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CHAPTER 10

The Tuberous Sclerosis Complex

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Tuberous sclerosis complex (TSC) is a disorder of cell migration, proliferation, and differentiation involving practically all tissues of the human body. Notable exceptions are the spinal cord, which is rarely if ever affected, the peripheral nerves, muscles, pituitary, and pineal gland, which has never been reported to be involved.

HISTORY

In 1835 under the title "vascular vegetations," Rayer's atlas of dermatological diseases depicted a patient whose facial lesions we can recognize today as typical facial angiofibromas. There is no additional information given with this colored drawing, most likely the first published case of the tuberous sclerosis complex. Von Recklinghausen, in 1864, presented to the Obstetrical Society of Berlin a newborn who had died after taking only a few breaths. At postmortem there were several cardiac "myomata" embedded in the myocardium or protruding into the cavities, and "several scleroses" of the brain. In 1880, D-M. Bourneville described the cerebral lesions he found at postmortem in a 15-year-old girl who had died in status epilepticus, for which he coined the term "tuberous sclerosis of the cerebral circumvolutions." Tuberous sclerosis or TS is the term used today not only for the cerebral pathology of the disease but also for the disease itself, manifested by the presence in multiple organs of a variety of hamartomas and hamartias. Tuberous sclerosis complex or TSC is a better name for the entire clinical and pathologic constellation of this protean disease, which is first clinically recognized by its skin lesions and particularly by facial angiofibroma (improperly called adenoma sebaceum). The association with renal tumors was first

reported by Bourneville in 1880 and again by Bourneville and Brissaud in 1881.

From early in this century until two decades ago, the association of facial angiofibroma with seizures and mental retardation, also known as the Vogt triad, was the only clinical indicator of cerebral tuberous sclerosis. The frequent observation that retinal hamartomas could be found in these patients added a new diagnostic feature of great value. With the successive introduction of simple radiography, pneumoencephalography, ultrasound (US), computed tomography (CT), echocardiography, and magnetic resonance imaging (MRI) as diagnostic techniques, cerebral, cardiac, and renal lesions of TSC have been recognized in patients and asymptomatic relatives, and the prevalence of TSC has been found to be 10 times greater than originally estimated. It is possible that currently we do not identify certain individuals with TSC because their lesions are not revealed with our present examination methods. The diagnostic criteria have gradually changed with the discovery of signs obtained by imaging the brain and other organs.

PROPOSED DIAGNOSTIC CRITERIA

We propose that the clinical and imaging features selected for diagnosing TSC be segregated according to their practical value into one of the following three categories: definitive, presumptive, or suggestive diagnostic features, as shown in Table 10.1.

It is impossible at this stage of our knowledge to completely exclude the diagnosis of TSC from any person, particularly from those at risk because of an affected direct relative. The absence of all the features listed in Table 10.1 in an individual at risk who has been properly examined makes it extremely unlikely that he or she is affected. In practice, the organs that need to be examined directly or by imaging are the skin, retina, brain, and kidneys. Less commonly, the bones are radiographically examined and the teeth are examined after staining their surface to detect enamel pits. It is not always practical to examine all organs and it is impossible to detect the smaller hamartomas in the organs examined.

For the purpose of genetic counseling it is accepted that subjects without any of the features listed in Table 10.1 have less than one percent (1%) probability of being affected even if they have a direct relative with TSC. Skipped (or bracketed) generations are a rarity in TSC; only a few families have been reported in which neither parent of two or more affected children had signs of TSC.

Table 10.1: Tuberous Sclerosis, Hierarchy of Diagnostic Features

Organ	*Definitive*	*Presumptive*	*Suspect*
CNS	*Cortical tubers* *Subependymal nodules* *Giant-cell astrocytoma*		Infantile spasms Generalized/ partial seizures
Retina	Hamartomas	Hamartoma	Iris/eyelashes depigment
Skin	Facial angiofibromas Ungual fibroma Fibrous forehead plaque	Confetti-like spots	Hypomelanotic macules
Kidneys	*Multiple angiomyolipomas*	Angiomyolipoma	Cysts
Heart		Multiple rhabdomyomas	Rhabdomyoma
Lungs		Lymphangio-myomatosis	Spontaneous pneumothorax Chylothorax Honeycomb image
Teeth			Enamel pits
Gingiva			Fibromas
Rectum		Polyps	
Thyroid			Adenoma
Adrenal			Angiomyolipoma
Gonads			Angiomyolipoma
Liver			Angiomyolipoma
Bones			Cysts

Listed vertically under each category are the majority of, but not all, clinical features of TSC. Horizontally to the right of the organ name are the lesions found in that organ. A feature's name printed in italics indicate these are lesions that sometimes may be in question; when only a CT, MR, or US image is available, this image should be unequivocally identified. The indirect viewing of a lesion as an image on a radiograph, US, CT scan, or MRI may not be distinct enough to establish the diagnosis. In this situation, two of these features listed in italics would be necessary to establish the diagnosis of TSC. This is to say that a single feature of those listed under the heading "Definitive" is sufficient for the diagnosis of TSC only if the image in question truly corresponds to the lesion technologically recognized as one of TSC.

