12 to 78 degrees, with a mean of 37 degrees.[9]

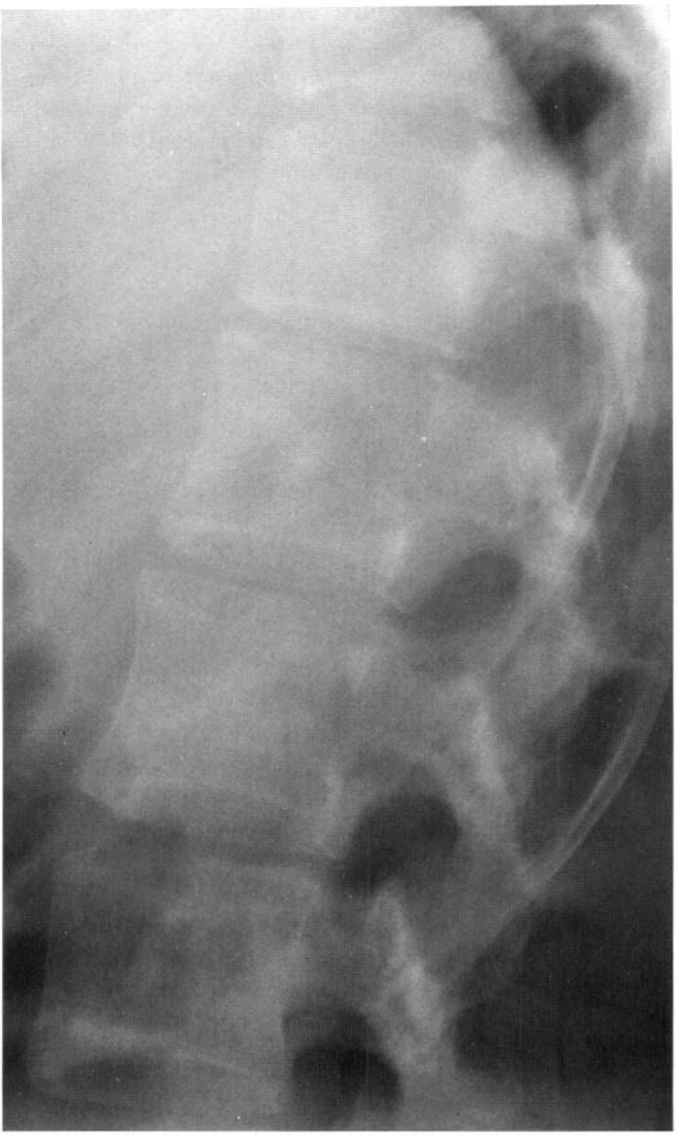


FIGURE 17.1. Localized lateral radiograph of the thoracolumbar junction demonstrating sclerotic patches in several vertebral bodies and in the neural arches. These findings are characteristic of the skeletal involvement with TSC. This patient clinically had manifestations of tuberous sclerosis.

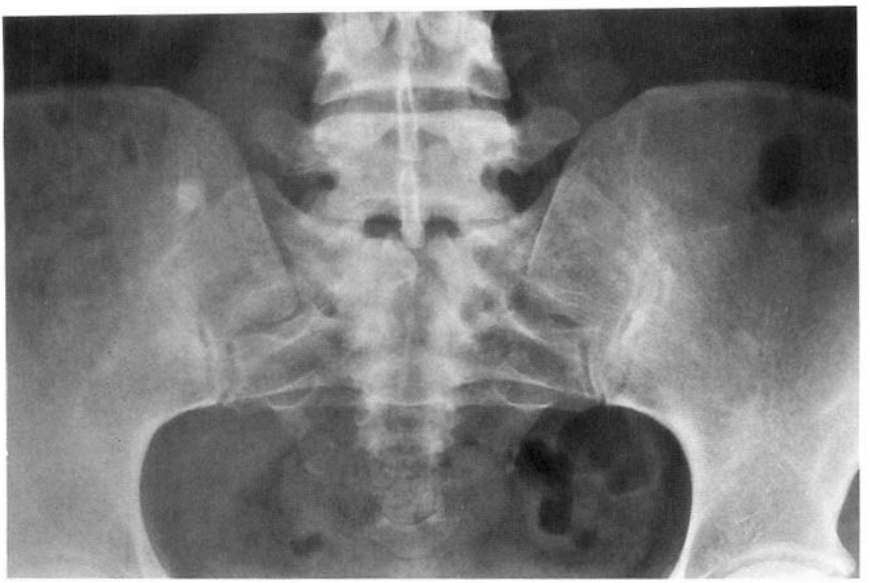


FIGURE 17.2. Localized view of the upper pelvis in a 50-year-old female with TSC. The sclerotic patch projected over the upper right sacroiliac joint and the more poorly defined sclerosis along the midportion of the left sacroiliac joint are characteristic findings of tuberous sclerosis.

Differential Diagnosis

The differential diagnosis of sclerotic lesions of TSC in various locations includes Paget disease, osteopoikilosis, sclerotic bone islands, and osteoblastic metastases. In the individual patient, this differential is usually not difficult. Patients with TSC will have normal serum alkaline phosphatase measurement, as opposed to those with Paget disease and metastatic bone disease. The findings, except for those in the long bones, are distinct from neurofibromatosis.[10] The metacarpal bone cysts could suggest sarcoidosis; however, the cysts in this condition have fine reticular bone destruction without periosteal new bone. Similar changes are present in many of the arthritides, but other abnormalities would also be present in the bones of the hands and wrists.

Vascular Lesions

A variety of unusual vascular abnormalities have sporadically been reported with TSC. Aneurysms of the aorta and great vessels are discussed in Chapter 16. Renal artery stenosis and coarctation of the aorta have been reported.[11]

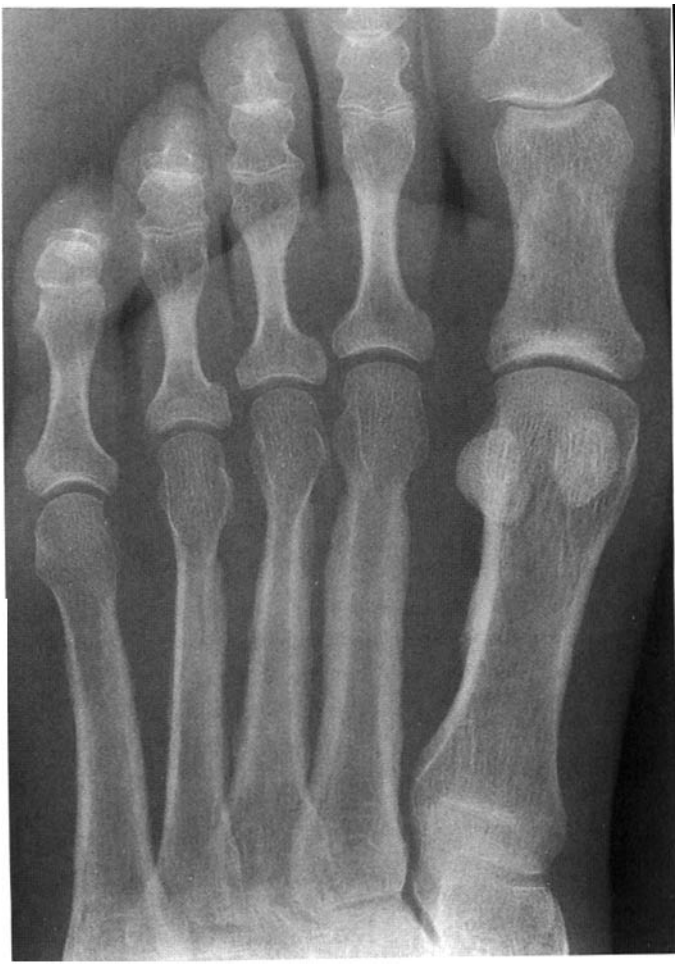


FIGURE 17.3. Localized radiograph of the anteroposterior view of the left foot in a 50-year-old female with TSC (same patient as in Fig. 17.2). There is characteristic periosteal new bone incorporated into the shafts of all of the metatarsals but particularly the second, third, and fourth.

There have been approximately 13 reported cases of aortic aneurysm, usually in the abdominal aorta and most often discovered in childhood.[12,13] However, in a large series of 204 cases of TSC, only 1 case occurred, suggesting only a rare or incidental association of aortic aneurysm with TSC.[14]

A 10-year-old boy with TSC was incidentally found to have an aneurysm involving the distal abdominal aorta and proximal right iliac artery. It was followed with abdominal radiographs and computed tomography over a 6-year period with little change and no perceptible symptoms (Fig. 17.4).

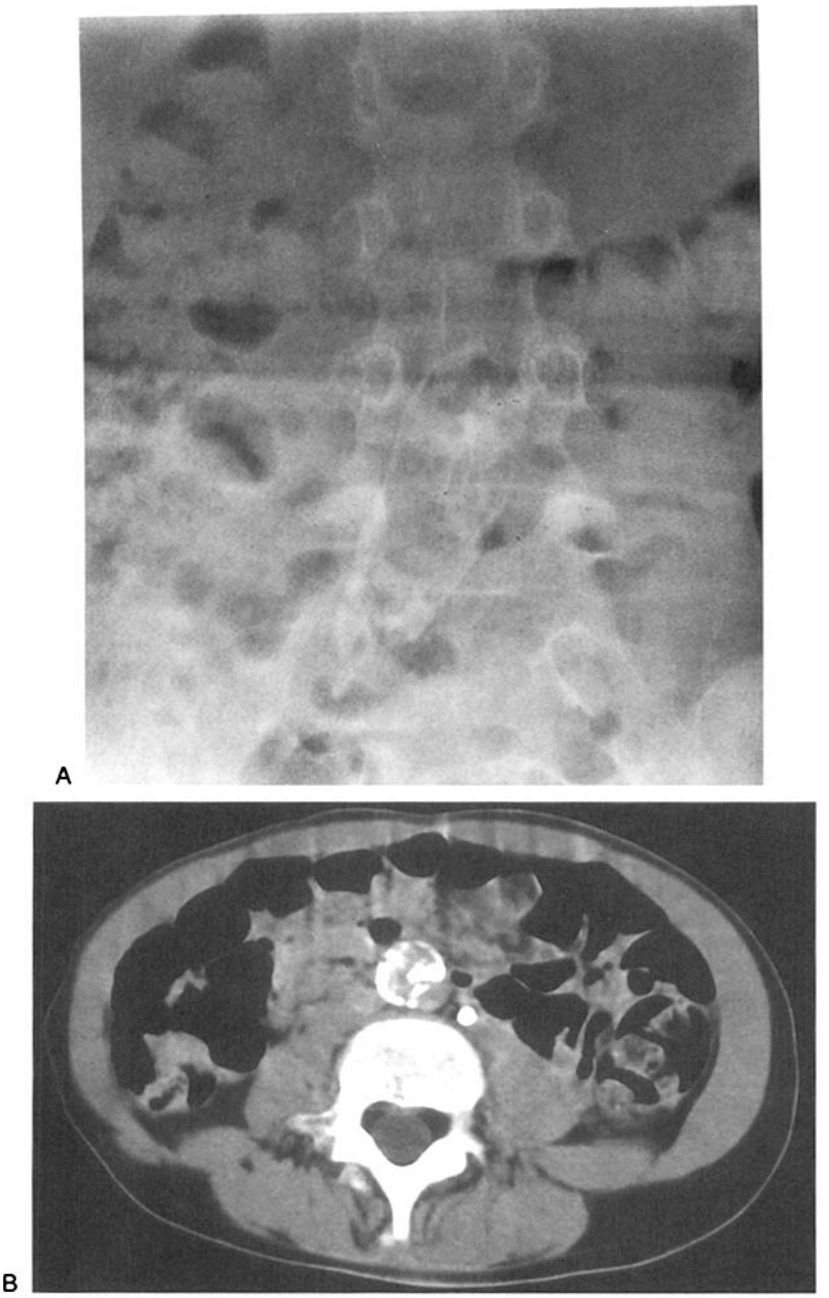


FIGURE 17.4. Ten-year-old boy with TSC. At age 4 he was incidentally found to have an abdominal aortic aneurysm involving the distal aorta and proximal right common iliac artery. He has been followed for 6 years without symptomatology or intervention. *A*, Radiograph showing calcification in the aneurysm at age 10. *B*, Computed tomography scan a few weeks later showing a slice involving the distal aorta. There is dense calcification of the aneurysm, which measured approximately 2.0 cm. There had been only slight increase in size over the period of observation. Contrast material is noted in the left ureter.

Localized Gigantism

Another unusual finding that has only rarely been seen in patients with tuberous sclerosis is focal overgrowth. This finding is more commonly seen with Klippel-Trenaunay syndrome or neurofibromatosis.

A 25-year-old woman with classic signs of TSC additionally had pain and deformity of the left arm. Radiographs demonstrated dysplasia, overgrowth, and sclerosis of the left humerus, the radius, and the three radial rays of the hand (Fig. 17.5). At least two similar cases have been reported in which angiograms showed arteriovenous malformation of the ulnar artery

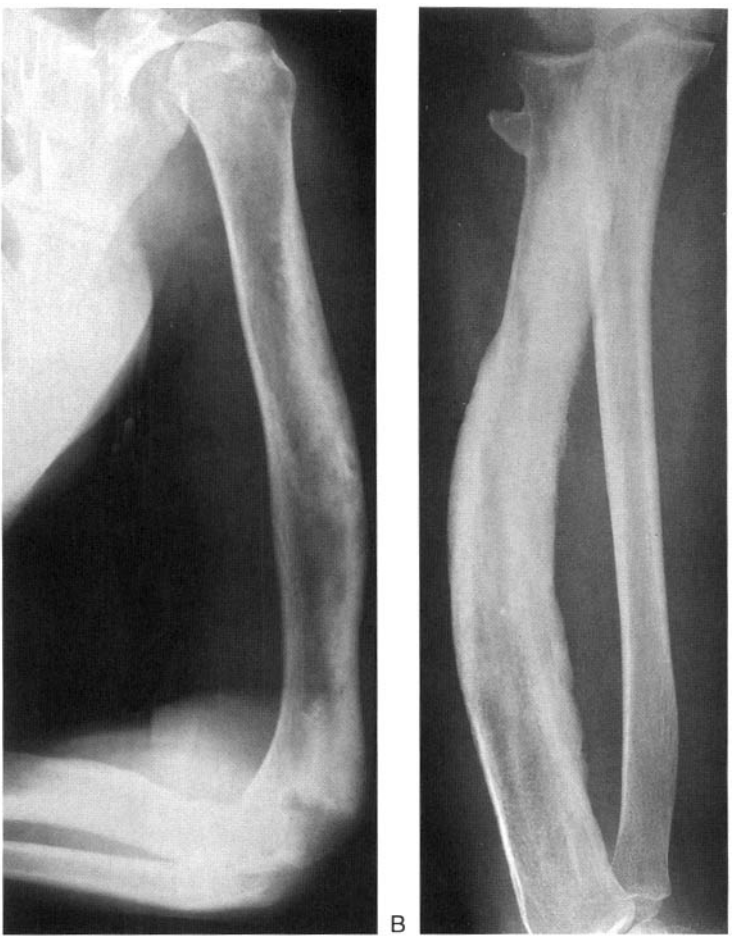
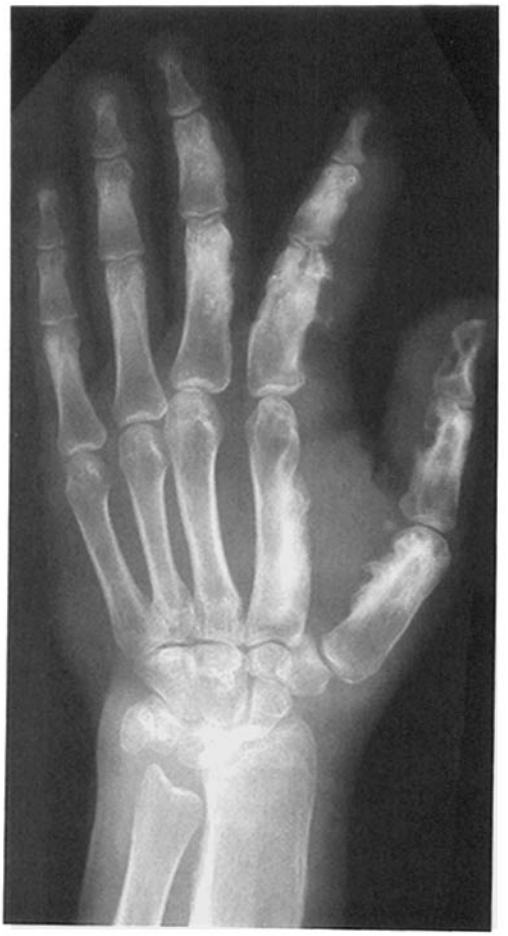


FIGURE 17.5. Twenty-five–year-old female with the typical signs of TSC. She complained of pain and deformity of her left arm. Radiographs of the humerus (*A*), forearm (*B*), and hand and wrist (*C*) demonstrate considerable dysplasia with sclerosis of the humerus, radius, and radial three rays of the hand. The ulna and the two ulnar rays are spared. *Illustration continued on opposite page*



C

FIGURE 17.5. (*continued*)

in one and agenesis of the radial artery in another.[15,16] Other cases have recently been noted.[17–19] The gigantism may be limited to a hand or just to one or more fingers of one hand.[20]

In the second edition of this book, I[21] described a young girl with TSC and Klippel-Trenaunay syndrome well documented by the marked hypertrophy of the right lower extremity, a large nevus of the skin, and varicosities all in the same extremity. She also had facial angiofibroma and a subependymal giant cell astrocytoma that required surgical removal. Since this brief report she has developed several bilateral renal angiomyolipomas and, at the young age of 19 years, pulmonary lymphangioleiomyomatosis. Although her head magnetic resonance imaging examination demonstrated many cortical tubers, she has had only one generalized seizure in her life, and this was immediately after having renal surgery under general anesthesia. She is of average intelligence and is self-supporting.

Imaging Implications

Because of the minor role of skeletal lesions in the diagnosis of TSC, it is unlikely that the role of imaging these lesions will change significantly in the future. The unusual occurrence of abdominal aortic aneurysm or overgrowth of an extremity in association with TSC should be appreciated so that proper diagnosis and treatment can be instituted.

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18

Lineages of Cells in the Central Nervous System

Value of Immunochemical Cell Markers and Classic Histologic Criteria in Determination of Lineages of Cells

The cells in the central nervous system lesions of tuberous sclerosis complex (TSC) appear sufficiently different from normal cells to make it difficult to determine from which cell type they are derived. This problem is not unique to the lesions of TSC. There is hardly a neoplasm that arises within the central nervous system for which there has not been a "lineage debate" in the literature. The differences between different cell types in the central nervous system are now known to be less clear than was once believed. Features that were considered to be specific for astrocytes[1] have subsequently been found in cells considered to be closer to oligodendrocytes or prospective oligodendrocytes.[2] Cells in vitro have even been shown to switch between astrocytic and oligodendroglial phenotypes depending on culture conditions.[3] Cells of neuronal and glial lineages may also be difficult to differentiate.[4] In TSC the problem is compounded by the fact that the central nervous system lesions are of several types, and that several types of atypical cells may be found in a single lesion.

Modern immunohistochemical techniques have significantly influenced the way in which we classify both normal and abnormal cells. They have added a new element of excitement to old speculations on the origin of cells, but they have also led to new controversies. It needs to be stressed that what is known about the distribution of immunohistochemical cell markers is based on classic histologic criteria. Problems arise when cells that stain with a marker for one cell type—for example, astrocytes—fulfill histologic criteria for another cell type, such as neurons. Is a cell that looks like a neuron but stains with astrocytic immunohistochemical markers a neuron or an astrocyte? This question is difficult to answer, short of redefining the astrocyte as a cell that contains one set of markers and the neuron as a cell with

another set of markers. The definition of a cell type based on the presence and/or absence of a set of markers is (or at least appears to be) more precise than a definition based on classic descriptive histologic criteria. It is relatively easy to decide whether a cell is "positive" or "negative" when it comes to immunohistochemical staining. This does not mean, however, that conclusions based on immunohistochemical staining are necessarily more correct than conclusions based on classic histologic analysis.

The above discussion underscores the problems involved with determination of the lineage of cells in the central nervous system in spite of modern technology, and perhaps also because of it. The following sections review the literature on the lineage of cells in the central nervous system lesions of TSC in the light of these observations.

Cortical Tubers

The cortical tuber is the lesion from which TSC derives its name. As the name indicates, the tuber is sclerotic or hard. Usually, lesions in the central nervous system are sclerotic because of the presence of significant numbers of fibrillary astrocytes. Accordingly, most light-microscopic and electron-microscopic studies have revealed a substantial contribution of astrocytes to the cortical tuber.[5-7] There is, however, disagreement on the relative contribution of neurons to this lesion.[6,8] Some investigators believe that the contribution of neurons is small,[6] but others maintain that it is substantial.[8] This discrepancy could be due to a segregation of cells within the tubers. Segregation invites a sampling error. In our studies on the immunohistochemical distribution of glial fibrillary acidic protein (GFAP) in cortical tubers,[9] we found that clusters of cells that contained GFAP were separated by areas devoid of GFAP staining (Fig. 18.1). In the central nervous system, GFAP is a marker for astrocytes.[10] Hence our results suggest that astrocytes—or at least GFAP-positive astrocytes—in cortical tubers form aggregates.

A Golgi study that we performed on some of the material used for the GFAP study showed that most of the cells located between the glial aggregates are probably neurons.[11] Staining with the Golgi-Cox method revealed that the tubers contained both neurons and astrocytes, which were unevenly distributed (Fig. 18.2). There were areas virtually devoid of neurons and dendritic processes interspersed among areas heavily populated by neurons and neuronal processes. The neurons in the tubers had a strikingly abnormal morphology (Fig. 18.3). In most areas, a random meshwork without discernible orientation was formed by multipolar or stellate neurons. They had unusual dendrites, often aspiny or oligospiny with beaded appearance (Fig. 18.4), suggesting decreased formation of synaptic contacts, as is also indicated in a recent report of synapsin I staining in cortical tubers.[12] Many of the neurons did not have identifiable axons. This may account for the sparsity of myelinated axons in the white matter below the tuber, which serves

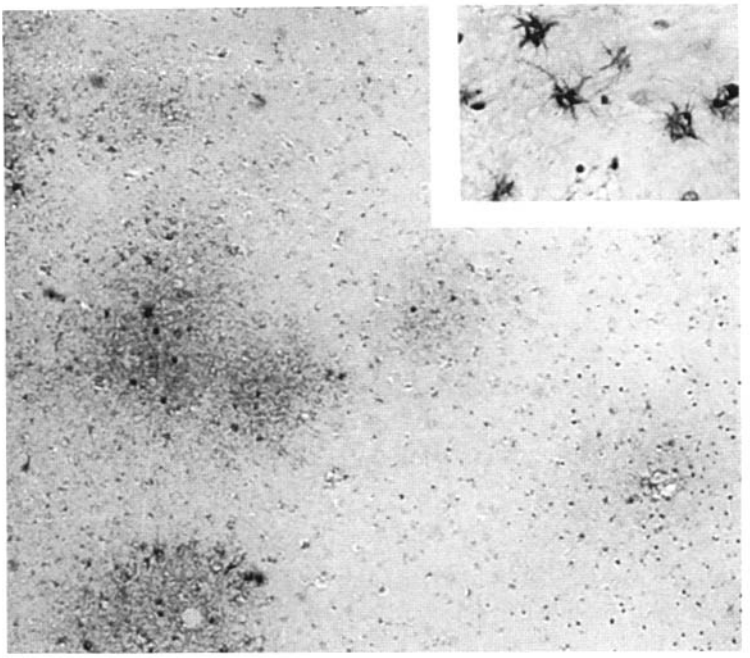

FIGURE 18.1. Cortical tuber stained with antiserum against GFAP and counterstained with hematoxylin. The dark areas are formed by aggregates of astrocytes staining darkly for GFAP. They are separated by areas devoid of GFAP-containing astrocytes. The inset shows a higher power view of cells in one of the dark areas staining darkly for GFAP. These cells have a morphology consistent with astrocytes.

as a marker for cortical tubers on magnetic resonance imaging of brain in TSC.