For a presumptive diagnosis of TSC, only one of the features listed under "Presumptive" is necessary. If an individual has two of the features listed under the heading "Presumptive" or only one of these features plus a direct relative with an established definitive diagnosis, the diagnosis of TSC is definitive. On the other hand, with one or more of the features listed under the heading "Suggestive," the diagnosis of TSC can only be suspected. It is prudent that subjects with one of the features listed under the heading "Suspect" should be classified as "Presumptive" when they have a direct relative with the diagnosis of definitive TSC.

CENTRAL NERVOUS SYSTEM INVOLVEMENT

The pathologic expression of the defective gene or genes of TSC is primarily in the form of well-circumscribed lesions within the involved organs. It is a unique characteristic of TSC that such pathology does not entirely involve the affected organ but is confined to only one or more regions in that organ, usually in the form of hamartias and hamartomas and rarely as hamartoblastoma. Small lesions usually cause no symptoms. Lesions may replace enough normal tissue to cause organ failure or dysfunction when numerous and large. Contrary to what occurs in malignant neoplastic processes, the hamartomas do not infiltrate or metastasize, although they may displace and compress normal adjacent tissue as they grow.

The great variation of clinical features among patients undoubtedly depends on which organ(s) is/are involved. Affected individuals from different families show greater discrepancy among them than individuals from the same family.

Symptoms

Seizures The most frequent clinical manifestation of patients with TSC is epileptic seizures, alone or in association with mental subnormality, abnormal behavior, or autistic features. Mental subnormality does not occur in patients with TSC who have never had seizures, but not all patients with TSC and seizures are mentally subnormal. The seizures may be partial or of any generalized type except for typical absences. Neonates with TSC most frequently present with partial motor, myoclonic, or tonic seizures, while infants older than two months present with infantile spasms or myoclonic seizures. After the first year of life complex partial seizures originating from frontal or temporal regions with or without generalization are common. Seizures starting after the first year of life may be complex partial, atonic, tonic, or myoclonic.

Arrest or regression of psychomotor development is not unusual in patients whose seizures started within the first five years of life and were frequent. A direct correlation exists between the presence of large and numerous cortical tubers and an early onset of seizures that increase in frequency and severity. These patients as a rule fail to attain normal mental development.

Intracranial Hypertension A different clinical form of presentation is with symptoms and signs of increased intracranial pressure. This is almost always due to a subependymal giant-cell astrocytoma (SEGA) growing into a lateral ventricle near the foramina of Monro and blocking one or both, thus causing dilatation of one or both lateral ventricles. A SEGA growing

into the ventricle may form an intraventricular cast and cause only partial obstruction for years before symptoms of increased intracranial pressure are recognized. With a more rapid and complete blockage there is an abrupt onset of symptoms.

Examination of the patient's eyegrounds may disclose chronic or acute papilledema. A head CT scan or MRI will reveal dilated ventricles. Generally, obstruction of the cerebrospinal fluid (CSF) circulation by a SEGA occurs between the ages of 5 and 22 years.

Cerebellar ataxia, hemiplegia, hemianopsia, choreoathetosis, autism, and progressive dementia are less common clinical features than intracranial hypertension. Autism and progressive dementia only occur when patients with TSC have had seizures.

Neuropathology

Four types of cerebral lesions may be found in this disorder: cerebral tubers or tuberosities, subependymal nodules, giant-cell tumors or astrocytomas (SEGA), and nests of heterotopic neurons within the white matter.

The cortical tuber is a cerebral hamartia, that is, dysplastic cerebral cortex and subjacent white matter. Tubers are multiple and visible on gross inspection as widened gyri protruding slightly over the cerebral surface. They are slightly pale and firmer to palpation than the surrounding normal brain. Their number, size, and location vary a great deal from one patient to another. When small, they are easier to locate visually than by palpation. Some tubers are large enough to involve two or more adjacent gyri and intervening sulci. They are less common in the cerebellum than in the brain.

On microscopic examination the tubers display a striking disorganization of the ganglion cell arrangement and loss of the normal laminal pattern. The neuronal nuclei are reduced and the astrocytic nuclei are increased in number. Some neurons are disoriented in relation to the cortical surface and positioned horizontally or even vertically, with the apical dendrite pointing away from the pial surface. Among the most prominent changes in pyramidal cells are shrinkage, chromatolysis, glycogen accumulation, and an excess of lipopigment. There are also large cells whose characteristics are neither clearly neuronal nor astrocytic and whose origin has been the subject of much controversy. Immunohistochemical studies have shown clusters of the large cells stained for glial fibrillary acidic protein (GFAP), a marker for astrocytes, next to clusters of other large cells that do not take the GFAP stain. With the Golgi-Cox method, it has been shown that the neurons of cortical tubers have an abnormal morphology, with unusual dendrites, that are often spineless or have a reduced number of spines and a beaded appearance or varicosities. The latter finding, although prominent in fetal neurons, is uncommon in the postnatal cerebral cortex.

Subependymal nodules are seen on the ventricular walls as rounded protrusions into the ventricular cavity that resemble candle gutterings. They are most often found along the course of the stria terminalis and near the foramina of Monro. They also occur, though less often, in the third and fourth ventricles and in the Sylvian aqueduct. These nodules are formed by abnormally appearing astrocytes of fusiform or plump appearance covered by intact ependyma. These astrocytes vary in size and may be extremely large and multinucleated. The subependymal nodules often have a prominent vascular stroma with thick vessel walls. In this gliovascular stroma there may be concentric microspherules or calcospherites. Hemorrhage and necrosis are uncommon findings.

The third pathological element found in the brain of TSC patients, the giant-cell astrocytoma (GCA), is a hamartoma with the same histological characteristics and often the same location as the subependymal nodule. The only difference between a nodule and GCA is continued growth of the latter, causing symptoms of CSF obstruction as aforementioned. Some GCAs are found within the white matter, presumably originating from clusters of undifferentiated heterotopic cells located along the migratory path of primordial glial cells and neurons. The GCAs contain few mitotic figures and are not malignant, although their appearance may suggest otherwise.

Neuroimaging

The calcified subependymal nodules and GCAs can be displayed on plain radiographs. Pneumoencephalography and ventriculography were used in the past to detect hamartomas within the ventricles. The image called "candle guttering" seen in the pneumoencephalograms became the most revealing sign when searching for cerebral hamartomas. The newer noninvasive imaging methods, CT and MRI, have replaced air encephalography.

CT scanning may demonstrate images of any of the three types of cerebral lesions: the subependymal nodules, GCAs, or cortical tubers. The subependymal nodules are best seen in noncontrasted head CT scans after they have become calcified, usually not before the patient is five months old. The calcifications are in the subependymal region on the lateral ventricles, protruding into them or imbedded into the caudate nucleus or thalamus. They are asymmetrical. Their location and type of image are so characteristic that it is easy to differentiate them from venous angiomas, cysticercosis, and prenatal inflammatory lesions caused by cytomegalovirus or toxoplasma capsulatum. Heterotopic gray matter along the external wall of the ventricles may simulate uncalcified subependymal nodules.

The MRI on T-1 weighted sequences reveals the subependymal nodules as small projections into the ventricles isointense to white matter and

slightly hyperintense to gray matter. On the T-2 weighted scans the nodules are isointense or hypointense to the white and gray matter and contrast well with the hyperintense CSF. After calcification the subependymal nodules give a less intense signal on both T-2 and T-1 weighted images, thus facilitating their recognition.

Subependymal giant-cell astrocytomas arise from subependymal nodules located on the inferior part of the head of the caudate nucleus, and may grow into the ventricular cavity to occlude one or both foramina of Monro. In CT scanning, these tumors enhance with intravenous injection of contrast media, thus appearing as bright white masses due to an intensely increased attenuation of the x-rays. In MR scanning, gadolinium injection enhances these tumors.

Cortical tubers may be detected on the uncontrasted CT scan by a focal decreased attenuation of the subcortical white matter. The cortex itself may appear as an area of increased attenuation if there has been some degree of calcification. In serial CT scans obtained through the years, the white matter underlying the cortical tubers will display an increase in attenuation and become less distinct from the surrounding normal white matter. Subcortical areas of decreased attenuation in the tubers are hypomyelinated histopathologically.

In the first months of the patient's life, when the normal white matter of brain is still insufficiently myelinated, the hypomyelinated subcortical white matter of the tuberous gyri does not stand out well with CT or MR scanning. As the patient gets older, and certainly by the end of the first year, some cortical tubers are detectable by their hypomyelinated area. With MRI in T-1 sequences, cortical tubers have the appearance of "empty gyri": a dark central core surrounded by an isointense ring. In T-2 sequences, there is hyperintensity of the tuber's subcortical region. In large tubers, the hyperintense area is extensive and may connect one gyrus with the next by involving the subcortical white matter of the intervening sulcus.

Radial low-attenuation bands or streaks extending from the ventricular region to the cortex, seen with the CT scan and better identified with MRI, indicate a migration disorder along these lines where clusters of heterotopic undifferentiated cells remain and myelination is impaired. Within these radial bands may be found a growing GCA.

EXTRANEURAL INVOLVEMENT

Extraneural expression of the TSC gene(s) occurs chiefly in skin, kidneys, heart, large arteries, and lungs. The endocrine glands, teeth, gums, gastrointestinal tract, and bony skeleton may also be affected.