The neurons in tubers are unusual in several respects. First, in normal mammalian isocortex, pyramidal cells are the most common neurons, which is in contrast to the predominance of stellate neurons in the tubers. Second, the neurons in the tubers do not have a major dendrite oriented perpendicular to the pial surface, which is expected of cortical neurons. Third, varicosities on dendrites are a prominent feature during fetal development of all neurons,[13] but they are uncommon on neurons in the postnatal cerebral cortex. These findings therefore suggest that the neurons in cortical tubers represent immature of "developmentally arrested" forms (see below).

Cortical tubers frequently contain a third cell type other than fibrillary astrocytes and dysplastic neurons, namely the giant cell. Much work has been devoted to the determination lineage of these cells. Ribadeau-Dumas et al.[6] concluded that giant cells have astrocytic properties, based on ultrastructural criteria. The opposite view is represented by Arseni et al.,[8] who concluded that all giant cells they analyzed in a cortical biopsy from a patient with TSC fulfilled electron-microscopic criteria for neurons. We stained cortical tubers immunohistochemically with antibodies against GFAP, as men-

FIGURE 18.2. Section from a cortical tuber stained with the Golgi-Cox method. The *arrow* points to an aggregate of cells with morphology typical for astrocytes. The center of the figure contains mostly abnormal neurons. There is no apparent organization of the neuronal processes in this area. The bottom third of the figure shows fairly normal cortex with apical dendrites oriented perpendicular to the pial surface.

tioned above, and with antibodies against neuron-specific enolase (NSE), which is a marker for neurons and cells of the neuroendocrine system.[9,14] All the giant cells we found were stained with antibody against NSE (Fig. 18.5) and none with antibodies against GFAP (Fig. 18.6). These immunohistochemical data support the notion that the giant cells are neuronal rather than astrocytic.

More recent work on the lineage of cells in tubers has provided important additional information but has not as yet resolved all controversy. Hirose et al.[15] have confirmed the presence of both dysplastic neurons and astroglia in tubers. They find glial markers (GFAP, vimentin, and S-100 protein) and, less consistently, neuronal markers (neurofilament protein and class III β-tubulin) on giant cells in tubers, and suggest that these cells have failed to undergo glial-neuronal differentiation during embryonic development. A very recent study by Crino et al.[16] comes to a somewhat different conclusion.

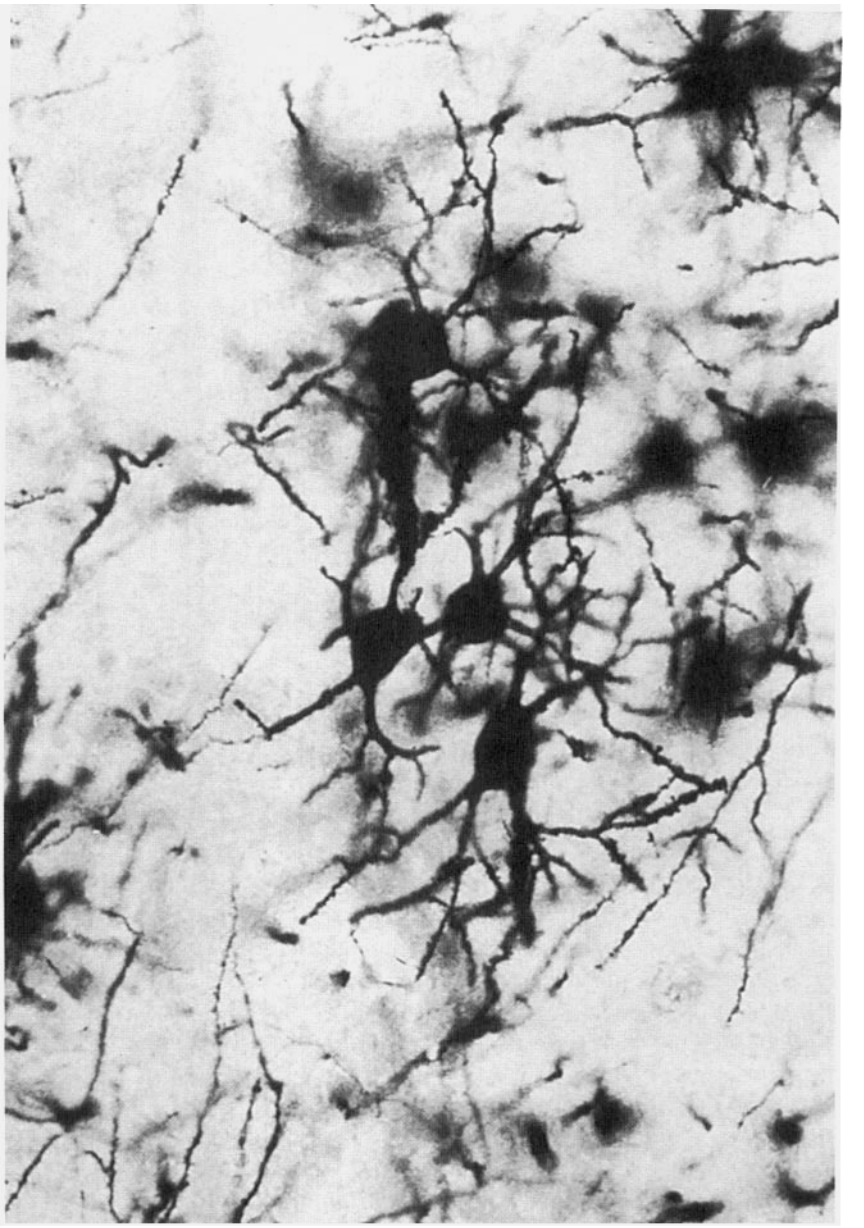

FIGURE 18.3. Small multipolar (stellate) neurons in a cortical tuber. (Golgi-Cox)

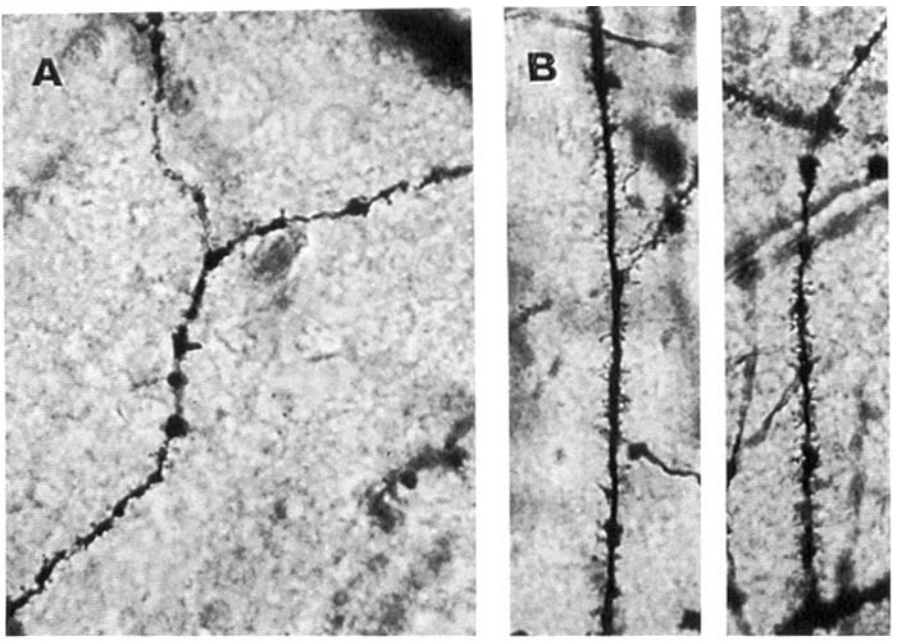

FIGURE 18.4. *A*, A beaded dendrite, as typically seen on multipolar neurons within tubers. Dendritic spines are sparse. *B*, Dendrites with normal spine formation in apparently normal cerebral cortex adjacent to a tuber. (Golgi-Cox)

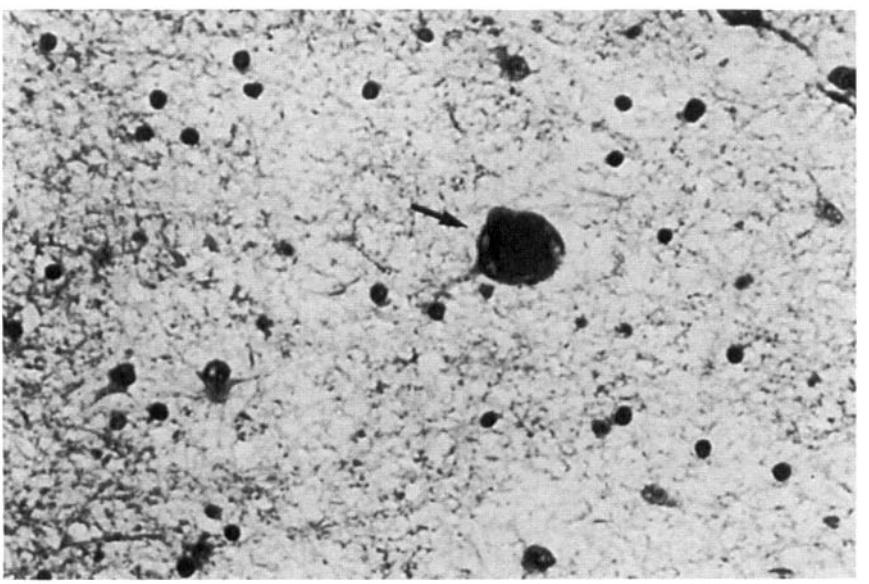

FIGURE 18.5. Section from a cortical tuber stained with antibodies against NSE and counterstained with hematoxylin. The *arrow* points to an intensely staining multinucleated giant cell typical for tubers.

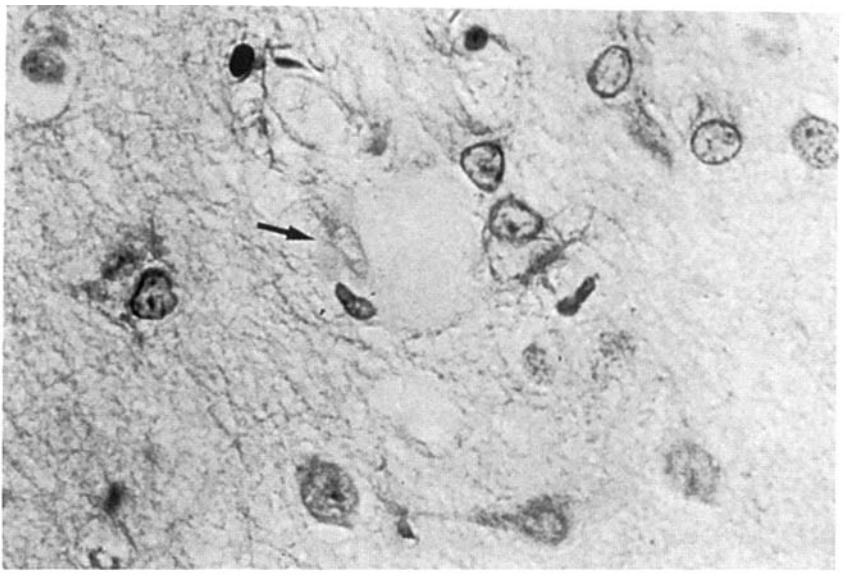

FIGURE 18.6. Section from a cortical tuber stained with antibodies against GFAP and counterstained with hematoxylin. The *arrow* points to a negative giant cell. The giant cell is surrounded by a few faintly staining astrocytes.

In an extensive immunohistochemical study of tissue from five tubers, these investigators found neuronal markers but not glial ones on giant cells. In addition, they found positive staining for three proteins (the embryonic intermediate filament protein nestin and two cell cycle markers, proliferating cell nuclear antigen and Ki-67) that are normally expressed only transiently by proliferating neuroepithelial cells in the embryonic germinal matrix. The same embryonic markers were found on dysmorphic neurons, but not on neurons in neighboring, histologically normal cortex. Utilizing a technique that involved dissection of single giant cells from formalin-fixed tissue and probing for messenger RNA in these cells, they were able to demonstrate absence of message for GFAP in giant cells, but presence of mRNA for several neuronal markers that are normally transiently expressed during or shortly after neuroblast division, as well as those that persist in mature neurons. There is at present no clear explanation for the difference in findings. One possibility is that giant cells in tubers are heterogeneous, some primarily neuronal in type, some astrocytic, and some undifferentiated, and that the differences in these studies are related to small sample sizes.

Subependymal Giant Cell Tumors

The subependymal giant cell tumor (SGCT) of TSC arises in the area of the foramen of Monro, either from the wall of the lateral ventricle over the basal

ganglia or, rarely, from the third ventricle.[17] Tumors with similar morphology occasionally arise in the same location in individuals without TSC. It is probably not wise to assume that the tumors that occur alone are of the same nature as the tumors that occur in the context of TSC. The SGCT seen in relation to TSC has different growth characteristics and probably also differences in immunohistochemical staining from its look-alike.[18,19]

The SGCT is well circumscribed, and the majority of cells in the tumor have eosinophilic cytoplasm, like gemistocytic astrocytes, but have nuclear features that resemble those of neurons.[20,21] Rarely, ganglion cells are seen in these tumors.[22] There is some controversy in the literature as to the cell of origin of SGCT. Globus et al.[21] concluded that the SGCT is a tumor of neurons. In contrast, Russell and Rubinstein[17] classified the tumor as an astrocytoma and coined the term *subependymal giant cell astrocytoma* (SEGA). Subsequent studies have provided some support for both of these views, as well as for a third view, namely that these tumors may be made of undifferentiated cells that express both glial and neuronal markers. It has also been suggested that SGCTs may contain two cell types, astroglial and neuronal.[23] The latter view received recent support from a cell culture study of SGCT in which both astroglia and dysplastic neurons have been described.[24]

Light-microscopic and electron-microscopic studies did not unequivocally point to the origin of the cells in the SGCT, and different investigators used the same observations to arrive at contradictory conclusions.[17,21,25] Therefore, it was reasonable to expect that immunohistochemical markers would play an important role in the analysis of this lesion. We studied the immunohistochemical distribution of GFAP and NSE in SGCTs from four TSC patients.[9,14] In none of the tumors did any of the tumor cells contain detectable GFAP, whereas the adjacent reactive glial tissue showed intense staining. Occasional straplike GFAP-containing cells were seen among the giant cells near the attachment of the tumor to the ventricular wall. These appeared to be astrocytes in fingers of reactive glial tissue entrapped within the growing tumor. The staining with antiserum to NSE gave results that were complementary to the results obtained with the anti-GFAP serum. All tumor cells in the four SGCTs were stained (Fig. 18.7), and the adjacent glial tissue was negative. We interpreted these data as supporting the view initially put forward by Globus et al.[21] that the SGCT is neuronal rather than astrocytic. However, this interpretation has not been universally accepted.

Bonnin et al.[18] found positive staining for GFAP in a great majority of 22 SGCTs and concluded that these are astrocytic tumors. However, only five of these lesions were from individuals with TSC. In these five, GFAP staining was observed in only two, and only in a small number of cells. Four of the five tumors occurring on a background of TSC contained NSE. The data in this study could therefore be reinterpreted as showing that SGCTs occurring in otherwise normal individuals are astrocytic tumors, whereas those seen in TSC are of neuronal lineage.

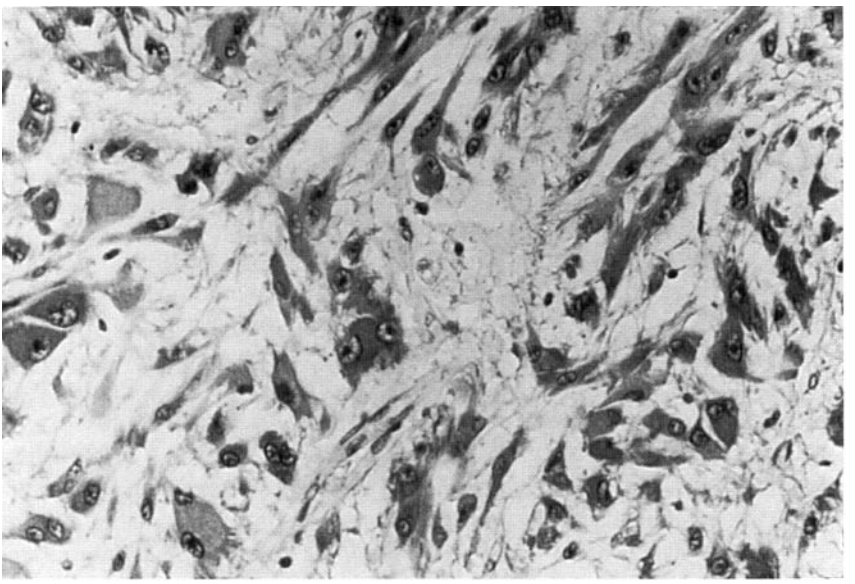

FIGURE 18.7. Section from an SGCT accompanying TSC. The section was stained with antibodies against NSE and counterstained with hematoxylin. All cells in the figure are intensely staining.

The interpretation of NSE staining is complicated by reports that NSE is not a faithful marker of neuronal tumors and may be found in various glial neoplasms.[26] Bonnin et al.[18] side with this view. There are, however, those who do not find NSE in glial tumors[27] and find it is tumors that may be derived from neurons.[28]

More recent immunohistochemical studies of SGCTs in TSC patients have employed multiple astrocytic and neuronal markers in addition to or instead of GFAP and NSE. Several investigators have found variable staining for neuronal and glial markers, with both types of markers in some tumor cells.[15,24,29] It therefore is possible that the tumor cells in SGCTs represent a heterogeneous population, some with abortive neuronal differentiation, some with glial differentiation, and some maintaining both glial and neuronal properties of primitive neuroectodermal stem cells.[15] Until these issues are resolved, it would seem proper to abandon the term *subependymal giant cell astrocytoma* and to simply call the tumor a subependymal giant cell tumor.

Subependymal Nodules

The subependymal nodules are responsible for the uneven surface of the lateral and third ventricles that have been likened to candle drippings and are so characteristic for TSC. They are also responsible for the periventricular calcifications seen on radiographs and computed tomograms. The con-

sensus is that they are composed mostly of astrocytes. In keeping with that, our studies of immunohistochemical distribution of GFAP and NSE in the lesions of TSC[9,14] revealed GFAP in a majority of the cells and NSE in a small minority of cells in the nodules. The cells containing NSE did not contain GFAP. Because it is a widely held opinion that the SGCT arises as a result of continual growth of cells of the subependymal nodule, it is tempting to postulate that the SGCT is derived from NSE-containing cells within the nodule. This is, however, entirely unproven. In summary, most of the cells in the subependymal nodules appear to be astrocytic both by classic histologic criteria and by immunohistochemical criteria. A few cells in each nodule contain NSE, which could reflect neuronal differentiation.

Retinal Tumors

A majority of retinal tumors that arise in TSC appear to be astrocytic as evaluated by classic histologic methods.[30] There are, however, descriptions in print of retinal tumors accompanying TSC that contain large cells that resemble ganglion cells, and there are those who have described them as being similar to the SGCTs.[31,32] Little has been written specifically about the lineages of cells in the retinal tumors, and the lineage of these cells has not been as controversial as the lineage of cells in the subependymal counterpart.

There was only one case in which the retinas were available in the material that we studied immunohistochemically for presence of GFAP and NSE.[9,14] The retinas in this case contained two tumors (phakomas). All of the cells in both tumors stained with antiserum against GFAP (Fig. 18.8) and not with antiserum to NSE. On hematoxylin-eosin–stained sections these tumors looked like astrocytomas. Therefore, in this one case, the classic histologic evaluation and immunohistochemical staining GFAP and NSE both point to astrocytomas.

Conclusions

As outlined above, there is still some controversy about the nature of the cells in the cortical tubers and in the SGCTs. One problem could be that investigators may be searching for (and finding) differences that do not exist between the cell types that may form these lesions. It is likely that future discussions about the lineages of cells in the central nervous system lesions of TSC will be influenced by recent work with cell markers showing that distinctions between the various cell types may not be as clear as previously thought.[3] It is also possible that the debate on whether the cells in the central nervous system lesions of TSC are derived from astrocytes or neurons or some other normal cell type may prove meaningless. The cells forming the TSC lesions may have been led astray very early in ontogeny, before astrocytes and neurons or their immediate precursors came into being. They may

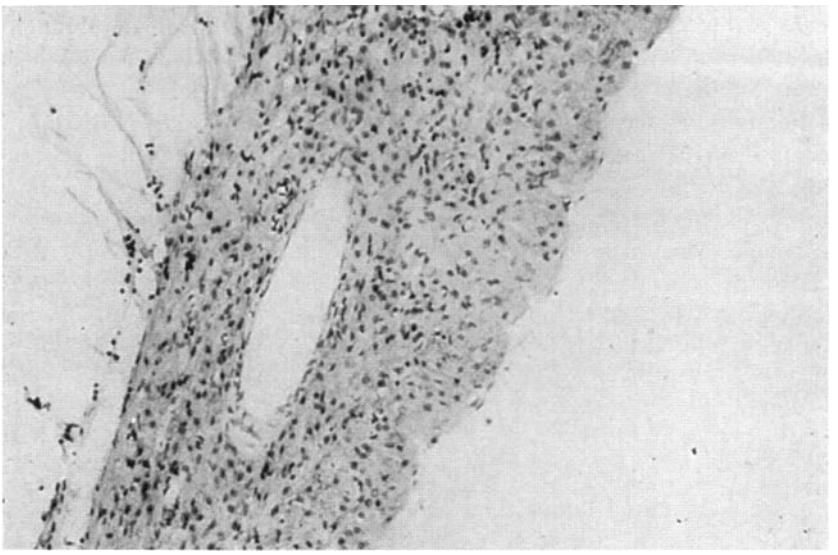

FIGURE 18.8. Section of a retinal tumor from a patient with TSC. The section was stained with antibodies against GFAP and counterstained with hematoxylin. All cells in the tumor are stained but not intensely.

be derived not from any cell type found in a normal postnatal (or even fetal) individual but from defective stem cells of the germinal epithelium in early embryogenesis. This view is supported by the fact that cerebral lesions of TSC have been found in a fetus as early as 28 weeks of gestational age.[33]

The central nervous system lesions of TSC contain cells that can be classified as neurons and cells that can be classified as astrocytes, as well as cells that are not easily classified. The neurons in the lesions are always primitive or aberrant. There is a suggestion that the astrocytes in the lesions are abnormal as well. They tend to form aggregates in the subependymal nodule and the cortical tuber rather than to infiltrate diffusely as they normally do. The genetic defect in TSC may be responsible for the appearance of defective stem cells in all germ cell layers of the embryo. In the neural epithelium these stem cells then evolve in both astrocytic and neuronal directions—as do normal neuroepithelial stem cells—but lack the ability to integrate themselves into a normal central nervous system. Some of the defective neurons and glia, perhaps the most immature, do not migrate from the periventricular germinal matrix but proliferate there and form the subependymal nodules and the SGCTs. Others, perhaps somewhat more differentiated, migrate to the mantle layers of the developing forebrain but fail to differentiate into pyramidal neurons and supporting glia and instead form cortical aggregates (tubers). More specific analysis of the lesions of TSC will probably have to await the discovery of the basic biochemical defect underlying the disease.

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The *TSC1* Gene: Part 1—Gene Mapping

Tuberous sclerosis complex (TSC) was first recognized as a genetic condition by Kirpicznik in 1910.[1] He reported a family with affected individuals in three generations and described the condition in identical and fraternal twins. Earlier studies[2,3] had noted that the facial lesions of TSC, erroneously called "adenoma sebaceum," were inherited in families. Berg, in 1913, reported that TSC was hereditary.[4] Gunther and Penrose first brought attention to the dominant inheritance pattern and were also first to suggest a high mutation rate as causal in TSC.[5] In 1951, Dickerson published an article describing three families with multiple members affected with TSC and reviewed the literature concerning all that was known about familial cases.[6] In 1967, Lagos and Gomez documented a family in which five generations were affected with TSC.[7]

Since the earliest descriptions of TSC at the end of the 19th century,[8,9] physicians and scientists have sought the underlying cause for the disease. Until the late 1980s, very little real progress was made toward uncovering the molecular basis of tuberous sclerosis. Fortunately, progress in the field of molecular genetics dramatically improved our ability to study genetic diseases. Tuberous sclerosis represents an excellent example of how far we have progressed.

Gene Mapping and Positional Cloning

For many years, investigators tried to determine the biochemical defect in patients with TSC. Unfortunately, these studies did not uncover the missing or malfunctioning protein in TSC patients. Knowledge about the protein product was the key in getting the first human genes cloned, the α- and β-globin genes.[10–12] The α- and β-globin genes code for the two protein chains that form hemoglobin. The knowledge that hemoglobin is abundant in re-

ticulocytes allowed isolation of the cDNAs of the globin genes from this enriched source. The protein sequence of the globin genes was utilized to verify that the correct genes had been found. Multiple other disease genes have since been cloned using their protein sequence to design probes for isolating cDNAs from libraries. For most genetic diseases, the malfunctioning or missing protein is not known. New methods were devised to circumvent this problem, which led to a new method for cloning disease genes based on their physical location in the genome (termed *positional cloning*) rather than knowledge of the gene product. The first gene to be isolated by positional cloning was the gene responsible for Duchenne muscular dystrophy.[13] In the ensuing decade, many other disease genes have been discovered by positional cloning.[14] A few examples include the genes that cause retinoblastoma,[15,16] cystic fibrosis,[17] and neurofibromatosis.[18,19]

In positional cloning, the rough location of a disease gene on a chromosome must first be determined. The term *locus* is used to denote the position of a gene on a chromosome. Sometimes investigators fortuitously have a chromosomal translocation or deletion detected through cytogenetic studies to start the search, as in the case of the retinoblastoma gene.[20–22] In other cases, cytogenetic studies lend no clues and genetic linkage studies are necessary to reveal which chromosome harbors a disease gene. In a genetic linkage study, DNA from individuals in families with multiple members affected by the disease is tested for known genetic variations (polymorphisms). A *polymorphism* by definition is the occurrence of two or more alternative genotypes (alleles) in a population.[23(p438)] An *allele* is one of the alternative versions of a gene that may occupy a given locus. A DNA sequence that displays variation is a polymorphic marker. Variation in sequence is known to occur approximately once every 300 to 500 base pairs along the 3 billion base pairs that make up one haploid copy of the human genome. Linked markers (stretches of DNA that are near one another on a particular chromosome) are transmitted together in meiosis. A polymorphic marker located near a disease gene will be passed from one generation to the next along with the disease gene. A genetic linkage study tests DNA from affected and unaffected individuals within a family for polymorphic variation from throughout the genome. When a particular allele of a polymorphism is passed from generation to generation along with the disease gene, linkage is established between the polymorphic locus and the disease gene (Fig. 19.1).

Statistical methods are used to determine when linkage is significant as opposed to chance. The primary test is the logarithm of the odds (LOD) score. The LOD score is the logarithm to base 10 of the odds in favor of linkage. By convention, a LOD score of 3 (odds of 1000:1 in favor) or greater is taken as proof of linkage and a LOD score of -2 (100:1 against) or less as proof that the loci are unlinked.[23(p435)] The *CFTR* gene, which is the gene mutated in the disease cystic fibrosis, was located to chromosome 7q exclusively by genetic linkage studies.[24–27] The *CFTR* gene was subsequently cloned without a chromosomal clue ever emerging.[17] The neurofi-

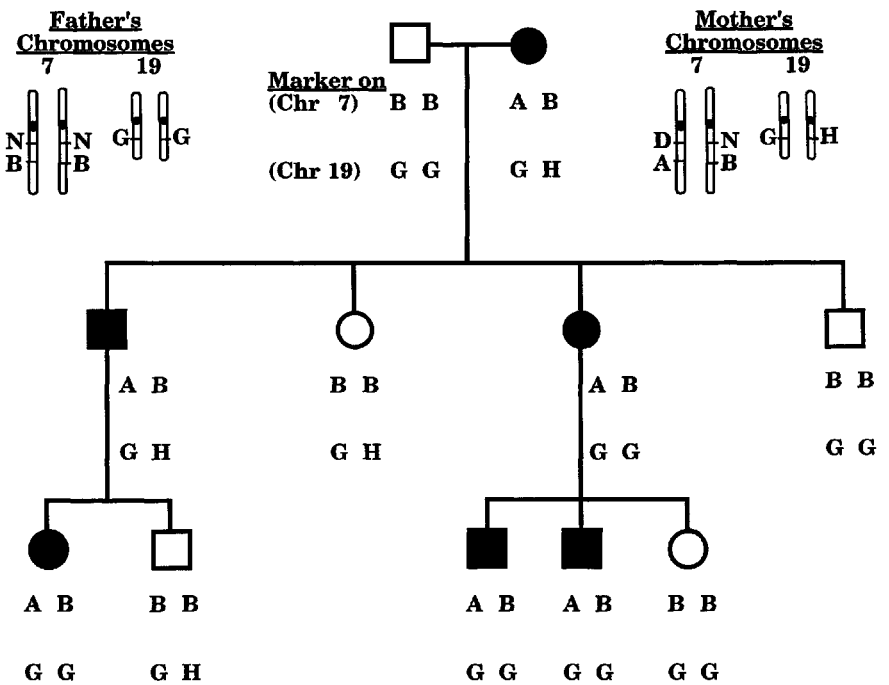


FIGURE 19.1. The polymorphic locus with alleles A and B illustrates segregation of allele A with the disease locus. The polymorphic locus with alleles G and H illustrates random inheritance in relation to the locus (D) of the disease gene. Locus N denotes the normal gene. The polymorphic locus with alleles A and B on chromosome 7 (Chr 7) is linked to the disease gene locus. The polymorphic locus with alleles G and H is located on chromosome 19 (Chr 19).

bromatosis type 1 (*NF1*) gene was first localized to chromosome 17 by genetic linkage studies,[28] with chromosomal translocations allowing further refinement of the gene's location.[29] The *NF1* gene was eventually isolated by positional cloning.[18,19]

Investigators turned to positional cloning technologies in the 1980s to solve the riddle of tuberous sclerosis. Fryer et al. undertook a genetic linkage study of 19 multigenerational TSC families in which 26 polymorphic markers were tested. Their report in 1987 of linkage between a TSC-causing gene and the ABO blood group gene on chromosome 9q34 was a breakthrough.[30] The locus was named *TSC1* for tuberous sclerosis complex type 1. Only 8 of the 19 families yielded information with the ABO blood group gene, because the variation among blood types in the population was limited; however, a LOD score of 3.85 was obtained, giving odds greater than 1000: 1 for linkage. A follow-up linkage study testing a DNA polymorphism within the *v-abl* gene (the *v-abl* gene was earlier shown to be very close to the ABO blood group gene) confirmed linkage and was utilized for prenatal

diagnosis of TSC.[31,32] Other groups subsequently found no evidence of linkage between TSC and polymorphic markers on chromosome 9q34 in the region of the ABO blood group gene in some of their familial TSC studies.[32–35] These conflicting results had to be resolved.

Genetic Heterogeneity

Investigators continued to test polymorphic loci on chromosome 9q34 in multigenerational TSC families. The studies indicated that investigators on both sides of the argument were correct: the *TSC1* locus on chromosome 9q34 must harbor a TSC-causing gene, and there was at least one additional locus (if not more) elsewhere in the genome. The observation of genetic heterogeneity was made independently by multiple groups[36–39] and by an international study that combined the data of many groups.[40]

Simultaneously, the search for the other TSC-causing genes continued. In 1988, a liveborn infant affected with tuberous sclerosis was found to have a chromosomal translocation involving chromosomes 11 and 22.[41] Smith et al. followed up on this report by testing polymorphic markers in multigenerational TSC families with polymorphic markers from the involved regions of chromosomes 11 and 22. The studies gave a positive result for linkage between the markers on chromosome 11 and a TSC-causing gene.[42] Follow-up studies found evidence that a few families with TSC may have a causative gene mapping to chromosome 11.[40,43,44] Others did not find evidence for a TSC locus on chromosome 11.[38,39] Meanwhile, another TSC patient who had a chromosomal translocation [t(3:12)(p26.3;q23.3)] was described.[45] Family studies of the two implicated regions on chromosomes 3 and 12 revealed a possible linkage to the phenylalanine hydroxylase locus on chromosome 12q.[46] Investigators were unable to substantiate the finding in other TSC families.

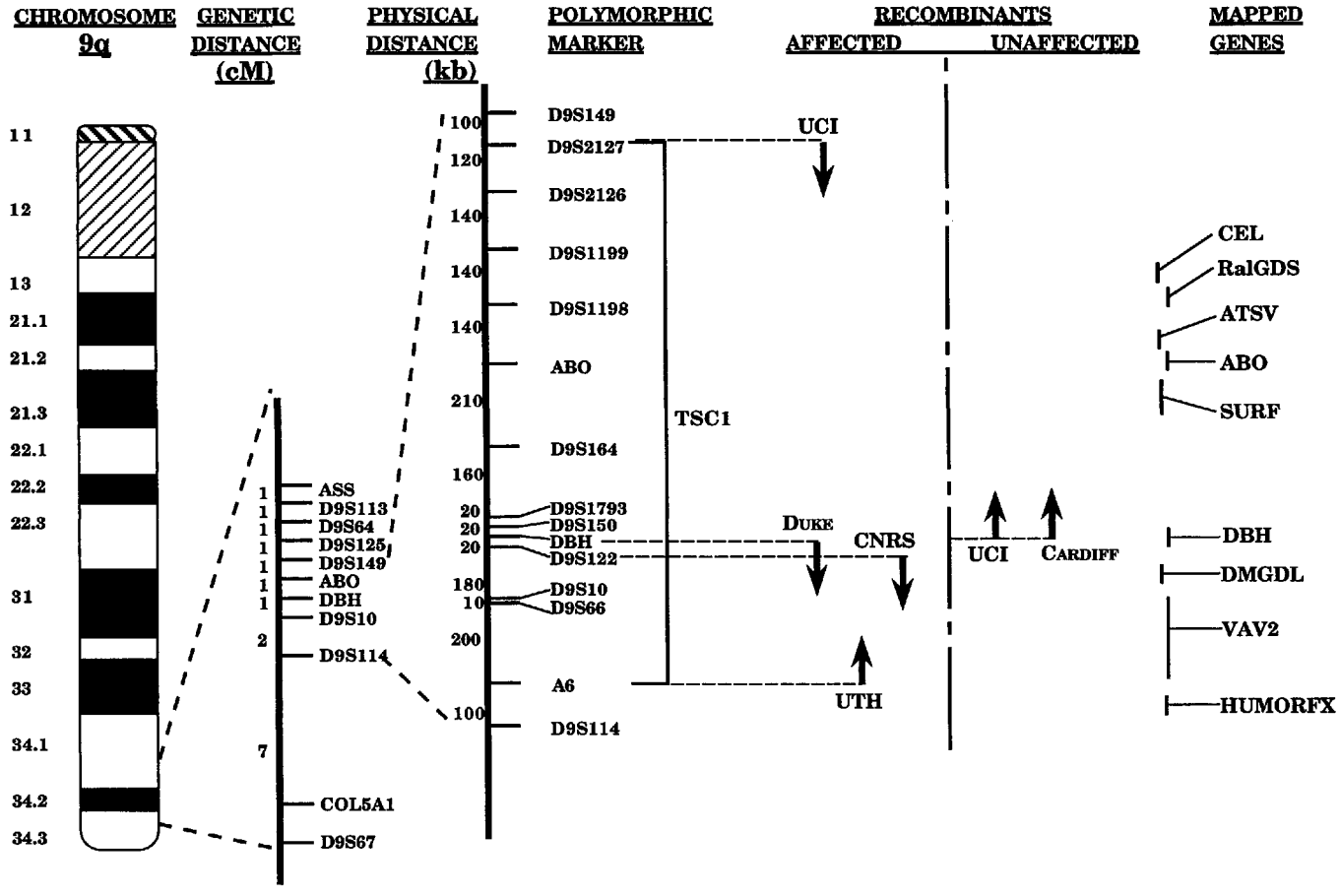
In 1991, multiple investigators in the United States formed a collaboration to find additional TSC gene loci. Five large families affected with TSC were studied. In all families, linkage between a TSC gene and markers from chromosomes 9q, 11q, and 12q had been excluded. A genome-wide search testing these five families revealed linkage between a TSC-causing gene and a polymorphic marker near the locus for a gene causing adult polycystic kidney disease type 1 (APKD1) on chromosome 16p.[47] The LOD score for linkage was 9.50, odds greater than 1 billion to 1 in favor of linkage. This finding set the stage for the cloning of the TSC gene on chromosome 16, termed *TSC2*. (The *TSC2* gene is the subject of Chapter 20). Subsequent studies indicate that, in the majority (>90%) of TSC families large enough to detect linkage, strong evidence of linkage can be shown to the *TSC1* or *TSC2* locus.[48,49] There is now little support for the existence of TSC-causing genes on chromosome 11 or 12.

Definition of the *TSC1* Candidate Region

Initial definition of a large *TSC1* candidate gene region on chromosome 9q34 was not difficult. Since at least 1993, investigators have been in agreement that the *TSC1* candidate region is between the polymorphic DNA markers D9S149 and D9S114[50] (Fig. 19.2). The region is approximately 5 cM in genetic distance but only 1.5 Mb of physical distance.[49,51] (Genetic distance is measured in centimorgans [cM] with 1 cM equivalent to a 1% chance that two loci next to one another on a chromosome will be separated during meiosis. During meiosis, the chromosomes replicate and then pairs of homologous chromosomes come together. While paired, segments of DNA from homologous chromosomes are exchanged, a process called recombination. The ability of the chromosomes to exchange pieces allows genetic diversity. The closer together two loci are on a chromosome, the less likely are they to become separated in meiosis and subsequently end up on different members of the pair of chromosomes. When the loci end up separated 10% of the time, this is interpreted as a 10-cM distance between them, 5% of the time is interpreted as a 5-cM distance, and so forth. The recombination frequency is averaged over the entire length of a chromosome; however, we know that there are areas on chromosomes where recombination occurs more frequently [e.g., near the ends of the chromosome, the telomeres]. Therefore, genetic distance does not always reflect physical distance between loci. Physical distance between two loci is a measurement of the actual base pairs separating the loci. Base pairs are often referred to in thousands [kilobases, abbreviated kb] or millions [megabases, abbreviated Mb]. A physical distance of 1 Mb on average contains 20 to 30 genes.)

In the *TSC1* candidate region, crossing over during meiosis occurs at a greater frequency than usual, leading to the discrepancy between the genetic and physical maps. By testing 9q-linked TSC families with polymorphic markers discovered since 1993, the region has been reduced slightly to 1.4 Mb. The critical region has recently been determined to be between markers D9S2127 (slightly telomeric to D9S149) (recombinant UCI in Fig. 19.2) and a marker named A6 (which is approximately 100 kb centromeric of D9S114) (recombinant UTH in Fig. 19.2).[52,53]

The critical region of the *TSC1* gene between D9S2127 and A6 is about 1.4 Mb and could easily contain 30 to 45 genes. It would be very difficult to test all available candidate genes within it because testing a candidate gene is a very labor-intense process. The best way to decrease the size of the critical region is to study large families with TSC who show strong evidence of linkage to the *TSC1* region, searching for individuals in the family who show a recombination event with polymorphic markers from within the critical region. In this situation, the individual inherits the chromosome with the disease gene but there has been an exchange during meiosis between the *TSC1* gene and the polymorphic markers that are nearby. The marker A6 near the *TSC1* gene illustrates such a recombination event in a chromosome 9–linked family (individual III-5 in Fig. 19.3).[52] The re-


CHROMOSOME
9q
GENETIC DISTANCE (cM)
PHYSICAL DISTANCE (kb)
POLYMORPHIC MARKER
RECOMBINANTS
AFFECTED
UNAFFECTED
MAPPED GENES
11
12
13
21.1
21.2
21.3
22.1
22.2
22.3
31
32
33
34.1
34.2
34.3
1 ASS
1 D9S113
1 D9S64
1 D9S125
1 D9S149
1 ABO
1 DBH
D9S10
2
D9S114
7
COL5A1
D9S67
100
120
140
140
140
210
160
180
10
200
100
D9S149
D9S2127
D9S2126
D9S1199
D9S1198
ABO
D9S164
20 D9S1793
20 D9S150
20 DBH
20 D9S122
D9S10
D9S66
A6
D9S114
TSC1
UCI
DUKE
CNRS
UTH
UCI
CARDIFF
CEL
RalGDS
ATSV
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SURF
DBH
DMGDL
VAV2
HUMORFX

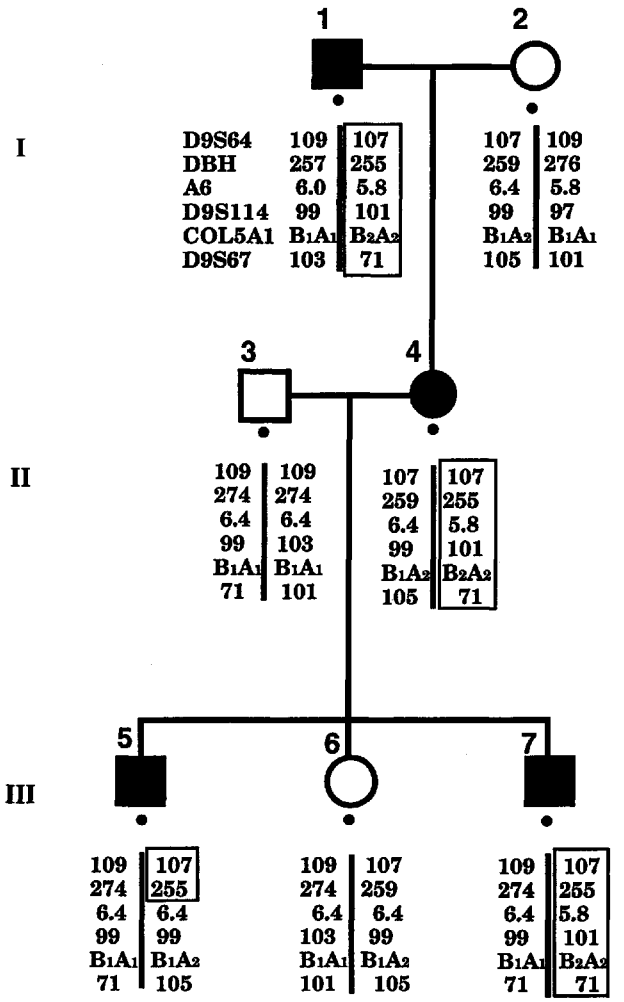

FIGURE 19.3. Pedigree with results of markers on 9q34.3 in the *TSC1* candidate region. Individual III-5 shows a recombination involving the chromosome 9s inherited from his mother (individual II-4). Individual III-5 has inherited the *TSC1* gene with allele 107 of marker D9S64 and allele 255 of marker DBH. There has been a recombination event between individual II-4's chromosome 9s. Individual III-5 has inherited allele 6.4 of marker A6, allele 99 of marker D9S114, allele B_1A_1 of marker Col5A1, and allele 105 of marker D9S67 from II-4s unaffected chromosome 9. Therefore, the *TSC1* gene is centromeric to marker A6.

combination during meiosis allows us to eliminate portions of the candidate gene region and exclude genes located between A6 and D9S114. Because there are a paucity of large multigenerational TSC families, there are not many individuals to test who could potentially narrow the region. A few families have been described with recombinant individuals who could sig-

nificantly narrow the region. Unfortunately, these reports conflict with one another. These critical recombinant events are summarized in Figure 19.2. Two recombination events in affected individuals reported by different groups would place the *TSC1* gene telomeric to the dopamine β-hydroxylase (*DBH*) gene.[54,55] In the report placing the *TSC1* gene telomeric to *DBH*, the affected individual (recombinant Duke in Fig. 19.2) has only minimal findings of TSC, but the individual has refused further testing which could clarify disease status. The diagnosis of TSC in the critical individual (recombinant CNRS in Fig. 19.2) placing the *TSC1* gene telomeric to D9S122 has recently been questioned by the investigators who reported the family.[55] Testing of two other large TSC families with unaffected recombinants would indicate that the *TSC1* gene is centromeric to *DBH* (recombinants UCI and Cardiff in Fig. 19.2).[56] Unaffected recombinants (termed *putative recombinants*) are always suspect because a diagnosis of TSC can be difficult to make in some individuals. Interestingly, these four recombinant events, while they point in different directions for the location of the *TSC1* gene, are all located within 40 kb of one another on chromosome 9q34.