Skin Skin lesions seen in TSC include facial angiofibromas, periungual fibromas, fibrous forehead plaques, shagreen patches, and hypomelanotic macules. The first three lesions are pathognomonic of this disease. When any of these three are found and are unequivocal, the diagnosis is unquestionable.

The facial angiofibromas usually appear around the age of 3 years and very rarely after puberty. Thus, they are present in more than 50% of adult patients only and in 30% of patients if children are included. Two other skin hamartomas, the ungual fibroma and the shagreen patch, appear in the second decade of life and are found in approximately 20–30% of TSC patients of all ages. Although the ungual fibroma is pathognomonic of TSC, the shagreen patch may not be so. The shagreen patch consists of a cluster of connective tissue hamartomas histologically similar to facial angiofibromas and to ungual fibromas; it may need histologic confirmation.

The fibrous plaque, another hamartoma most often found on the forehead, scalp, eyelids, or cheeks, is also histologically similar to the facial angiofibroma. Since it sometimes is seen in the newborn period, it is the earliest clinical sign to be found that is pathognomonic for TSC. These plaques may grow through the years and sometimes calcify.

The white spot, or hypomelanotic macule (HM), is the most prevalent of all the skin findings in TSC patients. They may be lance-ovate, round, or irregular in shape and vary in diameter between 2 mm and several centimeters. About 90% of subjects with TSC will have more than four HMs. The presence of one or two HMs alone is not sufficient to make the diagnosis of TSC on an individual, even if he/she is at risk by having a direct relative with a definitive diagnosis of TSC. Although not every subject with TSC has white spots and not every person with white spots has TSC, the spots are very convenient for making a presumptive diagnosis. A group of small confetti-like white macules has been reported to be sufficient for making a provisional diagnosis of TSC unless they appeared after prolonged and repeated sun exposure of the skin.

The histopathology of all four hamartomas is strikingly similar: dermal fibrosis with sclerosis and layering of the collagen fibers haphazardly arranged. Only the facial angiofibroma and fibrous forehead plaques have increased vascularity and dilated vessels, giving the lesions a reddish discoloration and fleshy appearance. In these hamartomas, the dermis contains spider-shaped cells with the appearance of glial cells. These cells proliferate in tissue culture and maintain the same stellate configuration and glial appearance.

Histopathologically, the hypomelanotic macule is characterized by the presence of a normal number of melanocytes. The melanosomes are reduced in size and the melanin is reduced or absent as determined by

electromicroscopic examination and histochemical reaction for dopa of melanocytes and keratinocytes.

Other skin lesions found in TSC patients are of lesser importance. Café-au-lait spots are seen with greater frequency in TSC patients than in the normal population, as are skin tags or molluscum fibrosum pendulum (soft pedunculated fibromas found on the neck of patients with TSC).

Kidneys Renal involvement is second to neural involvement as a cause of morbidity and mortality in TSC patients. There are three types of renal lesions in TSC: angiomyolipomas (AML), renal cysts, and renal cell carcinomas.

Angiomyolipoma, a renal hamartoma, is found in 80% of TSC patients who come to autopsy and has been detected in 45% of living individuals with TSC whose kidneys were examined with imaging methods, the majority of whom were asymptomatic.

Multiple renal angiomyolipomas are pathognomonic of TSC. However, a single renal angiomyolipoma is not sufficient for the diagnosis unless it is associated with multiple renal cysts.

Angiomyolipomas, whether associated or not with TSC, have a characteristic appearance. They often bulge from the renal surface as yellow rounded masses and are seen in cut sections of the kidney as yellowish solid tumors. Although they do not infiltrate the normal parenchyma, they displace it and may penetrate the renal capsule and extend into the perirenal tissues or into the renal vein. Microscopically, they are made up of fat, smooth muscle, and blood vessels. In addition to fat cells with compressed nuclei, there are scattered foamy polygonal cells with central nuclei, sometimes multinucleated, and prominent nucleoli. The smooth muscle cells are in disorganized sheets or clusters between fat cells or form concentric layers around the blood vessels. The blood vessels within the tumor are large, thick-walled, and resemble arteries except for the lack of an elastic layer.

Renal symptoms or signs of AML rarely appear before the third decade of life. Symptoms include flank pain, hematuria, hypertension, and uremia. The most dreaded event is sudden bleeding into the kidney from ruptured aneurysmatic vessels within the AML followed by bleeding into the retroperitoneal space. Prompt treatment of hypovolemic shock and nephrectomy may save these patients. Persistent AML bleed in the form of hematuria is treatable with arterial embolization.

The second most common renal finding in TSC is multiple cysts. These are also asymptomatic except when large and numerous. Renal cysts are found in at least 30% of patients with TSC. In rare exceptions, the cysts are so numerous and the displacement of renal parenchyma is so extensive that renal failure results. The few patients thus affected have been young

children who developed uremia and hypertension. When renal cysts are combined with even a single renal angiomyolipoma, it is unequivocal that the patient has TSC.

The cysts are lined with hyperplastic epithelium with large acidophilic cells containing hyperchromatic nuclei and occasional mitotic figures. Hyperplastic cells from this epithelium form small intratubular masses that are characteristic of TSC and not found in patients with autosomal dominant polycystic disease of the kidneys.

Renal clear-cell carcinomas have been reported infrequently in patients with TSC. It is believed that these are hamartoblastomas originating in the hyperplastic epithelium of the renal cysts. The clear-cell carcinomas may metastasize.

Lungs Infrequent and almost exclusively confined to women in the third or fourth decade of life, pulmonary lymphangiomyomatosis (LAM) is more common in patients with TSC than in the general population. Trapping of alveolar air leads to pulmonary cyst formation, which may result in spontaneous rupture and pneumothorax. Blockage of lymphatics by hyperplastic perivascular smooth muscle fibers may cause chylothorax. Vascular rupture causes hemoptysis. The progression of pulmonary cyst formation with loss of pulmonary elasticity causes lung hyperinflation, respiratory failure, and hypercarbia. At this stage, the radiologic image of the lungs, with cysts surrounded by sclerosed interstitial walls, is reminiscent of a honeycomb. Progressive respiratory failure due to LAM is fatal in less than five years. Treatment consists of progesterone or lung transplantation.

The gross pathology of the lungs is very characteristic: they are large and twice as heavy as normal. On cut sections, the normal parenchymal pattern has been replaced by multiple cysts a few millimeters to several centimeters in diameter, giving the lung a spongiform appearance. The cysts are usually empty and their thin walls lack epithelial lining. The septi between the cysts contain immature-looking smooth muscle cells with ill-defined interdigitating lymphatic spaces. There is no fibrous tissue. These lesions of TSC are indistinguishable from pulmonary LAM of patients not known to have TSC.

Heart and Vascular System Cardiac involvement is in the form of cardiac rhabdomyoma, a type of hamartoma that is usually multiple and most often clinically silent. The rhabdomyomas are more often ventricular than atrial. They may be strictly intramural, protrude into the cardiac cavity, or bulge on the cardiac surface. On gross examination they have a gray or yellowish-white color, measure a few millimeters to several centimeters in diameter, and are well demarcated from the surrounding myocardium.

With the use of light microscopy the tumors appear well circumscribed, have no capsule, and are composed of glycogen-filled cells. When glycogen is washed out during fixation, the stained tissue will show a characteristic "chicken-wire" or "spider-cell" image. The cells are similar to normal cardiac Purkinje cells and may even function as such, forming an abnormal conducting bundle that causes the pre-excitation pattern characteristic of the Wolff-Parkinson-White syndrome.

In approximately 50% of infants with TSC rhabdomyomas are discovered by echocardiography. When symptomatic in neonates, they are manifested by signs of obstruction of flow at the ventricular outlet or the atrioventricular foramen. This obstruction may lead to cardiac failure in neonatal life. There have been a few cases of hydrops fetalis resulting in stillbirth or neonatal death from cardiac rhabdomyoma. Intramural hamartomas may also be associated with cardiac arrhythmia secondary to interruption of the specific conduction system. Heart failure may result from arrhythmia, from obstruction, or from replacement of contractile myocardial fibers by noncontractile tissue. The majority of rhabdomyomas of newborns or young infants with TSC tend to involute or decrease in size as the patient grows, as demonstrated in serial echocardiograms. Prenatal diagnosis of TSC has been possible with echocardiography by demonstrating fetal cardiac rhabdomyomas as early as the 26th gestational week.

Aneurysms of the descending thoracic or abdominal aorta, the subclavian, internal carotid, anterior cerebral, middle cerebral, or vertebral arteries occur with more frequency in children with TSC than is expected in this age group from congenital defects in the arterial wall. Subarachnoid hemorrhage, hemothorax from ruptured aortic aneurysm, and visual loss from giant aneurysm are rare manifestations of TSC.

Strokes attributed to embolization of cerebral arteries in infants harboring cardiac rhabdomyomas have been reported without either arteriographic or pathologic evidence. The hypothesis that emboli result from fragments detached from a tumor seems improbable; if embolization has indeed occurred it is more plausible that it was due to thromboemboli originating from clots formed in the blood turbulence created by the intracavitary rhabdomyomas.

Other Organs Patients with TSC have dental enamel defects in the form of pits. Two or three pits per tooth surface is not unusual in these patients. These lesions are not pathognomonic of TSC and are only more frequently found in these patients than in the general population. The same may be said about gingival fibromas, a fibrous hamartoma. Macroglossia has been found infrequently. Fibromatous tumors may be found in the pharynx, larynx, and esophagus, and hamartomous polyps may be found in the

rectal mucosa. Other hamartomas found in TSC patients are hemangioma of the spleen, angiomyolipoma of the adrenal glands, fibroadenoma of the testes, and adenoma of the thyroid. A variety of bone lesions occur in TSC. There may be cystic formation in the metatarsal and metacarpal bones; and there may be osteomatous thickening or sclerotic patches in the calvarium, vertebral bodies, pelvis, and long bones. These lesions are asymptomatic and their presence does not indicate that the subject necessarily has TSC.