Physical Mapping of the *TSC1* Candidate Region

Several contigs, or parts, of the *TSC1* region have been constructed using clones from YAC, BAC, PAC, P1, and cosmid libraries.[57–61] YACs (yeast artificial chromosomes), BACs (bacterial artificial chromosomes), PACs (P1 artificial chromosomes), P1s (P1 bacteriophage), and cosmids are all vectors that can accommodate DNA of different sizes and maintain yeast or bacterial hosts.[62] YACs can hold the largest pieces of DNA (up to 2 Mb in size, with the average size of a piece of DNA contained by a YAC being 300 to 900 kb) and cosmids the smallest pieces of DNA (ranging from 20 to 45 kb; usually around 35 kb) with BACs, PACs, and P1s holding intermediately sized (70- to 130-kb) pieces of DNA. BACs, PACs, and P1s were developed because these vectors can hold larger pieces of DNA than cosmids but are more "faithful" than YACs during passages. DNA inserts in YACs often rearrange or have pieces of chimeric DNA from different chromosomes contained within the same YAC clone.

One method of finding candidate genes is to clone the candidate region harboring the gene into these vectors and then isolate all of the genes contained within that stretch of DNA. Almost all of the 1.4-Mb *TSC1* candidate gene region has been cloned in contigs except a gap of approximately 20 kb between markers D9S164 and D9S1793.[63] Clones representing the contigs of the candidate region are being utilized to isolate cDNAs (portions of the coding regions of genes) from within the *TSC1* region. DNA from TSC patients can then be tested for changes (mutations) in the candidate genes. If a candidate gene has mutations in the DNA from TSC patients, that candidate gene is the *TSC1* gene.

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The *TSC1* Gene: Part 2—Identification, Mutations, and Mosaicism

This part of the chapter describes the identification of the *TSC1* gene[1] and summarizes recent observations on mutations and mosaicism in TSC. Because of space limitations, references are given only to major reports, and the information presented builds upon the concepts and discussion given in part one of this chapter and in Chapter 20).

Identification of *TSC1*

The first report of genetic linkage analysis (made by some members of the TSC1 Consortium) identifying a probable TSC gene on 9q34 appeared in 1987. This gene was denoted *TSC1*. Subsequent studies indicated that not all TSC families demonstrated linkage to the 9q34 region, indicating that there probably were other TSC genes (locus heterogeneity). Later chromosome 16p13 was identified as the site of a second TSC locus (denoted *TSC2*),

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†The TSC Consensus Conference Panel 7 consists of the following researchers: David J. Kwiatkowski, Dicky Halley, Trevor Hawkins, Nicola Migone, Hope Northrup, Julian Sampson, Kit-Sing (Paul) Au, Moyra Smith, John Yates, Sue Povey, Sandra Dabora, Arupa Ganguly.

which led to the discovery of the *TSC2* gene. Genetic linkage studies performed by two groups on TSC family collections have indicated that the *TSC1* and *TSC2* genes each account for about half of all familial TSC cases.

Identification of the *TSC1* gene on 9q34 was a lengthy process and proved difficult for several reasons. Conflicting positional information was generated by the analysis of recombination events in TSC families; no large genomic rearrangements (e.g., chromosomal translocations) involving this region have been discovered (in contrast, these are common at the *TSC2* gene and accelerated its identification); and several parts of the region are unstable in multiple cloning vectors, making the analysis of the entire region for potential genes difficult. In addition, the region is gene-rich and there were several genes in it that were good candidates to be *TSC1*. The consortium surmounted these difficulties through development of a contig of cloned material for the region, by careful analysis of pedigrees available to us for positional information, by systematic gene identification throughout the entire region, by large-scale genomic sequencing of the contig, and finally by comprehensive mutational analysis of genes from the region.

Figure 19.4 shows the *TSC1* region, including limiting centromeric and telomeric markers based upon analysis of affected individuals (*solid arrows*, second level from bottom) from families with strong evidence that mutations occurring in *TSC1* were present (LOD scores >2). These limits were also consistent with limited information derived from loss of heterozygosity (LOH) analysis of TSC lesions. Two additional recombination events were identified in unaffected individuals (*open arrows*), who also were from families with LOD scores greater than 2. In each of these families two individuals from different generations carried the same recombinant chromosome, and all four had no evidence of TSC. Since the penetrance of TSC is nearly 100%, we concentrated our search on the 900-kb region between D9S2127 and *DBH*.

Several techniques were used to identify genes in the *TSC1* region, which proved to be relatively gene-rich. Using a combination of exon trapping, cDNA selection, expressed sequenced tag (EST) mapping, and whole-cosmid hybridization, we identified 142 exons (gene segments) and 13 genes between D9S1199 and D9S114. In all, 30 genes were identified or mapped to the 900-kb critical region. Several of the genes identified appeared to be good candidates based upon probable or defined roles in signal transduction pathways governing growth regulation. However, analysis of these genes did not reveal any evidence of mutation in patients with TSC.

In parallel, we had begun a strategy to sequence the entire 900-kb critical region using the contig clones. We expected that additional candidate genes would be discovered by this approach, and that the genomic sequence information would facilitate analysis of genes for mutations. After 208 kb of the genomic sequence of the region had been determined, we had discovered an additional four probable new genes in the region, and partial or complete genomic sequence information was derived for three genes identified by previous studies.

We used the polymerase chain reaction (PCR) to amplify putative and confirmed exons found in this 208-kb region on a screening set of 60 DNA samples from 20 unrelated familial TSC cases with linkage to 9q34 and 40 sporadic TSC cases. Amplification products were analyzed for mutations using a technique called heteroduplex (HD) analysis. The 62nd exon screened demonstrated HD mobility shifts in 10 of the 60 patient samples (Fig. 19.5*A*).

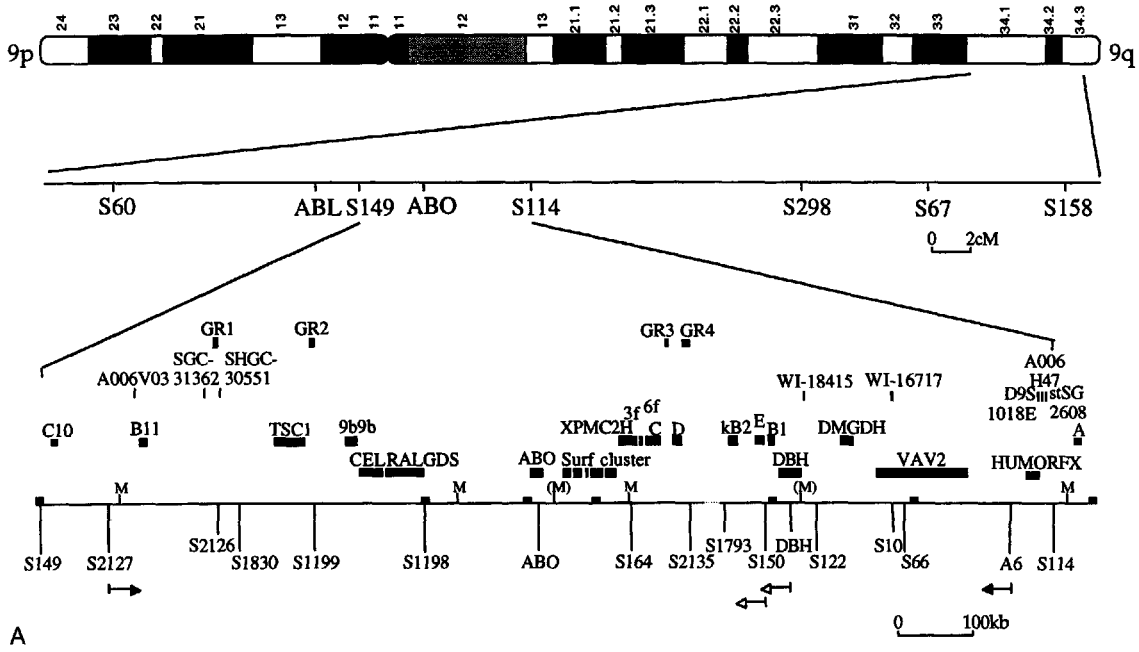
Sequence analysis of the DNA samples with HD shifts revealed seven small deletions (three identical) that would truncate the protein encoded by this exon, one nonsense mutation (also truncating), one missense change, and one polymorphism that did not change the encoded amino acid (Fig. 19.5*B*). Eight of the nine mutations were from the 20 familial cases tested, and only one mutation was seen among the 40 sporadic cases (Fig. 19.5*C*). Analysis of samples from other family members confirmed that each of the familial mutations segregated with TSC, and that a frameshift mutation had occurred de novo in the sporadic case (Fig. 19.5*D*).

The full sequence of the *TSC1* gene, containing the exon with mutations, was determined by comparison of genomic sequence and cDNA clone sequence. The *TSC1* gene consists of 23 exons; the last 21 contain coding sequence and the second is alternatively spliced (Fig. 19.4, bottom). There is a 221-bp 5′ untranslated region (exons 1 through 3), a 3492-bp coding region (exons 3 through 23), and a 4.5-kb 3′ untranslated region (exon 23). Northern blot analysis with a coding region probe (nt 1100 through 2100) revealed a major 8.6-kb message that was widely expressed and was particularly abundant in skeletal muscle.

Mutations in *TSC1*

Within the first 6 months of the discovery of *TSC1*, mutations had been found in all coding exons except exons 3, 16, 22, and 23 (Fig. 19.6). The lack of mutations in the last two exons (22 and 23) may reflect a lack of functional importance for this portion of the protein. Mutations are particularly frequent in exons 15 (28/91, 31%), 17 (13/91, 14%), and 18 (12/91, 13%), and several mutations have been identified more than once, including 2577C→T R786X, which has been seen in seven independent patients. All of the mutations identified are truncating in their effect on the protein, by virtue of a deletion or insertion of nucleotides or a nonsense or splice-site mutation. No genomic deletions or rearrangements in *TSC1* were detected by Southern blot or pulsed-field gel electrophoresis analysis of 250 TSC patients.

Current results in mutation detection for both TSC genes were reported by several investigators at the National Institutes of Health–sponsored TSC workshop (held in Annapolis in July 1998). About 300 unique *TSC1* or *TSC2* mutations have been identified in 399 distinct TSC patients/families (Table 19.1).[2–9] The types of mutation that occur in *TSC1* and *TSC2* are clearly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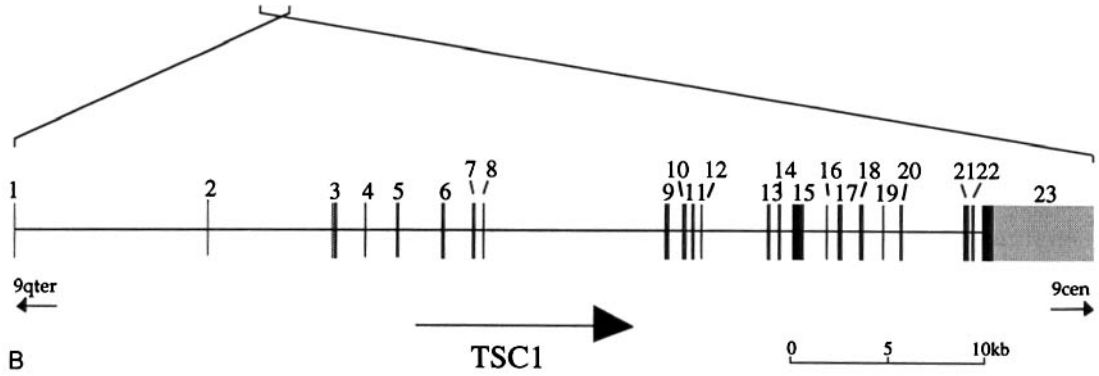

FIGURE 19.4. The *TSC1* regoin on chromosome 9. The ideogram (*top*) represents a normal G-banded metaphase chromosome 9, with the *TSC1* region located at 9q34. The male genetic map (*next line*) shows selected anchor polymorphic loci mapped to 9q34. The detailed physical map of the candidate region (*next level*) shows the positions of polymorphic markers and key recombination events in affected members (*solid arrows*) and unaffected members (*open arrows*) of families showing linkage of TSC to 9q34; the approximate positions of Mlu sites (M, with sites that partially cut in genomic DNA shown in *brackets*) and of probes used to screen the region for rearrangements in patients with TSC using pulsed-field gel electrophoresis (*boxes*); genes previously mapped to the *TSC1* candidate region; novel cDNAs isolated from the region; ESTs mapped to the region; and additional putative genes predicted by GRAIL analysis of genomic sequence (*boxes* at progressively higher levels). There was a single 20-kb gap in the contig near D9S1793. The map of the *TSC1* gene (*bottom*) shows the 23 exons, of which 3 through 23 are coding.

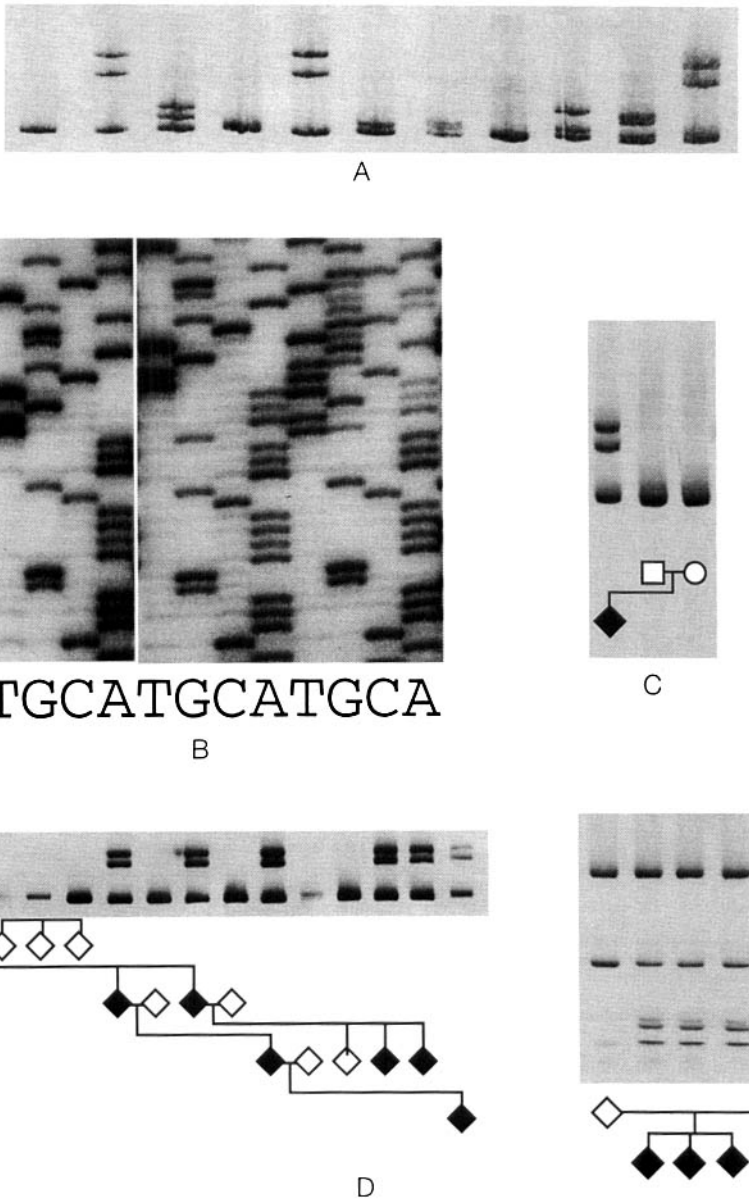

FIGURE 19.5. Identification of mutations in *TSC1* exon 15. *A*, Heteroduplex analysis. Control sample (*left*) followed by 10 samples with a shift. *B*, Sequence analysis demonstrating 2105delAAAG mutation. The sequence reactions were done in antisense orientation, so that, reading from the top down (bases 2083 through 2124 of the normal sequence is shown), the allele sequenced on the left has the deletion, the middle sequence is a normal allele, and the sequence on the right is the HD product with both alleles. *C*, In a sporadic case the HD mobility shift is not present in either parent. *D*, Segregation of HD mobility shifts in a large family with TSC, and digestion of amplification products with MwoI in another family, demonstrate segregation of the 2105delAAA mutation with the disease.

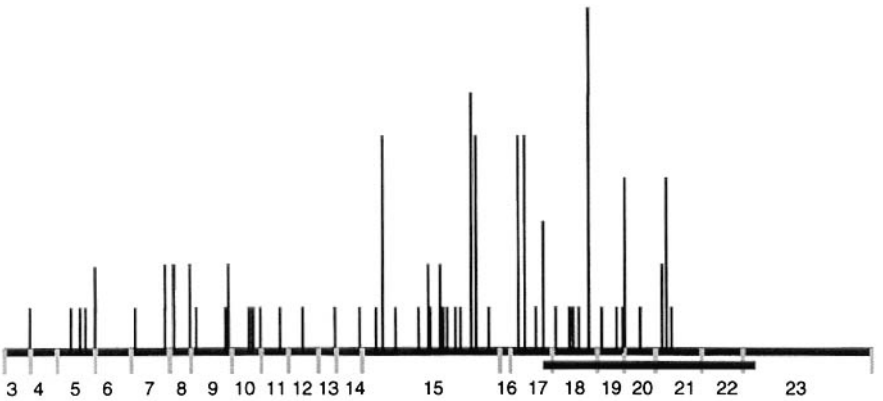

FIGURE 19.6. The mutation distribution in the *TSC1* gene. A diagram of the *TSC1* gene is given on the horizontal axis, with the limits of each coding exon marked by vertical gray lines and proportionally sized. Mutations are indicated by the vertical black lines, which are sized proportionally to the number of mutations seen at each nucleotide in the sequence. Exons 15, 17, and 18 are seen to be common sites of mutations. No mutations are seen in the last 23% of the gene, including exons 22 and 23. The black line under exons 18 through 22 indicates the coiled-coil region of hamartin.

different. In *TSC1* small deletions and insertions account for the majority of mutations, and point mutations that generate stop codons (nonsense mutations) are also common. Only three large (<3-kb) deletions have been seen in *TSC1* (N. Migone, personal communication 1998), and they are very rare. A few splice mutations have been seen (these affect the connecting together of the exons of the gene), and no clearly pathogenic missense mutations have been identified. In contrast, about 17% of mutations identified in *TSC2* are large deletions or rearrangements (>1 kb), and about half of these are very large mutations involving deletion or rearrangement of more than 50 kb of genomic DNA. Fifty-nine percent of *TSC2* mutations are small deletions or insertions, nonsense point mutations or splice-site mutations, all of which result in truncation of the protein product. Twenty-four percent of

TABLE 19.1. Mutational Spectrum in *TSC1* and *TSC2*

Mutation	*TSC1*	*TSC2*
Large deletions and rearrangements	3 (2%)	43 (17%)
Small deletions and insertions	81 (55%)	72 (29%)
Nonsense	57 (38%)	55 (22%)
Splice	7 (5%)	20 (8%)
Missense	0	61 (24%)
Total	148	251

identified *TSC2* mutations are missense mutations in which a single base substitution results in the change of a single amino acid in the protein.

Several methods have been used to identify mutations in the *TSC1* and *TSC2* genes, all of which are PCR based using genomic DNA or RNA transcribed to cDNA. The methods include HD and single-stranded conformation gel analysis, which are each predicted to be capable of detecting 50% to 90% of all point mutations, depending upon the size and sequence context of the mutation and technical aspects of the procedure. When used together, their combined predicted sensitivity is 75% to 90% for all small mutations. Improvements in the sensitivity of mutation detection assays for these types of mutations are clearly important because they account for 88% of all identified *TSC1* and *TSC2* mutations.

Comprehensive studies in several laboratories suggest that *TSC1* mutations account for only a minority of sporadic cases of TSC.[1,4,7–9] Although detection techniques are imperfect, *TSC1* mutations appear to be found in only about 15% to 20% of such patients. As noted in Chapter 20, a comprehensive mutational analysis for both *TSC1* and *TSC2* on a substantial group of TSC patients is nearing completion in the laboratory of Julian Sampson.[4–6] Preliminary estimates from this analysis are that *TSC1* mutations account for 15% of patients, *TSC2* mutations account for 65%, and 20% of patients have not yet had a mutation detected. This latter group could represent patients whose mutations have been missed because of technical reasons, might be mosaic patients whose mutations have gone undetected (see further below), might in fact have mutations in other (unknown) genes that cause a TSC syndrome, or may be misdiagnosed in some cases. Continuing study of these patients to distinguish among these possibilities is clearly of considerable interest.

The frequency of *TSC1* versus *TSC2* mutations in these sporadic patients (15:65) clearly contrasts with the frequency of *TSC1* versus *TSC2* mutations in familial TSC (50:50). This observation has suggested that the TSC phenotype might be milder in those patients with mutations in *TSC1* than in those with *TSC2* mutations, explaining their improved chances of having a family sizeable enough to be suitable for linkage analysis. Some direct evidence for this possibility has also been provided in that, in one series, sporadic *TSC1* patients had less mental retardation than sporadic *TSC2* patients.[4] However, this finding has not been confirmed[7] and additional study is required.

Function of Hamartin, the Product of the *TSC1* Gene

The predicted *TSC1* protein, termed *hamartin*, consists of 1164 amino acids with a calculated mass of 130 kDa, which has been confirmed by immunoblotting studies with hamartin-specific antisera. The protein is generally hydrophilic and has a single potential transmembrane domain at amino acids 127 through 144 and a probable 266–amino acid coiled-coil region begin-

ning at position 730 (Fig. 19.6). Genetic database searches identified a possible homolog of *TSC1* in the yeast *Schizosaccharomyces pombe*, a hypothetical 103-kDa protein, but there were no strong matches with vertebrate proteins. Thus these initial analyses gave no hint as to the function of tuberin.

It is clear that *TSC1* functions as a tumor suppressor gene. First, all mutations seen to date are likely to inactivate protein function because they result in production of a truncated protein. The absence of mutations within the COOH-terminal end of the protein (exons 22 and 23) is also consistent with this concept. Second, LOH studies have shown that there is a small subset of patients with sporadic TSC who in their hamartomas have undergone loss of the second *TSC1* allele. Although the frequency of this finding was quite low in most series, we can now appreciate that this was due to the low frequency of *TSC1* mutations in the sporadic patient population under study. Third, analysis of a renal cell carcinoma from a TSC patient with germline mutation 2105delAAAG revealed a somatic mutation 1957delG in the wild-type *TSC1* allele. Therefore, the current evidence strongly supports the tumor suppressor gene model in which hamartin is not expressed in an active form in hamartomas in *TSC1* patients.

The mechanism by which loss of hamartin expression produces TSC lesions is unknown. At the time of the identification of hamartin, it seemed likely that hamartin and tuberin participated in the same pathway of cellular growth control, since the clinical features of *TSC1* and *TSC2* disease are so similar. This hypothesis was verified in a recent report that hamartin and tuberin bind to one another within the cell.[10] Tuberin, the product of the *TSC2* gene, has modest GAP activity for both Rap1 and Rab5, but is likely to have other critical physiologic functions as well. A logical hypothesis worthy of exploration is that hamartin serves to regulate and/or direct tuberin function in the cell, such that a complex of the two proteins is required for a critical cell growth control function. Further investigation is clearly necessary.