Extraneural Imaging

Kidneys The renal angiomyolipomas, due to their large amount of fat, produce a strong echo on ultrasonographic examination. This echodense property is not specific for angiomyolipomas since renal carcinomas have given the same image. Renal cysts are well demonstrated on ultrasound. The combination of angiomyolipomas and renal cysts in the same patient makes the diagnosis of TSC definitive.

CT examination of the abdomen demonstrates the fat in the angiomyolipomas. Renal cell carcinoma, a rare tumor in TSC, lacks fat. MRI also reveals fat with an intense signal on T-2 sequences and has the advantage of showing the vascular components of angiomyolipomas even without the administration of contrast material.

Heart Two-dimensional echocardiography, MRI and, less often, angiocardiography are used for the detection of cardiac rhabdomyoma. Echocardiography can detect rhabdomyomas in the fetus after the 26th week of gestation.

Lungs Plain radiographs will display the honeycomb appearance of lymphangiomyomatosis with cyst formation, and pneumothorax or chylothorax when they occur in patients with pulmonary TSC.

Bones Cyst-like rarefaction of the metatarsal and metacarpal bones is a nonspecific finding in TSC, as is the osteomatous thickening or bone dysplasia found in the calvarium, vertebral bodies, pelvis, and long bones. The radiographic findings in the bone may be mistaken for Paget's disease and bone metastasis. If there is any question of diagnosis, the measurement of the serum alkaline phosphatase may be useful because it is normal in TSC and elevated in Paget's disease and bone metastasis.

GENETICS OF TUBEROUS SCLEROSIS

The population frequency of tuberous sclerosis was estimated as 1 in 10,000 by Wiederholt et al. in 1985. TSC is inherited as an autosomal

dominant disease. A large proportion of cases, possibly more than 50%, are thought to represent new mutations since manifestations occur in children whose parents show no evidence of TSC. It is clear that at this time the incidence of new mutations in TSC and the population frequency of TSC cannot be accurately determined since there is a very high degree of variability of penetrance in TSC. An individual who carries the TSC gene may have no manifestations on clinical examination. In order to have a high degree of confidence that a person did not carry the TSC gene, it would be necessary to perform cranial CAT scan and MRI, renal ultrasound or MRI, radiologic examination of the bones and lungs, and echocardiography, in addition to clinical examination. Despite the difficulties in assessing gene frequency and the incidence of new mutations due to nonpenetrance, the incidence of new mutations appears to be high.

Hypotheses to Explain the High Frequency of New Mutations

High frequency of new mutations could potentially be due to genetic heterogeneity, large gene size, or possibly, in certain sporadic cases of TSC, the interaction between two loci.

Hypotheses on Factors That Lead to Variable Penetrance in TSC

In order to understand the mechanism of penetrance versus nonpenetrance in TSC it will be necessary to determine the nature of the TSC gene mutation. One possible explanation for the TSC manifestations is that the TSC gene represents a tumor-suppressor gene and that an individual who has the TSC gene mutation does not develop a particular lesion unless a second mutation has taken place in a particular cell. The occurrence of extensive lesions in individuals who carry the TSC gene mutation may be due to a "second hit" occurring on the homologous chromosome in certain progenitor cells early in development. Subsequent migration of the daughter cells of this progenitor cell to distant parts of a particular organ or to different parts of the body would lead to the occurrence of widespread lesions. The genetic event that constitutes such a second hit may be a structural chromosomal change or a mutation event, such as has been described in retinoblastoma. Although chromosomal deletion events have most frequently been associated with loss of tumor-suppressor genes, e.g., in retinoblastoma, it is clear that any structural chromosomal change that leads to disruption of the tumor-suppressor gene may result in a lesion.

If one proposes that the situation in TSC is analogous to that in retinoblastoma, the tumor-suppressor gene would act as a recessive gene. Sporadic cases of TSC would represent individuals in whom two hits had taken place in a particular cell or progenitor cell at some time during development. In the case of neurofibromatosis (NF), Xu et al. postulated

that although a second mutational event may be required for development of neurofibromas, this second hit may not necessarily involve the NF gene but may involve another interactive gene, e.g., an oncogene.

There is evidence that suggests that other factors, e.g., hormonal factors, may affect expression of TSC manifestations. It is of interest to note that the lung lesions in TSC occur predominantly in women and are responsive to progesterone treatment. Certain TSC lesions are seldom seen before the onset of puberty, e.g., periungual fibromas. It seems possible that either hormonal factors or growth factors may influence the size of cardiac rhabdomyomas, since these lesions may be large in newborns and then decrease in size.