Mosaicism in TSC

Mosaicism is the phenomenon in which a fraction of, rather than all, cells making up the body contain a DNA mutation or chromosomal abnormality. Mosaicism for a TSC gene mutation occurs when only a fraction of cells in the patient contain a mutation in *TSC1* or *TSC2*. Because there is a high frequency of new mutations in the TSC genes (the clinical consequence of which is a high sporadic case rate), it has been anticipated for some time that mosaicism might occur in TSC. Indeed, as seen in Figure 19.7, when a new mutation occurs in a patient, it only occasionally occurs in the sperm, egg, or newly fertilized egg. If it occurs at any other time, then mosaicism for that mutation must be present. In many cases, the mutation may occur in such a small proportion of cells in the patient that there is no clinical

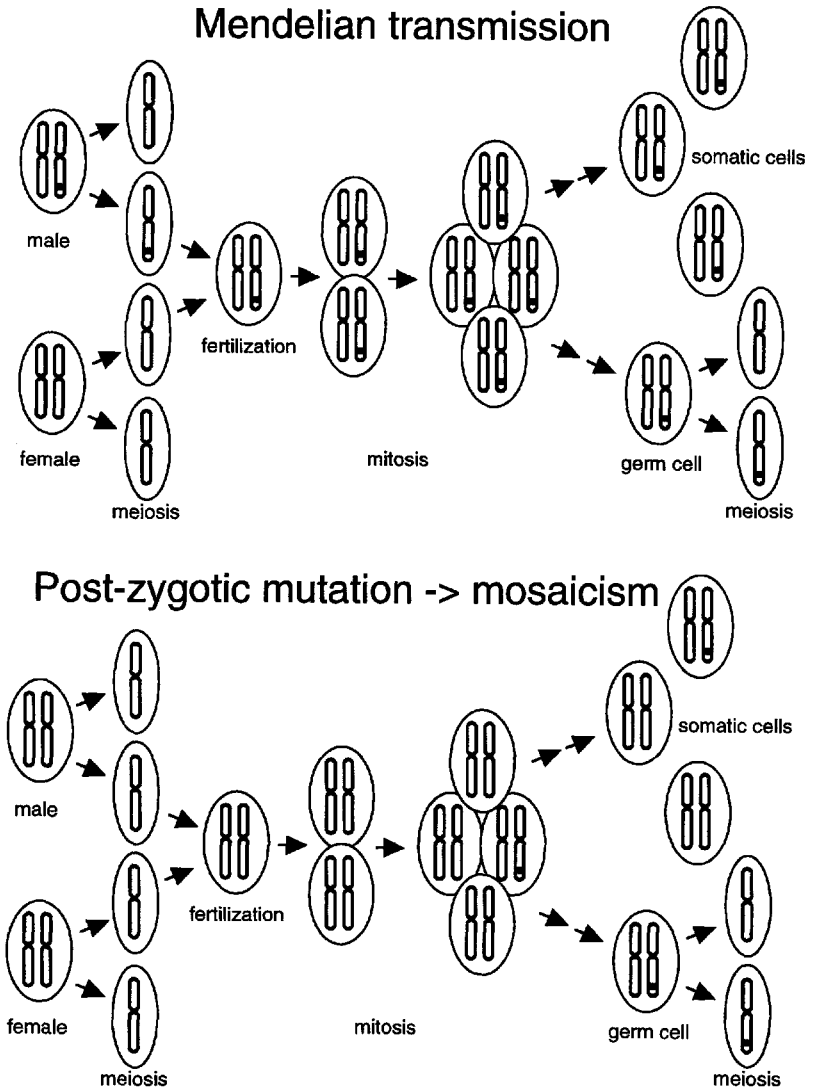

FIGURE 19.7. Mendelian transmission and mosaicism. The series of divisions that occur in cells during development is shown. Each oval represents a single cell, containing a pair of homologous chromosomes 9. A *TSC1* mutation is indicated by a black stripe on the chromosome. *Top*, Mendelain transmission. A mutation is present in all cells of the father, which he transmits to half of his sperm, so that each offspring has a 50% chance of inheriting the mutant *TSC1* gene. An offspring who inherits the mutant *TSC1* gene will pass on that same mutation to all cells. *Bottom*, A case of mosaicism. When the mutation occurs at the four-cell stage of embryogenesis, roughly 25% of cells in the child will also carry this mutation (all progeny from the cell with the original mutation), and 75% will not have a *TSC1* mutation. The types of cells in the body that contain the mutation determine which organs may have TSC lesions. If the mutation occurs at a later stage of embryogenesis (not shown here), then only germ cells may carry the mutation. This condition is confined germline mosaicism.

manifestation or consequence. Alternatively, when the mutation is present at somewhat higher frequency among body cells (approximately 5% to 50% mosaicism), one might anticipate clinical features of TSC to be limited in organ distribution or severity. Tissue- or organ-specific mosaicism might explain isolated but extensive facial angiofibromas or renal angiomyolipomas, for example. A second clinical situation in which mosaicism has been considered is that in which two or more children with TSC are born to parents who do not appear to be affected. In this case one suspects the possibility of germline or gonadal mosaicism, in which a fraction of the germ cells (priomordial cells giving rise to spermatozoa or eggs) of one or the other parent carries the mutation, but no other body cells. Although there has been suspicion of mosaicism in TSC for many years, only recently has molecular evidence for its occurrence been obtained.

TSC1 mosaicism was recently identified in a TSC patient with 30% mosaicism for a *TSC1* mutation in blood cells.[11] This patient had typical, severe manifestations of TSC, including two different types of seizure disorder, mental retardation, multiple cortical tubers and subependymal nodules on brain imaging studies, and facial angiofibroma. Analysis of DNA samples obtained from urine, blood, buccal mucosa, and hair indicated that the level of mosaicism varied from tissue to tissue, with a low of 0% representation in one buccal mucosa DNA sample and a high of 42% in one sample of urine cells.

This case is remarkable in that moderate-level mosaicism for a *TSC1* mutation is present, and yet the patient has relatively severe TSC. The observation has signficant implications for TSC in at least two respects. First, it indicates that, as long as there is a proportion of cells making up an organ that harbor a *TSC1* mutation, a hamartomatous lesion can develop. This concept is consistent with the two-hit model for hamartoma development in tuberous sclerosis.

Second, it suggests the possibilty that a substantial fraction of TSC sporadic patients could be mosaic. The mutation screening methodology (HD analysis, as in Fig. 19.5*A*) used to identify this patient's mutation was a relatively sensitive method for detection of mutations in the presence of mosaicism. This patient's mutation would likely not have been detected by direct sequencing of the exons of *TSC1*, which is often considered a gold standard for detection of mutations, because of to the low frequency of the mutant allele. Moreover, the extent of somatic mosaicism in different tissues may vary such that no mutant alleles might be present in blood cells, and yet a moderate frequency of the allele could be present in other organ systems affected by tuberous sclerosis, particularly the brain. Undetected mosaicism could well contribute to the substantial numbers of patients in whom serious efforts at mutation detection for both *TSC1* and *TSC2* have failed, as above.

Studies in which mosaicism for *TSC2* mutations has been identified[6,12] also indicate that mosaicism is relatively common in TSC. In contrast to the patient above, these patients have had milder clinical findings or were di-

agnosed with concurrent tuberous sclerosis and polycystic kidney disease (see Chapter 20). Nonetheless, Sampson et al.[6] provide a minimum estimate of the frequency of mosaicism in TSC in this same group of patients with the combined *TSC2-PKD1* gene deletion syndrome (see also Chapter 20). They demonstrated that 7 of 26 patients (27%) had mosaicism for genomic deletion of *TSC2* and *PKD1* in the first affected family member.

In addition, gonadal mosaicism has now been documented in 10 TSC families.[13,14] In these cases, two or more affected offspring were born to unaffected parents. The mutation in *TSC2* was identified in these affected children and shown not to be present in blood DNA samples from either of the parents. Gonadal biopsy or sampling has not been performed to confirm the occurrence of the mutation in germline tissues, but these analyses provide strong evidence for its occurrence. They also provide a basis for current estimates of the recurrence risk for a second affected TSC child to unaffected parents of 2%, and suggest that this recurrence risk might be minimized by molecular diagnostic studies.

Acknowledgments

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20

The *TSC2* Gene and Tuberin

When a novel gene such as *TSC2* is identified, a fundamental point in the associated disease process is defined. Using the gene as a resource, many areas of research can move from descriptive to experimental phases. Analysis of the sequence of the predicted protein product may reveal clues to the molecular and cellular processes that are perturbed in the disease state. In the case of tuberous sclerosis, descriptive research has been undertaken for over a century but experimental research is still in its infancy. With the progress that has been made in our understanding of the molecular basis of tuberous sclerosis, this area is now likely to grow and develop rapidly.

Positional Cloning of *TSC2*

Positional cloning is an elegant and conceptually simple approach to gene identification. The positions of our genes relative to one another on the 23 pairs of human chromosomes are the same in everyone, except for a small minority of individuals who have chromosome rearrangements. If one can work out the exact position of a specific gene relative to fixed points in the genome, then that gene can be found. The problem is one of scale. The haploid human genome comprises 3 billion base pairs (bp) of DNA, and many genetic diseases, including tuberous sclerosis, can result from change in as little as a single base pair. The search can usually be reduced approximately 3000-fold by linkage analysis. Just how difficult progress is from this point depends in large measure on whether further positional information can be generated. The identification of *TSC2* was relatively rapid because a series of unequivocal and increasingly precise positional clues were identified. These progressively pinned down the location of the gene.

The *TSC2* gene was localized to distal chromosome 16p (the end of the chromosome's short arm) in 1992.[1] Its isolation, on an entirely positional

basis, was reported in December 1993.[2] Two factors in particular helped to make such rapid progress possible. First, mapping at 16p13.3 was already advanced relative to many areas of the genome, and, second, chromosomal rearrangements were identified that defined a small region in which the gene could be sought. The distal short arm of chromosome 16 was an early focus for genetic mapping by groups interested in disorders of hemoglobin, because the α-globin gene lies in this region.[3] Further detailed mapping had also been undertaken during attempts to identify the *PKD1* gene, the major gene for autosomal dominant polycystic kidney disease, after this was genetically linked to the α-globin locus in 1985.[4] This work provided a map of fixed landmarks into which positional information on the *TSC2* gene could be rapidly integrated. Critical information on the position of *TSC2* was then revealed by analysis of deletions of the tip of chromosome 16. Initially very large deletions involving more than a megabase (Mb, or million base pairs) of DNA were characterized. Definition of the exact position of *TSC2* was then achieved by taking a gamble—hoping that a small but significant proportion of patients with tuberous sclerosis would have submicroscopic deletions of many thousands or tens of thousands of base pairs involving the gene. The gamble paid off. Unfortunately, a similar approach at the *TSC1* locus was not so rewarding.

During 1992–1993, linkage studies identified an approximately 1.5-Mb region of chromosome 16p as likely to contain the *TSC2* gene.[1,5] At the same time, a family with both tuberous sclerosis and autosomal dominant polycystic kidney disease was found to segregate a translocation between chromosomes 16p and 22q (family 77, Fig. 20.1). A mother (77-2) and her daughter (77-3) carried a balanced translocation involving 16p13.3. They had signs of typical autosomal dominant polycystic kidney disease. The karyotype in each was 46,XX,t(16;22)(p13.3;q11.21). At 48 years of age, patient 77-2 was hypertensive and had a raised serum creatinine level. Ultrasound scan revealed bilateral polycystic kidneys. Multiple bilateral renal cysts were also found in patient 77-3, who was then 17 years of age. Her blood pressure and creatinine were normal. Both were investigated for signs of tuberous sclerosis by dermatologic and ophthalmologic examination and by computed tomography of the brain. No signs of tuberous sclerosis were found. The son, patient 77-4, was severely mentally handicapped, suffered with seizures, and exhibited repetitive autistic behaviors. He had inherited an unbalanced karyotype, 45,XY,−16,−22,+der(16)(16qter→16p13.3::22q11.21→22qter) and was therefore deleted for the chromosomal regions 16p13.3→16pter and 22q11.21→22pter. Skin examination revealed facial angiofibromatosis and hypopigmented macules, and computed tomography of the brain revealed typical subependymal calcification, confirming a definitive diagnosis of tuberous sclerosis. Renal ultrasound showed a small number of cysts in each kidney, consistent with tuberous sclerosis or early autosomal dominant polycystic kidney disease. The translocation breakpoint on chromosome 16 in this family was eventually shown to disrupt the previously unidentified *PKD1* gene.[6] It was reasoned that patient 77-4 mani-

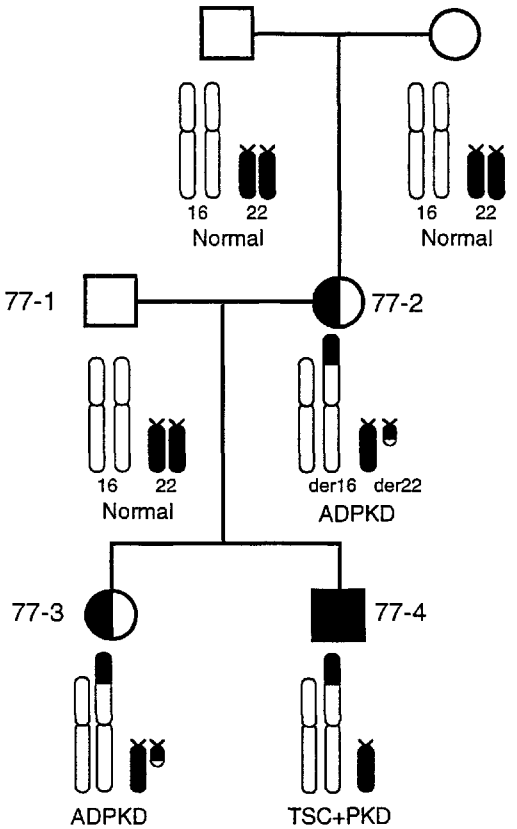


FIGURE 20.1. Pedigree of family 77. Chromosomal findings, with respect to chromosomes 16 and 22, are shown schematically for each family member. The index case, 77-4, with tuberous sclerosis and kidney cysts, has a maternally derived unbalanced rearrangement and is monosomic for 16p13.3pter and 22q11.21→22pter. His mother, 77-2, has a de novo balanced translocation and autosomal dominant polycystic kidney disease, as does his sister, who has inherited the balanced products of the exchange.

fested the tuberous sclerosis phenotype because of deletion of one copy of the *TSC2* gene, the implied location of which was distal to the translocation breakpoint on 16p13.3. The breakpoint was mapped by a combination of fluorescence in situ hybridization and pulsed-field and conventional gel electrophoresis. It was defined as lying some 150 kb (150,000 base pairs) distal to 16AC2.5, the most proximal flanking marker then identified for *TSC2* (Fig. 20.2). The position of the breakpoint in patient 77-4 therefore supported and marginally refined the proximal limit of the *TSC2* candidate region. The distal limit was greatly reduced by the position of a second chro-

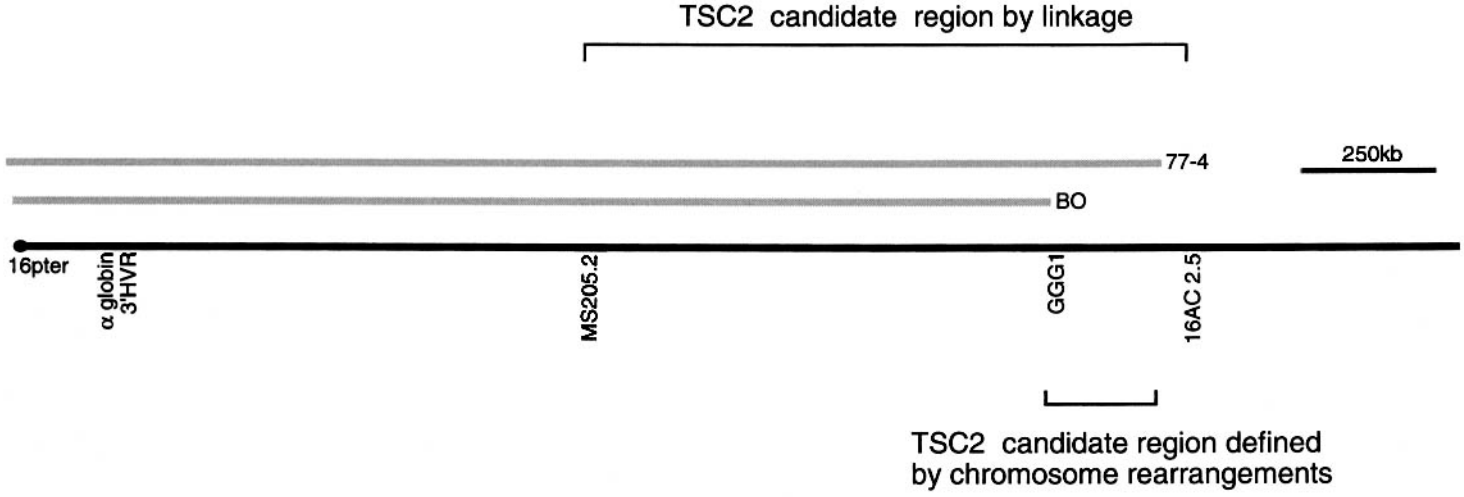


FIGURE 20.2. Map of chromosome 16pter. The positions of the polymorphic markers 3′HVR, MS205.2, GGG1, and 16AC2.5 are shown relative to approximately 2.5 Mb of DNA at 16pter. Linkage analysis defined an approximately 1.5-Mb candidate region, flanked by MS205.2 and 16AC2.5, that was likely to contain the *TSC2* gene. The deleted regions at 16pter in patients 77-4 and BO are indicated. Because patient 77-4 manifested the tuberous sclerosis phenotype, whereas patient BO did not, the *TSC2* gene was likely to map within that part of the region that was deleted in patient 77-4 but preserved in patient BO.

mosomal breakpoint in a previously reported patient (BO).[7] He had a de novo truncation of 16p that resulted in deletion of only marginally less genetic material than was the case for patient 77-4, but, crucially, he had no clinical or radiologic stigmata of tuberous sclerosis. The deletion in patient BO effectively excluded approximately 1.1 Mb of the remaining approximately 1.4 Mb *TSC2* candidate region (Fig. 20.2).

Together, the deletions at the tip of chromosome 16 in patients 77-4 and BO reduced the search for *TSC2* to approximately 300 kb, about one ten-thousandth of the genome. A region of this size is amenable to short-term cloning in overlapping DNA segments using vectors known as cosmids. These enable manipulation of stable pieces of DNA of approximately 40 kb in size. Using the cloned DNA, the region of interest can be studied in great detail. In order to further reduce the task of gene identification and characterization, the remaining approximately 300-kb *TSC2* candidate region was examined in a panel of 255 unrelated patients with tuberous sclerosis using pulsed-field gel electrophoresis. This technique enables the accurate measurement of stretches of DNA of this size. The approach revealed abnormal-length DNA fragments in five patients. Further investigation showed that each of these reflected the presence of a deletion mutation. Precise mapping of the deletions showed that they varied in size between approximately 30 kb and more than 100 kb and that all involved the same approximately 120-kb interval. Four genes were isolated from the 120-kb area by screening cDNA (expressed sequence) libraries with probes isolated from the cosmid clones. By determining the positions of each of the genes relative to the deletion mutations, it was possible to show that only one was disrupted by all five deletions, making it a strong candidate for *TSC2*. A search at higher resolution, using conventional gel electrophoresis, revealed four smaller intragenic deletions in patients with tuberous sclerosis, including a de novo deletion that was associated with production of a shortened RNA product or transcript from the gene. These findings confirmed the identity of the *TSC2* gene. A previously unknown protein product of approximately 198 kDa was predicted from the sequence of the approximately 5.5-kb *TSC2* transcript and named tuberin.[2]

Characterization of *TSC2*

Genomic Arrangement at the TSC2 *Locus*

The *TSC2* gene lies in a gene-rich region of chromosome 16p13.3, some 2.25 Mb from the telomere. *TSC2* has a complex genomic structure, being divided (in humans) into 41 separate protein-coding exons distributed over approximately 43 kb of genomic DNA. The sequence predicts a protein product of 1807 amino acids[8] (sequence available through Genbank using accession numbers L48517 through L48546). Exon 31, which is alternatively spliced, was not present in the cDNA clones that were characterized when

the *TSC2* gene was first identified.[2] Its presence was subsequently demonstrated in one of eight fetal brain *TSC2* cDNA clones spanning this part of the transcript.[8] A noncoding leader exon (exon 1a) has recently been defined.[9] Immediately centromeric to *TSC2* is the *PKD1* gene, which is mutated in autosomal dominant polycystic kidney disease. *TSC2* and *PKD1* are oriented 3′ to 3′, and their polyadenylation signals are separated by only 60 bp[10] (Fig. 20.3). Most of the *PKD1* gene, but not *TSC2*, is duplicated more proximally on chromosome 16 at 16p11.1.[6] Immediately distal to *TSC2*, and oriented 5′ to 5′, is a gene designated *OCTS3*.[2] This gene has recently been characterized and is a functional homologue of the *Escherichia coli Nth* gene, which encodes endonuclease III,[11] a major component of the base excision DNA repair pathway.[12] The *OCTS3* product has been termed *human endonuclease III homologue 1* (hNTH1), and it has been purified and characterized biochemically. hNTH1 possesses DNA glycosylase activity and AP lyase activity similar to *E. coli* endonuclease III. The immediate proximity of *OCTS3* to *TSC2* is of interest because the large somatic deletions revealed by loss of heterozygosity at 16p13.3 in tuberous sclerosis hamartomas must frequently delete one copy of *OCTS3*. Furthermore, a small proportion of patients with tuberous sclerosis also have constitutional deletions involving *TSC2* and *OCTS3*.[2] However, preliminary analysis of these patients has not revealed any phenotypic effect of hemizygosity for *OCTS3*.[11]

Possible Functional Domains

Determination of the nucleotide sequence of a novel gene enables the amino acid sequence of the encoded protein to be predicted. By searching databases for similarities between the predicted protein, or parts of it, and proteins about whose function something is already known, educated guesses can sometimes be made about possible functions of the new protein. This is not always the case, because the proteins encoded by some of the genes that have been identified by positional cloning have borne no resemblance to previously known proteins. However, if potential functional motifs can be identified from the sequence, clear directions for future research may be established.

In the case of tuberin, one very small region showed homology with the previously reported protein Rap1GAP, or GAP3. The homology extended over approximately 200 amino acid residues encoded by exons 34 through 38 of the *TSC2* gene.[2,13] Over a stretch of 58 residues, the criteria for structural homology defined by Sander and Schneider[14] were fulfilled. Rap1GAP is a GTPase-activating protein (GAP) that stimulates the hydrolysis of active GTP-bound Rap1a and Rap1b to their inactive GDP-bound forms.[15] Rap1a and b are members of the Ras superfamily of small GTP-binding proteins whose functions include transduction of mitogenic signals from plasma membrane receptors to the nucleus.[16–21] By catalyzing the conversion of GTP-binding proteins to their inactive state, GAPs can function as negative

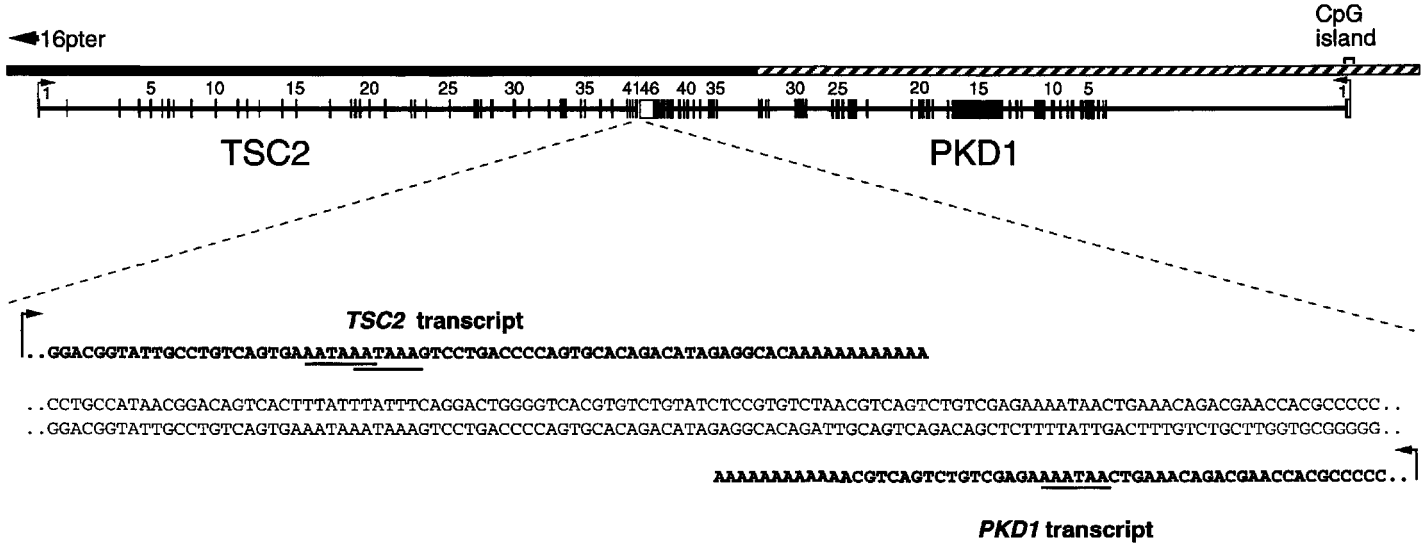


FIGURE 20.3. Map showing the *TSC2* and *PKD1* loci. The positions of the 41 coding exons of the *TSC2* gene and the 46 exons of *PKD1* are shown. *Arrowheads* indicate the directions of transcription of the genes. The hatched area is that part of the *PKD1* locus that is duplicated more proximally on 16p. The 3′ ends of the genes are shown expanded below. Although the genes cover a total of approximately 90 kb of genomic DNA, their polyadenylation signals (*underlined*) lie only 60 bp apart.

regulators of cellular proliferation. This role has been established for neurofibromin, the product of the neurofibromatosis type 1 (*NF1*) gene, which functions as a GAP for p21ras.[22] Loss of neurofibromin expression is associated with constitutional activation of p21ras in cell lines derived from tumors of patients with neurofibromatosis type 1.[23,24] The homology between tuberin and rap1GAP is considerably weaker than that between neurofibromin and p21rasGAP, but provisional evidence supporting tuberin's GAP activity has been obtained through biochemical investigation.

Other potentially important domains of tuberin include a region covering amino acid residues 81 through 102 which encompass a periodic array of leucine residues on an α-helix. The sequence complies with the leucine zipper consensus.[25] Leucine zippers are important mediators of protein-protein interaction, including dimerization. Also identified in the predicted protein were four potential tyrosine kinase phosphorylation sites that could be involved in signaling via proteins containing Src (SH2) homology domains. Toward the NH_2 terminal of the predicted protein are several regions rich in hydrophobic residues.[2] However, no transmembrane domains could be reliably predicted using the TMAP[26] or PredictProtein programs.[27]

In summary, analysis of the sequence of tuberin, the predicted protein encoded by the *TSC2* gene, revealed a number of potential functional domains toward which further research could be targeted. Investigation of these domains may reveal important regulatory pathways that are perturbed in tuberous sclerosis. Almost certainly, tuberin will possess additional novel cellular roles that cannot be inferred from the sequence of the predicted protein. There will be a place for more speculative experiments in order to identify these.

Interspecies Comparisons

Since its identification in humans, the *TSC2* gene has also been characterized in mouse,[28,29] rat,[30] and the Japanese pufferfish, *Fugu rubripes*.[8] It is highly conserved across these species. The murine *TSC2* homologue maps to mouse chromosome 17, in a conserved synteny group with human 16p13.3.[31] The nucleotide identity of the human and mouse *TSC2* genes is approximately 85%, and deduced amino acid identity of the products approximately 91%, with most amino acid differences representing conservative substitutions. The 58-amino-acid core of the Rap1GAP homologous domain of tuberin shows only one conservative amino acid change.[28,29] The rat *TSC2* gene shows a similar level of conservation.[30] Its genomic structure has been determined, and all intron-exon boundaries are positioned as in the human gene. Maheshwar et al.[8] undertook a comparative analysis of the *TSC2* gene in humans and the Japanese pufferfish, which has a much more compact genome than mammals.[32] These species have diverged over some 400 million years of evolution, and conservation of specific regions of the gene is likely to reflect constraints imposed by critical cellular functions. Overall

amino acid identity with human was 60% (similarity 79%), but four regions of the gene were particularly highly conserved. These included a region spanning the putative GAP domain, the hydrophobic NH_2-terminal portions of both predicted proteins, and two internal regions that did not show homology to other known proteins. These areas represent potentially important functional domains that can now be further investigated.

Alternative Splicing

Alternative splicing involving exon 25, the first codon of exon 26, and exon 31 has been documented in humans,[8,33] mouse,[31,33] rat,[30] and *Fugu*.[8] Other possible splice variants have been suggested by analysis of murine mRNA.[29] Variation in expression of the different transcripts has been observed in different tissues and at different developmental stages. However, from the preliminary data so far obtained, no clear pattern of developmentally regulated expression has been defined. The observation of the alternative splice forms in all organisms so far studied, particularly *Fugu*, points to the likelihood of functional significance. Many cell lines, including human lymphoblastoid and fibroblast lines, appear to exclusively express the isoform lacking exon 25. Within this exon the residue Ser946 is potentially a site for casein kinase phosphorylation, and Ser970 and Ser981 are potential protein kinase C phosphorylation sites. If these sites are variably phosphorylated, expression of the isoform lacking exon 25 could be a mechanism for regulation of intermolecular signaling.[33]

Mutations at the *TSC* Loci

The identification and characterization of mutations at the *TSC* loci has been, and will continue to be, important for several reasons. During the search for the *TSC2* gene by positional cloning, large mutations helped to define the position of the gene and smaller mutations were essential for distinguishing the gene from others close by.[2] Since the gene was identified, work has continued to define the mutational spectrum at the *TSC2* locus. This is critical to the design of efficient and comprehensive molecular diagnostic strategies. Furthermore, characterization of mutations in patients with specific complications can lead to the identification of relationships between genotype and phenotype. These can provide useful diagnostic and prognostic information and insights into the etiology of the disease process. The detection of specific classes of mutation can also be very revealing in functional terms. Missense mutations are most helpful in this regard. These are subtle mutations that result in exchange of a single amino acid in the protein product for another. In some cases the change leads to the production of a protein that is stable but has lost (or sometimes gained) crucial functions through structural alteration of biologically active sites. Identification of missense

mutants can provide an invaluable resource with which to investigate putative or novel cellular roles.

Germline Mutations of TSC2

The association of tuberous sclerosis with clearly inactivating mutations, such as deletion of the entire tip of the short arm of chromosome 16 in patient 77-4, suggests that a wide range of mutations might be expected at the *TSC2* locus. This is because many different types of mutation can lead to functional inactivation. The mutations of *TSC2* so far reported in patients with tuberous sclerosis fully support this expectation. In addition, a number of possible missense mutations, which could lead to the production of stable but functionally altered proteins, have been reported. Among the inactivating mutations so far identified are several deletions that remove one copy of the *TSC2* gene in its entirety.[3] Many other large deletions that remove substantial parts of the *TSC2* gene have also been characterized,[34–36] and at least 5% of unselected sporadic patients with tuberous sclerosis have deletions at the *TSC2* locus of 0.5 kb to 200 kb. Frameshift, nonsense, and splicing mutations have been characterized by using a variety of mutation detection techniques with higher resolution.[37–41] Wilson et al. used an approach based on reverse transcriptase–polymerase chain reaction to analyze the expressed coding region of *TSC2* comprehensively (excluding the alternatively spliced exon 31).[42] Five possible missense mutations were identified among nine putative mutations in 30 unrelated patients.[42] Missense changes have been shown to be a major class of mutation in the GAP-related domain of tuberin.[13] Single-strand conformation polymorphism analysis of exons 34 through 38, which span the GAP-related domain, revealed mutations in 14 of 171 unrelated patients with tuberous sclerosis. Eight of these, including one recurrent mutation, were missense changes. Each missense change was shown to occur de novo in a sporadic case of tuberous sclerosis, supporting a pathogenic role. These naturally occurring mutants will provide a valuable resource for the investigation of the proposed GAP activity of tuberin. The wide spectrum of mutations at the *TSC2* locus, and the complex structure of the gene itself, have made comprehensive approaches to mutation detection labor intensive. However, a study of both *TSC1* and *TSC2* in a large series of sequentially ascertained families and sporadic cases with tuberous sclerosis has now been reported.[43,44] This revealed that *TSC2* mutations are much more frequent than *TSC1* mutations in medically ascertained sporadic cases of tuberous sclerosis. The mutational spectra at the *TSC1* and *TSC2* loci were also shown to differ. Large rearrangements, including whole gene deletions, and missense mutations accounted for a significant proportion of all *TSC2* mutations but appeared unusual at the *TSC1* locus. These findings have major implications for the development of molecular genetic diagnostics for tuberous sclerosis and suggest that no single mutation detection technique (even direct sequencing) is likely to provide a comprehensive test.

A Contiguous Gene Syndrome Involving TSC2 *and* PKD1

The only genotype-phenotype correlation so far established in tuberous sclerosis is the association between large deletions involving both the *TSC2* gene and the immediately adjacent *PKD1* gene and severe renal cystic disease. This is an important complication of tuberous sclerosis, accounting for significant morbidity and mortality (see Chapter 12). When *TSC2* was localized to the same region of chromosome 16 as *PKD1*, it was suggested that *PKD1*, the major gene for autosomal dominant polycystic kidney disease, might play a role in the etiology of renal cystic disease in tuberous sclerosis.[1] During studies that led to the identification and characterization of *TSC2* and *PKD1*, a large deletion involving both genes was identified. The patient had tuberous sclerosis and severe polycystic kidney disease of early onset.[6] Further studies revealed similar contiguous deletions of *TSC2* and *PKD1* in five more patients with tuberous sclerosis, all of whom had been found to have severe polycystic kidney disease within the first few months of life.[34] Analysis of a more extensive series of patients has recently shown that contiguous deletions involving *TSC2* and *PKD1* can be found in the majority of patients with tuberous sclerosis and polycystic kidneys[35] (Fig. 20.4). Mosaicism for such deletions was a common phenomenon. Mosaicism is the presence, within the same individual, of two or more genetically distinct populations of cells, and is usually the result of postzygotic mutation. Patients mosaic for *TSC2/PKD1* deletions were found to have mild cystic disease when compared to constitutionally deleted patients.

Despite considerable evidence that *PKD1* plays a role in the etiology of renal cystic disease in patients with tuberous sclerosis, there are significant differences from autosomal dominant polycystic kidney disease. First, renal cystic disease in patients with tuberous sclerosis is often present in childhood.[45] In contrast, manifestations of autosomal dominant polycystic kidney disease are very unusual during childhood.[46] Second, the cystic epithelium in tuberous sclerosis comprises strongly eosinophilic, hypertrophic, and hyperplastic cells, which are considered unique.[47] The differences in natural history and pathology may reflect a contribution of *TSC2* mutation to the process of renal cystogenesis, acting additively or synergistically with the pathologic changes that result from mutation of *PKD1*.

Mosaicism

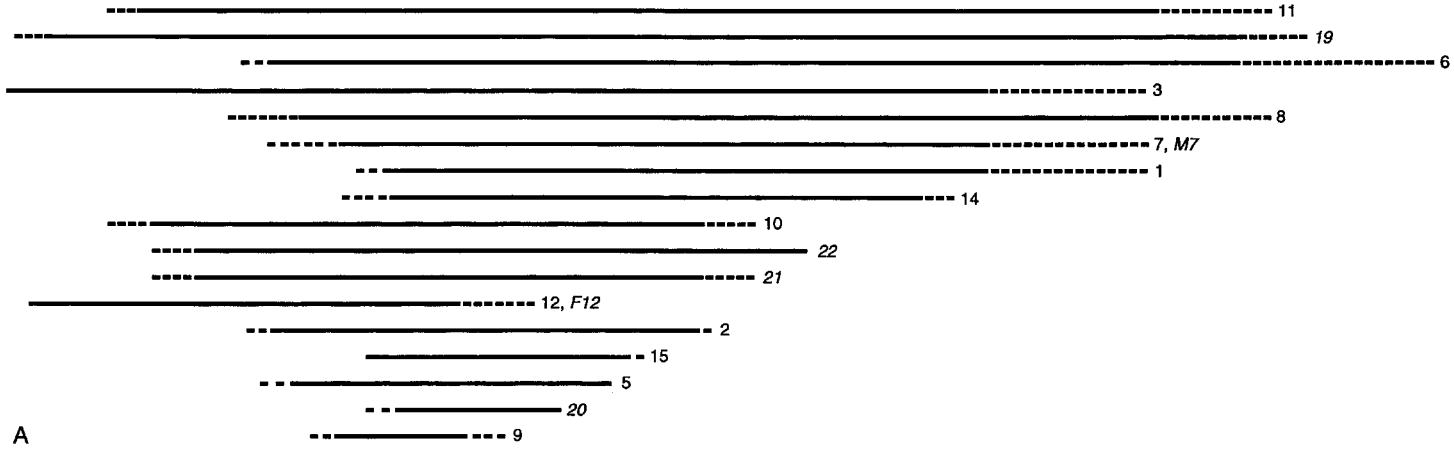
In principle, postzygotic mutations at the *TSC* loci could lead to occasional patients having markedly asymmetrical signs of tuberous sclerosis or manifestations limited to specific body segments. Alternatively, if the mutant cell line were dispersed, a mild phenotype might be anticipated. Verhoef et al. reported evidence for somatic mosaicism associated with mild disease in a father whose 2-year-old son was more typically affected.[48] The son presented with seizures and was found to have hypopigmented macules and typical

periventricular calcification on computed tomography of the brain. Southern blot analysis revealed a deletion of approximately 1.5 kb in his *TSC2* gene. The father was of normal intelligence and had never had seizures. Examination revealed only unevenness of the skin of the nasolabial folds, suggesting mild facial angiofibromatosis, and a few dental pits. Computed tomography of the brain showed a single calcified periventricular nodule. Southern blot analysis revealed the same mutant *TSC2* allele as in the son, but at reduced dosage relative to the normal counterpart, indicating mosaicism. Mosaicism has also been demonstrated for large contiguous deletions of *TSC2* and *PKD1*.[35] Some of the patients with mosaicism for these deletions manifested less severe renal cystic disease than typical patients with the contiguous gene syndrome. Three were particularly mildly affected adults who were diagnosed only after having a child with typical tuberous sclerosis and severe polycystic kidney disease. Tissue-specific mosaicism in segmental or asymmetrical tuberous sclerosis has not yet been reported.

A number of sets of siblings affected by tuberous sclerosis but with apparently normal parents have been reported.[49] One explanation for these observations is the occurrence of gonadal mosaicism in a parent, that is, the presence of a significantly prevalent mutant cell line in the germ cells of the gonad, but without somatic evidence of tuberous sclerosis. Molecular genetic analysis has recently confirmed that this phenomenon does occur in tuberous sclerosis.[50,51] In consequence, it is likely that there will be some demand for prenatal genetic testing from the parents of apparently sporadic cases.

Loss of Heterozygosity in Hamartomas

Most individuals who have tuberous sclerosis carry a mutant tuberous sclerosis gene in each of their somatic cells. However, it is clear that a huge majority of these cells proliferate, differentiate, and function normally. The hamartomas and other tumors that develop in people with tuberous sclerosis represent focal abnormalities in an otherwise normal body. This suggests that further localized events are a prerequisite for tumorigenesis. In his classic paper, Knudson[52] proposed that inherited predisposition to tumors might reflect the germline mutation of "tumor suppressor genes" and that tumor development might be the result of somatic "second hit" mutations. Molecular genetic analysis, which was unavailable to Knudson at that time, has subsequently enabled confirmation of his hypothesis, not only in relation to retinoblastoma, the model inherited tumor syndrome he first studied, but also in other analogous conditions. Many of those working on tuberous sclerosis have recognized the potential relevance of the Knudson hypothesis to the etiology of the disease (Fig. 20.5). Recent investigation of somatic mutation in a variety of tuberous sclerosis hamartomas supports classification of the TSC genes as tumor suppressor genes. Several groups have reported evidence for large somatic deletions across the *TSC1* or *TSC2* regions,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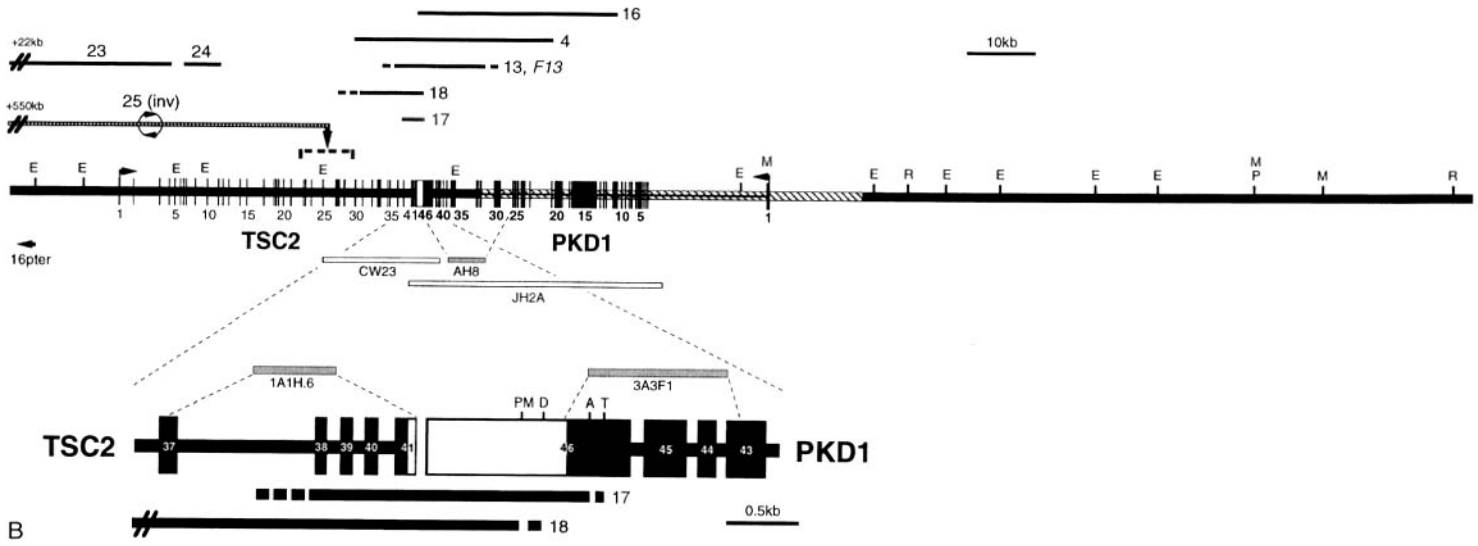

FIGURE 20.4. Mutations in patients with tuberous sclerosis and renal cystic disease. The positions of deletions and an inversion are shown relative to the *TSC2* and *PKD1* genes. Twenty-five mutations at the *TSC2* locus were identified among 27 unrelated patients with tuberous sclerosis and cystic kidneys. In 22 cases (1 through 22), deletion mutations were identified that involved all or parts of both *TSC2* and *PKD1*. Large deletions involving *TSC2* but not *PKD1* were identified in patients 23 and 24, and an inversion of approximately 600 kb with one breakpoint in *TSC2* was identified in patient 25. (Reproduced from Sampson et al.[35])

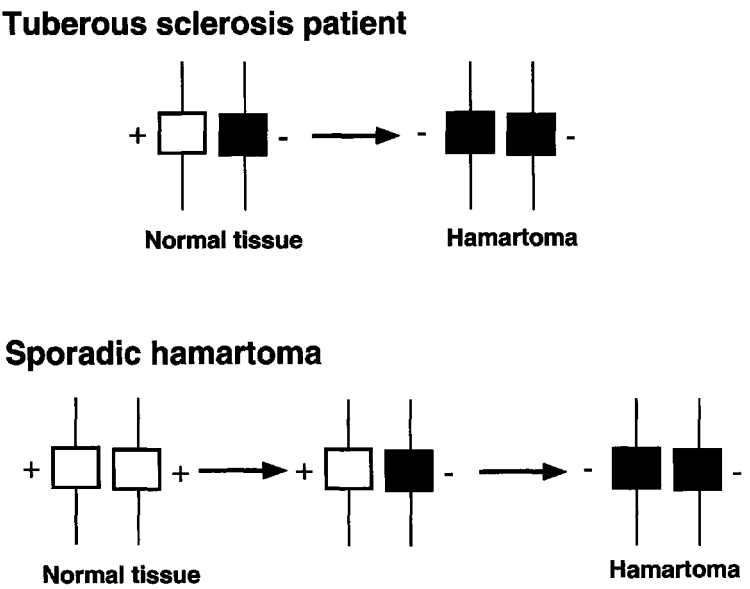

FIGURE 20.5. The Knudson hypothesis applied to hamartoma formation in patients with and without tuberous sclerosis. Two alleles of each *TSC* gene are present in each somatic cell. Wild-type (normal) alleles are shown as open boxes and designated +. Mutated or deleted alleles are shown as shaded boxes and designated −. A (nonmosaic) patient with tuberous sclerosis inherits one mutated allele, which is then replicated to every somatic cell at mitosis. Loss of the second allele through somatic mutation results in clonal proliferation and hamartoma formation. Typically, many such somatic mutations occur in susceptible cells and the patient develops many hamartomas. Individuals without tuberous sclerosis inherit two normal alleles. Hamartomatous transformation can only occur if somatic mutation inactivates both alleles in a susceptible cell. This is statistically unlikely, and sporadic hamartomas are seen only occasionally and are usually solitary.

manifested as "loss of heterozygosity" (or "loss of alleles").[53–55] In these studies, loss of heterozygosity has been revealed by parallel assay of polymorphic marker loci close to the *TSC1* or *TSC2* genes in hamartomatous and normal tissue from the same patient. The observation of loss of heterozygosity implies clonality of hamartomas, and this has now been supported by demonstration of nonrandom X chromosome inactivation in hamartomas from female patients with tuberous sclerosis.[56] Many hamartomas contain several distinct cell types—for example, disorganized blood vessels, fat-containing cells, and smooth muscle cells in angiomyolipomas. If all these elements are really the result of a single somatic mutation in a common progenitor cell, the regulatory role of *TSC* genes in the differentiation process is likely to be complex. Although loss of heterozygosity has been documented in a wide variety of tuberous sclerosis hamartomas, and in more aggressive tumors including giant cell astrocytomas and renal cell carcino-

mas, it appears to be a more consistent finding in some types of lesion than others. Henske et al. reported loss of heterozygosity in approximately half of angiomyolipomas and cardiac rhabdomyomas but in none of 14 cortical tubers and only 1 of 11 subependymal giant cell astrocytomas.[57] The findings could reflect a different mutational spectrum, or even alternative mechanisms of tumorigenesis in the central nervous system. Alternatively, they could reflect a mixture of normal and abnormal cells in lesions of the central nervous system.