Studies Aimed at Determining the Basic Genetic Defect in TSC

To date no consistent protein or enzyme abnormality has been identified in TSC. In the past few years a number of investigators have initiated genetic linkage studies in TSC for the purpose of mapping the TSC gene or genes to a specific chromosomal region. It will then be possible to apply the so-called reverse genetics approach to isolating the TSC gene. This approach has been applied successfully to the isolation of the cystic fibrosis gene. In addition, an effort has been made to identify individuals with TSC and dysmorphic features suggestive of a chromosomal abnormality, since the presence of a chromosomal deletion or translocation in a sporadic case of TSC may indicate a chromosomal region that is important in the pathogenesis of TSC. Chromosomal deletions and translocations have greatly expedited the regional mapping and disease-gene isolation in Duchenne muscular dystrophy and neurofibromatosis.

Genetic Linkage Studies in TSC

In 1987, Fryer et al. published evidence for linkage of TSC and the ABO blood group locus on human chromosome 9q34. This evidence was a peak cumulative lod score of 3.85 at $\theta = 0$, between TSC and *ABO*. Connor et al. (1987) reported results of linkage analyses in three Scottish families that revealed a peak lod score of 3.18 at $\theta = 0$, between TSC and another chromosome 9q34 marker, *ABL*.

Following these initial reports, there were a number of reports from other investigators of linkage analysis data that did not support linkage of TSC and the chromosome 9q34 region. Results of two-point and multipoint analysis of TSC and three chromosome 9 markers, *ABO, ABLK2,* and *MCT136,* by Kandt et al. (1989) led them to conclude that location of the TSC locus could be excluded for a distance of 20 cM adjacent to the ABO locus.

In 1990, Smith et al. published results of two-point and multipoint

linkage analysis in 15 TSC families. In the pairwise linkage analysis, using a penetrance value of 90%, a significant positive lod score was observed between TSC and the chromosome 11q22–11q23 marker *MCT128.1* (*D11S144*): 3.26 at $\theta = 0.08$. A probe for tyrosinase (mapped in the 11q14–q22 region), gave a maximum lod score of 2.88 at $\theta = 0$. Results of multipoint analysis indicated that the most likely order was (*TYR TSC*)—*MCT128.1*–*HHH172*.

Janssen et al. (1990) investigated nine multigenerational families with TSC. For linkage analysis they utilized an approach that combined multipoint linkage analysis and heterogeneity testing. Their results supported a model with two different loci determining TSC. Results of their studies mapped the *TSC1* locus in the vicinity of the *ABL* locus on chromosome 9q34 and supported assignment of a *TSC2* locus in the interval between the *D11S29* locus and the locus for the dopamine D2 receptor, on chromosome 11.

Analysis of Genetic Heterogeneity in TSC

A number of investigators have recently pooled their linkage data obtained in TSC families so that a large data set may be available for analysis of genetic linkage and linkage heterogeneity. Haines et al. (1990) analyzed 111 families in a collaborative data set. They used the LINKAGE and LINKMAP programs to determine two-point lod score and multipoint location scores. In addition, they used the HOMOG programs to test for homogeneity of linkage. Using the chromosome 9 data, they obtained highly significant evidence for rejection of homogeneity ($\chi^2 = 21.54$, $p = 0.0001$). When the 9q and 11q data were used simultaneously, the analysis again rejected homogeneity ($\chi^2 = 39.74$, $p = .0001$). They also examined the combined data for evidence of a third locus; results of this analysis were suggestive but not significant. Haines et al. (1990) determined that the maximum likelihood estimate of the proportion of chromosome 9–linked families was 0.38, of chromosome 11–linked families was 0.47, and of unlinked families was 0.15. Results of multipoint analysis revealed a location score for chromosome 9q of 6.51. In the case of chromosome 11q, the peak location score was 2.77. The most likely position of the 9q TSC gene was near the ABL oncogene.

Future Prospects

From the studies described above it is clear that there are at least two TSC-determining genes, one located on chromosome 9q34 and the other located possibly in the chromosome 11q22–11q23 region. For further progress in linkage mapping it will be important to identify additional highly polymorphic markers in these gene regions and to design analyses that will allow one

to clearly determine whether a particular TSC family represents a 9-linked or an 11-linked family.

Further progress is also dependent on the development of more accurate maps of chromosome 9 and 11 markers, since the power of multipoint linkage analyses is greatly diminished by inaccuracies in the map relationships of genes. Linkage maps of chromosome 9 and of chromosome 11 have been published, and these maps will provide a basis for further expansion. Linkage studies using markers on chromosomes other than 9 and 11 will be necessary given the suggestion in certain analyses that there may be a third TSC locus.

SELECTED REFERENCES

Baraitser M, Patton MA. Reduced penetrance in tuberous sclerosis. J Med Genet 1985;22:29–31.