Loss of heterozygosity has been documented infrequently at the *TSC1* locus as compared to the *TSC2* locus. In the largest study reported to date, loss of heterozygosity was observed in hamartomas from 14 of 25 patients informative for markers at the *TSC2* locus, but in only 1 of 27 patients informative at the *TSC1* locus.[57] Smaller studies have reported similar results.[58,59] This finding was unexpected because roughly equal proportions of multigeneration families appear to segregate for markers linked to the *TSC1* and *TSC2* loci.[60] The different rates of observed loss of heterozygosity at the *TSC1* and *TSC2* loci might reflect the relative frequencies of large and more subtle mutations at the two loci, differences in the growth characteristics of the resultant tumors (and hence the likelihood of their being excised), or unequal representation of *TSC1* and *TSC2* germline mutations among sporadic cases. One corollary of the Knudson model is that sporadic counterparts of the tumors that characterize tuberous sclerosis are expected in individuals without the condition. Sporadic tumors are expected to arise if both *TSC1* alleles or both *TSC2* alleles are subject to somatic mutation in a single susceptible cell (Fig. 20.5). Consistent with this expectation, isolated sporadic tumors, including angiomyolipomas and cardiac rhabdomyomas, are recognized. Recent studies have demonstrated loss of heterozygosity at 16p13.3 in 3 of 29 angiomyolipomas analyzed from patients without other signs of tuberous sclerosis,[61] lending support to classification of the TSC genes as tumor suppressor genes. In addition to revealing evidence for the mechanism of tumorigenesis in tuberous sclerosis, loss of heterozygosity can provide positional data useful to the identification of the causative genes themselves. However, loss of heterozygosity studies did not help with the identification of *TSC1*, perhaps because it is infrequently seen at this locus. At the *TSC2* locus, sufficient positional information was generated through characterization of rare germline deletions to allow identification of the *TSC2* gene prior to recognition of loss of heterozygosity in tuberous sclerosis.

Mutation of TSC *Genes in Sporadic Tumors*

It is likely that somatic mutation of the *TSC* genes is involved in the etiology of some sporadic tumors. Loss of heterozygosity in the *TSC2* region has been reported in angiomyolipomas removed from patients who had no other signs of tuberous sclerosis.[61] Studies of other sporadic tumors are underway, but none has been reported at the time of writing.

A *TSC2* Animal Model

The Eker Rat

The Eker rat develops multiple spontaneous renal adenomas and other tumors including pituitary adenoma, uterine leiomyoma, and splenic hemangioma.[62–64] Eker demonstrated that tumor predisposition in this laboratory animal could be attributed to transmission of a single mutant gene.[65] Matings between heterozygotes produce progeny in the ratio 1:2:0 (normal/heterozygote/homozygous mutant), consistent with early in utero lethality of the homozygote. Examination of homozygous conceptuses reveals death at approximately 10 days with malformations including abnormal brain segmentation.[66] Most reported studies have focused on the renal tumors, which are the most consistent part of the phenotype. They are usually visible by 10 months of age, but histopathologic examination reveals focal atypia in scattered proximal tubules much earlier.[67] Many of the tumors develop the histopathologic features of renal cell carcinoma, although metastasis has not been observed. Most are nonpapillary and some exhibit cystic change.[66] Ionizing radiation has been shown to induce the tumors with a linear dose-response relationship, suggesting that a single somatic mutation is required for tumor induction.[66] Similar tumors derived from the renal tubular epithelium are also induced by transplacental exposure to the mutagen *N*-ethyl-*N*-nitrosourea. In contrast, non-Eker rats develop nephroblast-derived tumors, similar to Wilms tumors, in response to transplacental exposure to this agent.[68] These differences suggest that the Eker mutation may affect late or terminal differentiation of renal tubular cells, rather than earlier steps.

Mutation of TSC2 *in the Eker Rat*

The Eker RC (renal carcinoma) locus was mapped to rat chromosome 10q12 by linkage analysis.[69,70] Analysis of renal tumors from Eker rats revealed loss of heterozygosity for markers mapping to 10q12, supporting both the localization of the Eker RC gene and its putative function as a tumor suppressor gene.[66] After the human *TSC2* gene was identified by positional cloning,[2] the Eker phenotype was shown to be tightly linked to its rat homologue, *Tsc2*.[71] This observation, and the similarities between the Eker phenotype and tuberous sclerosis, suggested that *Tsc2* itself might be the Eker RC gene. Examination of RNA from a variety of nontumor tissues in the Eker rat revealed normal and aberrant *Tsc2* transcripts, while analysis of some Eker tumors revealed only the aberrant transcripts,[72,73] consistent with *Tsc2* being the Eker RC gene and its function as a classic tumor suppressor. Detailed analysis has confirmed that the Eker RC mutation is an inserted retrotransposon in intron 30 of the *Tsc2* gene.[30,74] The retrotransposon is a novel 6253-bp intracisternal-A particle (IAP) whose *gag*, *pol*, and *env* genes have been inactivated through acquisition of multiple stop codons. Tran-

scription of the mutant allele generates chimeric *Tsc2*-IAP transcripts that lack the Rap1GAP homologous domain of *Tsc2*. No stable protein product has been demonstrated from the mutant allele, indicating that the Eker mutation is functionally null.

Functional Analysis Using the Eker Model

The Eker rat model has facilitated direct study of the tumor suppressor properties of the *Tsc2* gene. Introduction of the wild-type *Tsc2* gene into cell lines derived from tumors of the Eker rat has been shown to result in restoration of contact inhibition, to induce a degree of normalization of cell morphology and to reduce tumorigenicity of Eker tumor lines in SCID mice.[75,76] Overexpression of wild-type *Tsc2* has also been shown to reduce the growth rate of normal Rat1 fibroblasts.[76] These experiments provide formal support for the tumor and growth suppressor functions of *Tsc2*. Perhaps surprisingly, constructs expressing only the COOH terminal of rat tuberin, including the GAP-related domain, have been reported to display similar, though less marked properties.[76] In very elegant experiments, introduction of the wild-type *Tsc2* gene into the Eker germline has also been shown to correct the Eker phenotype.[77]

Studies of Expression and Function

Expression of TSC2 *and Tuberin*

Initial assessment of *TSC2* expression by Northern blot analysis indicated the presence of transcript in cell lines derived from a wide variety of human tissues.[2] Similar studies in normal rodent and human tissues also indicated widespread expression.[78] More detailed assessment of tissue-specific and developmental expression has been undertaken by in situ hybridization studies utilizing antisense riboprobes. These have demonstrated ubiquitous expression throughout the 9-day mouse embryo and, by 14 days, particularly high levels in brain, heart, and kidneys. In adult tissues, high levels of transcript were noted in the brain, especially the cerebellum, hippocampus, olfactory bulb, and dentate gyrus, and also in tissues that are not commonly involved in tuberous sclerosis, including spinal cord, testis, and ovary.[28,78]

The availability of antibodies to tuberin has enabled immunohistochemical studies to be initiated. These have confirmed expression of tuberin in many tissues, particularly brain, heart, kidney, and skin. High levels of protein expression have been noted in cortical neurons, Purkinje cells of the cerebellum, and vascular smooth muscle in many organs.[79] Apparently high levels of tuberin expression have recently been reported in cortical tubers and subependymal giant cell astrocytomas from patients with tuberous sclerosis.[80] However, the nature of the germline mutation (*TSC1* or *TSC2*) had not been determined in the small number of patients studied to date.

Subcellular Localization

Indirect immunofluorescence using affinity-purified polyclonal antibodies to tuberin revealed punctate perinuclear staining in cell lines derived from several human tumors.[81] The possibility of localization to the Golgi stacks was supported by co-localization with the Golgi stack marker mannosidase II. Furthermore, brefeldin A, a drug that disrupts the Golgi apparatus, abolished the perinuclear staining. Studies with the same antibodies revealed staining throughout the cytoplasm of neuronal cells, eccrine sweat glands, and the islets of Langerhans.[79] The subcellular localization of tuberin may be influenced by expression of hamartin, the *TSC1* product. van Slegtenhorst et al. noted that, whereas diffuse cytoplasmic staining was seen when tuberin was overexpressed alone in COS cells, recruitment to possible vesicular structures was seen when coexpressed with hamartin.[82]

Possible Functions of Tuberin

The putative function of tuberin as a GAP was initially suggested by a region of homology between tuberin and Rap1GAP.[2] Subsequently the GTPase-activating properties of tuberin have been investigated biochemically. Modest GAP activity toward Rap1a, but not Rap2, Ras, Rho, or Rac, has been demonstrated for native tuberin immunoprecipitated from K-562 cells.[83] GST fusion proteins incorporating the GAP-containing COOH-terminal part of tuberin and expressed in *E. coli* and in Sf9 insect cells showed similar activity, though at a lower level.[83] Although these data support the hypothesis that tuberin may function as a GAP, the reported activity for Rap1a is marginal and, because Rap1 was originally identified as an antagonist for p21ras, it was difficult to see how a deficit in down-regulation of Rap1 could lead to increased cell growth. However, more recent evidence has pointed to mitogenic and oncogenic properties of Rap1[84] and also to a likely role in MAP kinase–mediated neuronal differentiation.[85] Dysregulation of these processes would be consistent with the abnormalities that characterize the TSC phenotype.

Immunoprecipitates of native tuberin and the recombinant protein have also been reported to have modest GAP activity for the GTPase Rab5, which serves a role in regulating endosome fusion.[86] Increased fluid-phase endocytosis as measured by horseradish peroxidase has been reported in cells deficient in tuberin, and reexpression of tuberin appears to normalize this.[86] However, the relevance of these observations to the etiology of tuberous sclerosis remains speculative.

The possibility that tuberin might contain transcriptional activation domains was unexpectedly raised during attempts to identify interacting proteins using the yeast-based two-hybrid system.[87] This system exploits the fact that certain yeast transcription factors such as GAL4 comprise two separable domains: a site-specific DNA-binding domain and a transcriptional

activation domain. For functional activity the domains must be brought together. A hybrid gene containing (part of) a gene of interest coupled to the DNA-binding domain can be used to screen a library of genes coupled to the transcriptional activation domain. Interaction between the product of the gene of interest and the product of a clone in the library localizes the two domains of GAL4 together. The activity of the functional dimer can be used to drive expression of a reporter gene, such as *LacZ*, enabling the putative interacting clone to be identified. Tsuchiya et al. found that a construct expressing the C′ 581 amino acids of tuberin coupled to the DNA-binding domain of yeast GAL4 autoactivated expression of the *LacZ* reporter in the absence of an activation domain hybrid.[87] A series of deletion mutants was generated, and analysis of these suggested that regions comprising amino acids 1163 through 1359 and 1690 through 1743 of tuberin were both capable of GAL4-dependent *LacZ* activation in yeast. Similar results were obtained in CAT assays in HeLa and NIH3T3 cells with a construct comprising amino acids 1163 through 1359, but only weak activity was detected with amino acids 1690 through 1743. Further investigations are required to establish whether these preliminary results reflect a true cellular function of tuberin.

A role for the TSC gene products in cell cycle control is implied by the abnormal proliferation of cells that results in hamartoma growth. Experimental approaches to address the possible mechanisms through which tuberin might influence the cell cycle have now been initiated. Antisense inhibition of *TSC2* expression has been found to induce quiescent G_0-arrested fibroblasts to undergo CDK-dependent reentry into the cell cycle.[88] Further work should clarify whether there is a link between tuberin's likely GAP activity and its apparent role in progression from $G_{0/1}$ to S phase.

The recent identification of the *TSC1* gene has enabled investigation of the relationship between the *TSC1* and *TSC2* gene products. Several lines of evidence support a direct interaction between these proteins.[83] They interact together in the yeast two-hybrid system, coimmunoprecipitate from cell lysates, and co-localize when coexpressed in COS cells. These data strongly suggest that hamartin and tuberin participate in at least some shared cellular functions.

Summary

The *TSC2* gene was rapidly identified by positional cloning through the characterization of a series of naturally occurring mutants. The gene has a complex structure, and a diverse spectrum of germline mutations are found in patients with tuberous sclerosis. The demonstration of somatic mutations in hamartomas indicates that *TSC2* acts as a tumor suppressor gene. Direct demonstration of the tumor-suppressing properties of *TSC2* has been achieved by transgene expression in the Eker rat model. The regulation of cell growth by tuberin, the protein product of *TSC2*, may be mediated in

part through down-regulation of a small GTP-binding protein of the Ras superfamily. Hamartin, the product of the *TSC1* gene, interacts with tuberin and probably participates in at least some of the cellular functions performed by tuberin.

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Genetic Counseling

The hereditary nature of tuberous sclerosis complex (TSC) was suggested in the early 1900s based on its familial aggregation.[1,2] Individual components of the disease ("adenoma sebaceum") had been recognized in parent and child even earlier.[3] In 1935, Gunther and Penrose[4] recognized the inheritance pattern as autosomal dominant. Since that time, much has been learned about the variable expression of the disorder, and new mutation cases have been recognized. More recently, two chromosomal localizations have been identified, on chromosomes 9q34 and 16p13.3, that contain genes that can cause TSC. Linkage studies suggest that each locus accounts for about 50% of multigenerational TSC families. Both the gene on chromosome 9 (*TSC1*) and the gene on chromosome 16 (*TSC2*) have been identified, and their products have been named *hamartin* and *tuberin*, respectively. Much current research is directed at understanding the role of these gene products in the normal cell and the mechanism by which a genetic abnormality coding for these proteins results in disease. In spite of these remarkable advances, and the insights they provide, the factors that determine the exact pathogenesis of the TSC lesions, penetrance and variable expression, even within the same family, are still uncertain. The advances in the molecular understanding of tuberous sclerosis ultimately will open new opportunities for diagnosis in equivocal cases and for prenatal diagnosis if desired by the parents. It is hoped that these advances may also lead to improved treatment or even prevention of the disease in the future.

Although historically TSC was grouped with neurofibromatosis, von Hippel–Lindau disease, and Sturge-Weber disease (SWD) as one of the "phakomatoses," this term has been discarded for these clinically and genetically distinct entities. One patient with TSC and neurofibromatosis was determined to have inherited both gene defects from different sides of the family; all other members had either TSC or neurofibromatosis, consistent with the fact that the disorders are separate genetic entities.[5] The cause of

SWD remains unknown. However, it is of note that neurofibromatosis types 1 and 2, von Hippel–Lindau disease, and TSC all appear to involve loss or abnormal function of tumor suppressor genes.[4] Furthermore, it is intriguing that tuberin, the product of the *TSC2* gene, functions as a GTPase-activating protein (GAP) for Rap1, while neurofibromin (the product of the *NF1* gene) has GTPase activity for p21ras.[6]

Autosomal Dominant Inheritance

TSC was reported in three generations of a family as early as 1910[1] and was recognized to be a hereditary disorder by Berg in 1913.[2] Since then, many kindreds with multiple generations have been reported.[7] The pattern of inheritance, with a 50% chance for affected persons transmitting the disorder to each of their children, led to the conclusion by Gunther and Penrose that TSC is a single-gene defect following Mendel's principles of autosomal dominant inheritance.[4] The implications of autosomal dominant inheritance have been supported by subsequent observations: males and females are affected equally in number and severity; the disorder can be transmitted from persons of either sex to children of either sex; the numerical risk for each child born to an affected parent is 50%; the disorder can arise by new mutation; and, when a new mutation occurs, the risk for siblings of the affected person is low. A recurrence risk figure of 2% for siblings when the family history and evaluation are negative has been suggested to take into account variable expression, incomplete penetrance, or mosaic germinal mutation in an unaffected parent.

Germinal mosaicism refers to the occurrence of a new mutation in the progenitor of one or more germ cells, so that there is the potential for a systemically unaffected person, in whom most cells do not have the deleterious mutation, to have one or more affected children. This presumably is an uncommon event, although one study suggested gonadal mosaicism in 8 of 100 families based on affected siblings and well-studied unaffected parents. A mutation was verified in one of these cases.[8]

Variable Expression

Variable expression refers to the degree to which a person with a single-gene defect is phenotypically affected by that gene; that is, it is a measure of severity and implies that the person has some evidence of the disease. In some autosomal dominant disorders (e.g., achondroplasia), the degree of severity is relatively uniform; there is no question, even by casual observation, whether or not a person has the gene defect. However, for other diseases, such as TSC, the degree of expression is extremely variable; it is not always obvious that a person is affected. This variable expression, with some gene carriers having minimal signs or symptoms of the disease, can make genetic counseling and epidemiologic studies on the proportion of new

mutation cases very difficult. This is so because, in spite of advances at the molecular level, there still is no biochemical marker for the gene defect or gene product that is readily available on a clinical basis to determine whether or not a person has the gene defect. For example, even on a research basis, only nine *TSC2* mutations were found in a series of 30 TSC patients after screening by single-strand conformation polymorphism mobility shifts.[9] Thus the ability to assign mild cases as affected still depends on factors such as the age of the person, the development of symptoms, the thoroughness of the physician examination, and the availability of investigative techniques. For example, an asymptomatic child may later develop facial angiofibromas, or may only have a subependymal nodule detected by computed tomography (CT) or magnetic resonance imaging (MRI) of the head. For large families who have been studied thoroughly on a research basis and show strong evidence of linkage to either *TSC1* or *TSC2*, some individuals with questionable clinical findings may be designated as having or not having the abnormal gene on the basis of linkage data. Similarly, for those patients with TSC in whom a specific mutation has been identified on a research basis, direct mutation analysis of DNA can be used to study relatives. However, these approaches apply to only a minority of families at the present time. It is anticipated that improved DNA diagnostic techniques or protein assays for the gene product will become available in the future.

The genes for TSC are variably expressed not only in terms of severity but also in terms of pleiotropic effects. Pleiotropy refers to several distinct phenotypic results of a single gene—for example, hypopigmented skin macules and cortical tubers. Thus an affected person may exhibit one, several, or all the manifestations of TSC. In some cases, somatic mosaicism may account for a milder phenotype.[10]

The new molecular data on the *TSC2* gene provide some insight that relates to the pleiotropic effects of this gene. Evidence suggests that the *TSC2* gene acts as a "tumor suppressor." Thus, in any given cell, the inherited abnormal gene is paired with a homologue that was inherited from the unaffected parent. If this normal homologue is lost or damaged through somatic mutation, the cell no longer has any normally functioning *TSC2* gene product, and the normal cell cycle and/or function is disrupted. This random "loss of heterozygosity" (LOH) has been observed in various types of hamartomas from *TSC2* patients. A lower rate of LOH seems to exist for the *TSC1* locus, and the significance of this remains uncertain.[11] Furthermore, allele loss appears to be more common in kidney and heart lesions as compared to brain lesions, suggesting different mechanisms.[12]

Clinical differences have been sought between having *TSC1* and having *TSC2*.[10] The *TSC2* gene may be deleted along with the autosomal dominant polycystic kidney disease 1 gene, as part of a contiguous gene syndrome resulting in more severe polycystic kidney disease. Furthermore, it has been reported that mental retardation is less frequent among carriers of *TSC1* than *TSC2* mutations. It has been suggested that this results in ascertainment bias and explains the relative paucity of *TSC1* mutations in sporadic TSC, as

compared to *TSC2* mutations.[13] Theoretically, it is possible that variable expression may be influenced by the environment and/or other genes at other loci. These "modifier" genes could act in a general way or could act in a tissue-specific fashion, such that some TSC patients may be more apt to have symptomatic renal angiomyolipomas, for example.[14]

For a few autosomal dominant disorders, gender is one of the variables that can modify expression of the disease. In general, there is no evidence for difference in severity of TSC in males as compared with females except for pulmonary lymphangioleiomyomatosis and renal angiomyolipoma. Hormonal, immunologic, or other genetic factors may play a role in these differences. There is no clear evidence for a difference in severity of TSC in cases inherited from the mother versus the father.[15,16]

Incomplete Penetrance

Incomplete penetrance means that a person has an autosomal dominant gene defect but shows no detectable signs or symptoms of the disease. Obviously, the clinical assignment to this category depends on the thoroughness of the investigation and the diagnostic tools available. Before CT of the head was available, a person with TSC who had only cranial lesions not evident by head radiograph would have been considered an example of incomplete penetrance but now would be classified as having mild expression. Thus some reputed cases of "incomplete penetrance" reported before the availability of CT, for example, can no longer be considered valid.[14]

A person without clinical evidence of TSC can be strictly considered to represent an instance of incomplete penetrance only when shown to have a specific TSC-causing mutation that is present in other family members. Until recently it has not been possible to formally demonstrate this, and no such cases are recorded. A number of unusual pedigrees are reported, but these do not necessarily indicate nonpenetrance. Baraitser and Patton[17] reported first cousins with TSC in whom the parents were normal by physical examination, including Wood lamp examination of the skin, ophthalmologic examination, and CT of the head; however, no study of the kidneys was performed. A set of identical twin sisters, one with a single periungual fibroma and the other with no signs of the disease, each has an affected child. Both twin mothers had negative examination of skin and ocular fundi, CT and MRI of the head, ultrasound of the kidneys,[18] and chest radiograph. An alternative explanation to incomplete penetrance is that a new mutation occurred in the first few cell divisions after zygote formation and before twinning occurred, resulting in both somatic and germinal mosaicism. There are a few reports of affected siblings and half-siblings with apparently unaffected parents.[19–21] Alternative explanations for affected siblings with apparently unaffected parents are gonadal mosaicism or nonpaternity.[22]

Thus incomplete penetrance appears to be rare in TSC. However, it is still reasonable to assume that a gene that sometimes results in very mild clinical expression may occasionally cause no clinical signs or symptoms at all.

New Mutations

Genetic material is composed of DNA that must replicate each time a cell divides. A mistake in this replication process that results in a change in the sequence of nucleotide bases or in a loss, duplication, or rearrangement of genetic material is referred to as a mutation. Although some mistakes are repaired, others remain encoded in the genetic material and are passed on to all future derivatives of that cell. Thus a mutation in an egg or sperm affects the entire individual arising from these germ cells and becomes a heritable mutation that can be transmitted to future generations. A somatic mutation occurs in a nongerm cell during or after the development of an individual; although it is transmitted to daughter cells, it generally cannot be transmitted to future generations unless it occurs very early before the zygote develops into an embryo and also affects the germ lineage. A somatic mutation may have either widespread, localized, or no effect on the individual organism, depending on the stage in development at which the mutation occurs.

The proportion of cases of TSC that arise by new mutation is difficult to determine because of problems relating to incomplete family studies, variable expression, and possibly incomplete penetrance. Therefore, the estimate of the proportion of new mutation cases varies in reported series, and ranges from 50% to 75%.[4]

Sampson et al.[21] studied parents in 84 cases only by clinical examination, including funduscopy and Wood lamp examination of the skin, and concluded that up to 60% arose by new mutation. Fleury et al.[22] studied the parents of 48 patients by ophthalmologic and physical examinations (usually with Wood lamp), CT of the head, photographs of the teeth to look for dental pits, electroencephalogram, and radiographs of the skull, spine, hands, and feet, but unfortunately did not routinely include evaluation of the kidneys. Siblings also were examined. Sixty-six percent had apparently sporadic disease, a figure within the range of previous series that relied on questionnaires or less thorough family evaluations. This suggests that the familial nature of the disease may be readily apparent in many cases, although this should not be taken to negate the critical importance of thorough evaluations in individual cases, because of the importance in genetic counseling of a correct assignment of familial versus new mutation.

Although the exact cause of new mutations in humans is unknown, there is a correlation of advanced paternal age with new mutations for some autosomal dominant disorders. Most studies of paternal age in sporadic TSC cases showed no significant increase. Maternal age and birth order[23] also appear to have no effect. The accuracy of these conclusions is dependent on the correct assignment of cases to the sporadic or familial category, a factor that also influences estimates of the new mutation rate.

Gunther and Penrose estimated a new mutation rate of 1:60,000 per individual per generation,[4] which is equivalent to eight mutations per million genes per generation.[23] Other estimates range from 2.5 to 16 $\times$ 10^{-6} mu-

tations per gamete per generation.[21,22,24,25] The mutation rate in the Rochester study[26] was calculated from the observed prevalence of 10.6×10^{-5}; only two patients had a positive family history and six were presumed to represent new mutations. Therefore, the mutation rate was $3/4 \times 1/2 \times 10.6 \times 10^{-6}$ or 39.75×10^{-6}. Because estimates of new mutation rates depend on complete ascertainment of sporadic cases within a population, these numbers may represent minimal estimates of the new mutation rate.

Genetic Heterogeneity

There is no compelling evidence that TSC can be clinically inherited as anything but an autosomal dominant disorder. However, at the cellular level, the mechanism of gene dysfunction or loss may have autosomal recessive effects. This is analogous to other disorders, such as retinoblastoma, that involve genes with tumor suppression action. The suggestion that the few instances of affected siblings with apparently normal parents may result from an autosomal recessive gene defect with a similar clinical phenotype is theoretically possible, but more plausible explanations exist.[27] However, more than one nonallelic autosomal dominant gene defect can cause this clinical entity, as documented by linkage analysis described elsewhere in this text. Gene defects at different loci, or different mutations within *TSC1* or *TSC2*, could conceivably account for the tendency of some families to show a preponderance of specific organ involvement. For example, in one family three of four members with TSC had rhabdomyomas.[14] The determination of whether or not locus or intragenic heterogeneity accounts for some of the clinical variability of TSC awaits additional genotype-phenotype and locus-phenotype studies. Thus far, no definite conclusions have emerged.[11]

Genetic Counseling

Family Evaluation

Every patient with TSC, or in whom the diagnosis is being considered, should have a thorough investigation of family history. The health status of grandparents, parents, uncles and aunts, cousins, siblings, and offspring should be considered. The health status of more distant relatives should be questioned generally, with a more detailed history when appropriate. Specific attention should be given to a history of mental retardation; autism; seizures; obstructive hydrocephalus; brain or cardiac tumors; cardiac dysrhythmias (especially in infants and children); stillbirths (especially with hydrops); kidney, lung, or bone cysts; pulmonary failure; spontaneous pneumothorax; renal angiomyolipomas; renal failure, or renal cancer; fibromatous growths around or under the nails or on gums; enamel pits; retinal phakomas; skin lesions, including hypopigmented macules, facial angiofibroma, and sha-

green patches; poliosis or canities of scalp, lashes, or brows; and iridis depigmentation.[28-30] These questions should be phrased to include possible misdiagnosis of family members' lesions as vitiligo, refractory or atypical acne, or autosomal dominant polycystic kidney disease. Medical records should be reviewed on family members considered to have, or suspected to have, TSC. In some cases, a review of the medical records, autopsy reports, or pathology specimens may be helpful.

A family history is insufficient family evaluation for patients with TSC, even when apparently negative. In one study, the parents of 13 patients with a negative family history were investigated by history and by physical examination, including examination of the skin under a Wood lamp; ophthalmologic examination; roentgenograms of the skull, hands, and feet; renal ultrasound; and head CT. Four patients had previously undiagnosed parents: three with skin lesions, three with characteristic CT abnormalities, and one with renal cysts.[31] Several investigators have concluded that the most helpful family investigations are a thorough skin examination (including use of a Wood lamp) and CT, MRI, or both of the head.[22,31] However, ophthalmologic examination and renal investigations (CT, intravenous pyelogram, or ultrasound) may be positive in approximately 50% of cases[31-33]; therefore, these plus a chest radiograph also are recommended routinely. The teeth and oral mucosa should be examined for pits or gingival fibromas.[34] An echocardiogram should be considered for the parents and children of affected patients when all other studies are negative.[22,35] No patient has had lesions of the hands or feet by radiograph as the only finding[31]; furthermore, bone lesions are nonspecific and of little value in making a diagnosis when no other signs of the disease are present.[36,37]

It is sometimes difficult to determine the significance of a single nonspecific lesion, such as a single calcified brain nodule in an atypical location, a single retinal hamartoma, or a single angiomyolipoma.[17,28] A single renal cyst in adults is so common that it is of little or no significance. Single renal angiomyolipomas have been reported in up to 2% of autopsies.[38] Similarly, hypopigmented macules can be difficult to interpret without other diagnostic criteria. In various studies, 20% of adults, 12% of schoolchildren, and 0.4% to 0.8% of infants have had hypopigmented macules.[39] In another study, 4.7% of all individuals under 45 years had one to three hypopigmented macules.[40] Gomez[18] found that the most common cause of a false diagnosis of TSC was accepting hypopigmented skin macules as a definitive sign of the disease. Although enamel pits of the teeth may be seen in many adult patients with TSC, many adult controls also have enamel pitting.[41,42] In one study, not only 2 of 60 parents of TSC patients, but also 3 of 60 controls had a cardiac lesion detected by echocardiography that could have represented rhabdomyoma.[43] However, in the presence of a positive history in a first-degree relative, any of the following should be sufficient for a provisional diagnosis: facial angiofibroma, a periungual or subungual fibroma, central nervous system hamartoma, a shagreen patch, a retinal hamartoma, one renal angiomyolipoma, or one cardiac rhabdomyoma.[3] The presence of infantile

spasms and one large or two or more small hypopigmented macules, with a positive family history, is highly suspicious of TSC, but the diagnosis needs to be confirmed by finding a cortical tuber or a hamartoma anywhere in the brain, kidney, heart, lung, skin (facial angiofibroma), or any other site.

Genetic counseling should be provided for all patients with tuberous sclerosis and their at-risk relatives. Any person with TSC has a 50% chance of transmitting the gene to his or her children. However, the risk for clinically severe disease is obviously less than 50% because of variable expression. Thus affected persons who are contemplating having a child should be informed of the percentage of patients who exhibit severe manifestations of the disease, such as mental retardation and seizures. For example, to inform a prospective parent that approximately one-half of patients are retarded,[3] resulting in a risk of 1/2 × 1/2, or 1/4, for mental retardation in each future child, is more meaningful than simply quoting a 50% risk for presence or absence of the gene defect. Reproductive alternatives—for example, adoption or remaining childless—should be discussed so that prospective parents may make a fully informed decision. They should realize that the severity of their own disease is not necessarily predictive of the severity in their future children. For couples in whom the male is affected, artificial insemination of the wife with anonymous donor semen should be offered as an alternative. When the woman is affected, in vitro fertilization of a donor egg with the husband's sperm for transfer to the affected woman is possible.

Affected women who elect to reproduce should be informed that there is no adverse effect of pregnancy on the course of the disease for many patients.[44,45] However, renal complications, such as hemorrhage of an angiomyolipoma, can be aggravated by pregnancy. Pregnant women with TSC should be monitored for renal function and by renal ultrasound.[15] Of course, women with seizures as a component of their disease may have aggravation of their seizures and should be informed about possible teratogenic effects of anticonvulsants. There has been one reported case of uterine rupture in a pregnant woman with TSC.

Prenatal diagnosis is not uniformly possible, but the disease has been detected as early as 22 weeks' gestation by ultrasonographic detection of cardiac tumors.[14] A brain tumor has been detected by ultrasound in a 25-week fetus,[46] and a fetal cardiac dysrhythmia has been detected prenatally.[47] It has been suggested that fetal MRI after premedication to reduce fetal movements can detect typical brain lesions in a significant number of cases.[48] However, these studies were done at 30 to 34 weeks' gestation, and the usefulness of the information at that stage of pregnancy should be considered before routinely embarking on such tests. For those families with confirmed linkage to either *TSC1* or *TSC2*, or for whom a specific TSC2 mutation has been discovered, prenatal diagnosis utilizing DNA from amniotic fluid cells or chorionic villi is an option. However, caution must be exercised to avoid misinterpretation of neutral polymorphisms as causative mutations.[49]

Parents of an affected child who have a negative family history and no signs of the disease by the evaluations recommended above probably have

a child whose disease arose as a new mutation. Their risk of having another affected child is very small, perhaps less than 2%. Certainly, it should not be predicted that they cannot have another affected child, because it is possible there is incomplete penetrance or germinal mosaicism in one of the parents.

Genetic counseling should be nondirective. This means that people should be provided the appropriate information to assist them in family planning and other lifestyle decisions. The role of the physician or counselor should be to provide support for the patient's personal decisions. DNA-based testing for presymptomatic and prenatal diagnosis is likely to become increasingly available on a clinical basis. This may create additional dilemmas for some persons. If a person does not meet clinical criteria for a diagnosis of TSC but is found to have the mutation, will he or she be denied health or life insurance, or discriminated against in employment or career opportunities? These types of problems are already being faced by persons with other genetic conditions. Legislation to prevent discrimination varies widely between states and countries. Although many medical and nonmedical organizations are trying to help society address these issues, much remains to be done. Additional difficult decisions will be faced when a DNA-based prenatal diagnosis test is made available. The test may indicate that a fetus is affected but will not provide a definitive answer on the severity in that particular fetus. Thus more research and information and more societal planning are needed to overcome these concerns.

Before counseling for TSC is provided, it must be certain that the diagnosis is correct. The differential diagnosis includes pigmentary skin defects caused by several different chromosomal mosaicism syndromes, which can also be associated with seizures and mental subnormality. X-linked dominant periventricular heterotopia with seizures also must be considered.[49,50]

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The Diagnosis of Tuberous Sclerosis Complex: The Impact on the Individual and Family

The diagnosis of tuberous sclerosis complex (TSC) may be made at any point in an individual's life, young or old. A newborn infant with white spots on her skin may be diagnosed with TSC when she has her first seizure and the tubers in the brain are seen on magnetic resonance imaging. A gifted and talented 5-year-old with facial angiofibromas may be diagnosed with TSC when his mother takes him to see a dermatologist because of the "rash" on his face. An adult may be diagnosed with TSC when his child is diagnosed with the disease. No matter when the diagnosis comes, however, it is a diagnosis that can be very difficult to understand and to accept, and it can be devastating for both the individual and the family.

Why is this diagnosis so difficult for a family to accept? First, it can be difficult to diagnose TSC, and it may have taken the individual with TSC several weeks or months, and visits to several physicians, before he or she received the correct diagnosis. In a survey of families associated with a TSC support group in North Carolina, the average delay from the onset of infantile spasms to proper diagnosis of the seizure type and of TSC was 65 days (W. G. Ward, unpublished data, 1994). A median of three physicians were visited before the diagnosis of TSC was established, usually by a neurologist (in 71% of the cases) or a pediatrician (21%). Only 57% of infantile spasms were diagnosed by the initial physician as a seizure, and it was the most likely seizure type to lead to an inappropriate physician response to the family. Families may have been told that it was colic, indigestion, or gas, or just an overreactive infant. Inappropriate physician response to initial inquiries from the family, usually the mother, can cause a breakdown in the patient-physician relationship that will lead to anger and distrust. Parker[1] pointed out that, for many parents, the lack of professional support in their caregiving crisis began early. Most parents interviewed in her study stated that it was immediately apparent to them at birth that there was something different and possibly wrong with their child. However, physicians and nurses ignored their input.

TSC is still considered a rare disorder, even though recent estimates place the birth incidence for the disease at 1 in 5800.[2] It is very frightening for a family to be told that their child has a disease that they have never heard of. If the physician has never seen an individual with TSC, the family may feel as if they are on their own to find information about the disease and the resources they will need. The family needs to know that there are other people with the disease, and that there are resources they can and should access.

It is also difficult to tell an individual what to expect because of the broad spectrum of symptoms seen in individuals with TSC and the extreme variability between individuals with the disease, even within the same family. Attempting to understand what the disease is, that the disease is extremely variable, and that there are significant differences in opinion regarding how to treat the various manifestations of the disease can be overwhelming. How does the family know who to turn to for guidance? Whom do they trust? Whom should they listen to? Why does everyone have a different opinion about how to treat the various manifestations of TSC? Can no one give them a straight answer? Where do they find the resources they need?

The best thing that a physician can give a family at the time of diagnosis is information. If the physician does not have information on TSC to provide to the family, the physician should know where the family can get information and support. It is crucial at the early stages that the family be provided with the tools they will need to learn about the disease, how it may affect their child, and how they can access the services they will need. Some families were given no information about TSC, but were told by their physicians that TSC was too difficult to describe, that they did not need to know all about it right away, that it might frighten the family too much to tell them what to expect in the future, and that the disease is too variable from one individual to the next to even begin to describe to the family. It is important to provide the family with a knowledge of the scope and extreme variability of the disease, the various organs that may be affected, and the tests that should be done at the time of diagnosis and as screening and follow-up. If the physician does not have this knowledge, then he or she should be willing to help the family find the information, and be willing to learn along with the family.

An increasing problem for individuals with TSC and their families, as well as for others with chronic diseases, is negotiating the health care system. It is often necessary for an individual with TSC to see several specialists if the individual has, for example, seizures, cardiac involvement, kidney involvement, learning disabilities, and behavioral or psychiatric problems. In a managed care setting, the referring physician or the decision makers in this setting may or may not have a knowledge of the various manifestations of TSC and the extreme variability, or an appreciation of the ongoing screening and follow-up testing that should be done. It may be very difficult for the individual with TSC to get the necessary testing because of difficulty

accessing the specialists or services that he or she needs. It is not unusual for the parent of a child with TSC or the individual with TSC to become the "manager and coordinator" of medical care, as well as educational needs and various therapies. It can be very difficult for a family to cope with the stress of taking care of their child with TSC, as well as having to be in contact with various physicians, teachers, and therapists. Because TSC is a complex disease, the care of an individual with the disease can include a whole team of individuals. Each of these individuals has to be willing to work together as a team for the sole purpose of making life as good as possible for the individual with TSC. If one member of the team does not do his or her part, the whole team can break down, and the one who suffers is the individual with TSC.

Support for the Individual with TSC and The Family

The experience of other individuals with TSC and their families can be invaluable. Talking to another family who have dealt with the same issues the individual or his or her family are facing, who understands the frustrations they have, who knows how to access the resources they need, and who can provide the knowledge that they are not alone can make all the difference in how well an individual and his or her family learn to cope. However, every individual and every family will approach support in their own way. For some families, joining a TSC support group helped alleviate their feelings of isolation in caring for their child with TSC.[1] However, many parents interviewed for Parker's survey had reservations about joining support groups.[2] If their child was mildly affected with TSC, they were afraid to see children who were more severely affected. Conversely, the parents of severely affected children stated that they resented seeing families whose children were basically asymptomatic, happy, and healthy.

Impact on the Family

Caring for an individual with a chronic disease has a dramatic effect on all of the members of a family. The parents may feel anger, guilt, and frustration caring for a child with severe medical and developmental problems. Because TSC is a genetic disease caused by a defect in one of two genes, either the *TSC1* gene on chromosome 9 or the *TSC2* gene on chromosome 16, the parents may feel guilty because they passed on the gene to their child, or did something to "cause" the disease.

One of the parents may be forced to leave his or her job either temporarily or permanently to provide care for the child with TSC, including transportation to or from school, therapists, physician visits, and the like. It is very difficult to find an employer who will be flexible enough to cope with the demands that the varying medical and educational needs of a child with TSC put on the parents. There can be significant consequences for the

family if one of the parents is no longer employed. There may be less disposable income and the family may find they are now under extreme stress coping with financial issues, especially if the individual with TSC has extensive medical or educational expenses that are not covered by health insurance or the public school system. There may be resentment on the part of the parent who is now providing care for the individual with TSC because he or she is no longer in the work force and may be exhausted from full-time care of an individual with multiple disabilities.

Parker[1] pointed out in her study that some parents she interviewed were worried about the effect on their marriage of caring for a child with TSC. Often, the parents were so focused on caring for the child with TSC that they put their personal lives and relationships on hold. They worried about higher divorce rates for parents with chronically ill children. Parents may also worry about how the stress of caring for the child with TSC affects their own health, and how it affects their relationships with their other children.

Siblings of children with chronic disorders are sometimes lost in the shuffle of caring for the child with the chronic disease and/or disability. Their lives may be totally disrupted because of the presence of a sibling with severe medical and/or behavioral problems. If a severely affected child with TSC requires frequent visits to therapists or physicians, or one-on-one attention while in the home, it may mean that the siblings are expected to take care of themselves, assist in the care of the child with TSC, be left with a babysitter for extensive periods of time, not be allowed to bring friends into the home, or requested to modify their behavior. Siblings can feel neglected because so much attention is paid to the child with TSC, sad or depressed because they are both fearful that something might happen to their sibling with TSC and because they do not feel as though their lives are fair or "normal," or angry and resentful because so much is expected of them or because they do not feel it is fair that they have to cope with having a brother or sister with a disability. Conversely, siblings of children with chronic diseases are often very compassionate and caring, and learn much from having a sibling with special needs.

The lack of spontaneity in families caring for a child with TSC can create significant problems in a family.[1] Life is more unpredictable when there is a child with TSC in the family than for families with a member with some other chronic diseases because of behavioral problems, physical symptoms, and the unpredictable progression of the disease for each individual with TSC. Having TSC has been described as being similar to walking through a mine field. Some days you make it through just fine, but other days you step on a mine and off you go the emergency room, or suddenly there is another medical crisis to cope with. The difficult thing is that you never know when you are going to have a good day or a bad day, and you know that this will continue throughout the life of the individual with TSC. The ups and downs of having TSC and caring for an individual with this disease can be extremely difficult for everyone in the family.

How Can Physicians Help Individuals with TSC and Their Families?

Physicians can help families who are caring for an individual with TSC in the following ways:

1. Provide compassionate care for the individual with TSC, and treat this individual with dignity and respect.
2. Listen to what the parents and other caregivers are saying. They know the individual with TSC better than anyone. They can tell when the individual is not acting in his or her usual way, when there are problems, and when he or she is in pain even when the individual with TSC is nonverbal.
3. Be willing to learn as much as possible about TSC, and not be afraid to say, "I don't know the answer to that question, but let's work together to find the answer."
4. Explain in very clear and simple terms the genetic aspect of TSC and what it may mean for the rest of the family.
5. Provide information to the family about national or local support groups that may benefit them. The family should decide whether or not to contact any organization or support group, but they have the right to know that these groups exist and are there to provide information and support.
6. Provide compassionate care for the family of the individual with TSC, and help the family to seek support and counseling and to take care of each other. A strong family working as a team will provide the best environment for any individual with a chronic disease and for unaffected siblings.
7. Provide hope. It is important to be realistic about the possible future for an individual with TSC, but it is important to give the family hope.
8. Understand that caring for an individual with TSC can be difficult because of the unpredictability of the disease and the extreme variability between individuals with the disease, even within the family. A physician should be willing to spend the time required to care for the individual with TSC, to seek the advice of colleagues when it is needed, and to work as a member of a team that includes not only the family of the individual with TSC, but the therapists, teachers, and other caregivers.

Tuberous Sclerosis Organizations and Support Groups

TSC organizations and support groups exist in many different countries. These groups have jointed forces to form Tuberous Sclerosis International (TSI), an umbrella organization of all the groups throughout the world. The

TABLE 22.1. Tuberous Sclerosis Organizations

Tuberous Sclerosis International
Secretariat: Mrs. P. J. M. den Hollander
Prins Bernhardlaan 36
3722 AG Bilthoven
The Netherlands
Telephone: 31-30-281821
World Wide Web address: http://crystal.feo.hvu.nl/Groepen/TSI/TSI.htm

Tuberous Sclerosis Association
127 Boxley Drive
West Bridgford
Nottingham NG2 7GN
England
Telephone/FAX: 44-115-923-4301
e-mail: 106264,734@compuserve.com

National Tuberous Sclerosis Association
8181 Professional Place, Suite 110
Landover, MD 20785-2226
Toll-free in the U.S.A.: 1-800-225-6872
Telephone: 301-459-9888
FAX: 301-459-0394
e-mail: ntsa@ntsa.org
World Wide Web address: http://www.ntsa.org

TSC organizations provide information about TSC to individuals with TSC, their families, health care professionals, care providers, and educators, among others. In addition, they provide information about the latest research on TSC, and some provide support group meetings, newsletters, conferences for families and health care professionals, pen pal programs, physician referrals, contact with other parents or individuals with TSC, and other information and programs. All of the TSI member organizations share information and are there to support each other. The Tuberous Sclerosis Association (TSA) in the United Kingdom and the National Tuberous Sclerosis Association (NTSA) in the United States have active research grant programs that provide support for research on the genetics of TSC and on the clinical manifestations of the disease. The list of all TSC organizations is too extensive to provide here. A complete list can be obtained by contacting either the Great Britain TSA, NTSA, or the TSI office in The Netherlands (Table 22.1). Information can also be accessed via the Internet.

Summary

The diagnosis of TSC can have a significant impact on the individual with TSC and his or her family. It is important that the health care professionals who interact with the family provide accurate information at the time of the

diagnosis, and refer the individual or family to either the local, national, or international TSC organizations so that they can receive up-to-date information about TSC, as well as support for other individuals with TSC and their families.

References

1. Parker M. Families caring for chronically ill children with tuberous sclerosis complex. *Fam Community Health.* 1996;19:73–84.
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