Bourneville D-M. Sclérose tubéreuse des circonvolutions cérébrales: idiotie et épilepsie hémiplégique. Arch Neurol (Paris) 1880;1:81–91.

Bourneville D-M. Encéphalite ou sclérose tubéreuse des circonvolutions cérébrales. Arch Neurol (Paris) 1881;1:390–412.

Cavenee WK, Dryja TP, Phillips RA. Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature 1983;305:779–784.

Connor JM, Pirritt LA, Yates J, Fryer EA, Ferguson-Smith MA. Linkage of tuberous sclerosis to DNA polymorphism detected by vABL. J Med Genet 1987;24:544–546.

Fryer AE, Chalmers A, Connor JM, et al. Evidence that the gene for tuberous sclerosis is on chromosome 9. Lancet 1987;1:659–661.

Gomez MR. History. In: Gomez MR, ed. Tuberous sclerosis. New York: Raven Press, 1988.

Gomez MR. Criteria for diagnosis. In: Gomez MR, ed. Tuberous sclerosis. 2nd ed. New York: Raven Press, 1988:9–19.

Gomez MR. Neurologic and psychiatric features. In: Gomez MR, ed. Tuberous sclerosis. 2nd ed. New York: Raven Press, 1988.

Gunther M, Penrose LS. The genetics of epiloia. J Genet 1935;31:413–430.

Haines J, and the Tuberous Sclerosis Collaborative Group. Genetic heterogeneity in tuberous sclerosis: study of a large collaborative dataset. Tuberous sclerosis and allied diseases. Ann N Y Acad Sci 1990;615:256–264.

Houser OW, Nixon JR. Central nervous system imaging. In: Gomez MR, ed. Tuberous sclerosis. 2nd ed. New York: Raven Press, 1988.

Huttenlocher PR, Heydeman PT. Fine structure of cortical tubers in tuberous sclerosis. Ann Neurol 1984;16:595–602.

Janssen LAJ, Sandkuyl LA, Merkens EC, et al. Genetic heterogeneity in tuberous sclerosis. Genomics 1990;8:237–242.

Julier C, Nakamura Y, Lathrop M, et al. A detailed map of the longarm of human chromosome 11. Genomics 1990;7:335–345.

Kandt RS, Pericak-Vance M, Hung W, et al. Absence of linkage of ABO bloodgroup locus to familial tuberous sclerosis. Exp Neurol 1989;104:223–228.

Kunkel LM, Monaco AP, Middlesworth W, Ochs HD, Latt S. Specific cloning of DNA fragments absent from the DNA of a male patient with an X chromosomal deletion. Proc Natl Acad Sci USA 1985;82:4778–4782.

Lathrop M, Nakamura Y, O'Connell P, et al. A mapped set of genetic markers for human chromosome 9. Genomics 1988;3:361–366.

Michel JM, Diggle JH, Brice J, et al. Two half-siblings with tuberous sclerosis, polycystic kidneys and hypertension. Dev Med Child Neurol 1983;25:239–244.

Nixon JR, Miller GM, Okazaki H, Gomez MR. Cerebral tuberous sclerosis: postmortem magnetic resonance imaging and pathologic anatomy. Mayo Clin Proc 1989;64:305–311.

Reagan TJ. Neuropathology. In: Gomez MR, ed. Tuberous sclerosis. 2nd ed. New York: Raven Press, 1988:63–74.

Rommens J, Ianuzzi M, Kerem B, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 1989;245:1059–1065.

Shepherd CW, Gomez MR, Lie JT, et al. Causes of death in patients with tuberous sclerosis. Mayo Clin Proc 1991;66:792–796.

Shepherd CW, Scheithauer BW, Gomez MR, Altermatt HJ, Katzmann JA. Subependymal giant cell astrocytoma. Neurosurgery 1991;28:864–868.

Smith M, Smalley S, Cantor R, et al. Mapping of a gene determining tuberous sclerosis to human chromosome 11q14–11q23. Genomics 1990;6:105–114.

Van der Hoeve J. Eye symptoms in tuberous sclerosis of the brain. Trans Ophthalmol Soc UK 1920;40:329–334.

Viskochil D, Buchberg A, Xu G, et al. Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type I locus. Cell 1990;62:187–192.

Vogt H. Zur Diagnostik der Tuberösen Sklerose. Z Erforsch Behandl Jugendl. Schwachsinns 1908;2:1–12.

von Recklinghausen F. Ein Herz von einem Neugeborenen welches mehrere theils nach ausen, theils nach den Holden prominirende tumoren (Myomen) trug. Verh Ges Geburtsh 1862;20:1–2.

Wiederholt WC, Gomez MR, Kurland LT. Incidence and prevalence of tuberous sclerosis in Rochester, Minnesota, 1950 through 1982. Neurology 1985;35: 600–603.

Xu GF, O'Connell P, et al. The neurofibromatosis type 1 gene encodes a protein related to GAP. Cell 1990;62:193–201.