THE TUBEROUS SCLEROSIS COMPLEX

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Introduction

Tuberous sclerosis complex (TSC) is the now preferred name for the autosomal dominant condition also known as tuberous sclerosis (OMIM # 191100). The addition of the term *complex* (first introduced in 1942 by the pathologist Moolten) emphasizes the multisystem involvement and variable expression of the disease, which "may affect any human organ with well-circumscribed, benign, non-invasive lesions known as hamartias and hamartomas" (Gomez 1999). The skin, brain, retina, heart, kidney, lung and liver are the organs most often involved, usually with the lesions called *hamartomas* (i.e., well-circumscribed groups of disorganized/dysplastic cells that, in addition, have a propensity to multiply excessively, thus growing as benign tumours that may or may not cause symptoms e.g., cardiac rhabdomyomas and renal angiomyolipomas) (Wilson et al. 2005) or with the other characteristic TSC lesion, the *hamartias* (i.e., well-circumscribed, misaligned or misarranged groups of dysplastic cells that nevertheless are appropriate for the organ or tissue involved and do not multiply or grow more rapidly than the normal cells of the affected organ e.g., hypomelanotic maculae in the skin, depigmented spots in the retina and cortical tubers in the brain). Other tissues that may be affected include bone, dental enamel, gums, oral, nasal and rectal mucosa, pituitary gland, thyroid, adrenals, thymus, gonads, uterus, vagina, pancreas, spleen, lymph nodes, lymphatics, synovia, aorta, and other large-caliber arteries. The spinal cord is rarely involved (e.g., spinal cordoma). Neither the skeletal muscles nor the peripheral nerves have been reported to be affected in TSC individuals so far. Except for the limited dysplastic lesions, the remaining parenchyma of the affected organs is normal in TSC (for reviews see Curatolo 2004, Gomez et al. 1999, Huson and Korf 2002, Osborne 2006). Central nervous system tumours are the leading cause of morbidity and mortality below 20 yrs, while renal disease is the main cause of death after the second decade (Northrup and Au 2006).

TSC is best known for its association with seizures, cognitive and behavioural impairment and skin manifestations. Until recently it was regarded as a rare disease and always associated with neuropsychiatric impairment. It is now apparent that TSC is not so rare and that only half of symptomatic mutation carriers have cognitive and behavioural difficulties, a further quarter have seizures but not intellectual impairment while the remainder have neither, being asymptomatic neurologically but having skin and/or visceral lesions (Osborne 2006, Schwartz et al. 2007).

Recent advances in molecular genetics have shed light into the pathogenesis and complex nature of this intriguing disease, and have been instrumental in the development of new treatment modalities. TSC results from mutations in one of two genes, *TSC1* (encoding *hamartin*) (OMIM # 605284) or *TSC2* (encoding *tuberin*) (OMIM # 191092). The activity of *TSC1* and *TSC2* is regulated by both inhibitory and activating phosphorylation events at specific amino acid residues. The TSC1-TSC2 protein complex interacts with several proteins: the clinical relevance of these interactions however is not yet well understood.

Historical background and eponyms

For thorough reviews see also Curatolo (2004), Gomez (1987, 1995) and Wikipedia (2007).

19th century: the earliest pathological descriptions

The earliest writing on TSC is a brief necropsy description made by the German pathologist **Friedrich Daniel von Recklinghausen** on March 25th *1862* at the Obstetrical Society of Berlin: a newborn infant who (had) "died after taking a few breaths", had "several myomata" protruding "on the cardiac surface…into the cardiac chambers…and embedded in the ventricular walls" and "a great number of scleroses" of the brain.

An earlier (possible) illustration of TSC is given by the French dermatologist *Pierre François Rayer* in his *1835* atlas of skin disorders: a young man's face is dotted with clusters of small, erythematous papules with a characteristic distribution and similar appearance to the typical TSC facial angiofibromas.

It was not until *1880* however that the French physician, writer and politician **Désiré-Magloire Bourneville** (1840–1909), gave the first detailed report of the typical skin and neurological TSC abnormalities and gross cerebral and renal pathology in a 15–year-old epileptic and mentally handicapped inmate girl at *La Pitié Salpetrièr* who died in her bed at 3 o' clock in the morning on May 7th 1879: clinically she presented with skin tags (of the "molluscum pendulum" type) of the neck and "confluent vesiculopapular eruption of the nose, cheeks and forehead"; she had suffered of (partial and generalised) seizures most of her life and frequent episodes of status epilepticus and developed right spastic hemiplegia. On post-mortem examination of the brain Bourneville found "hard, raised, whitish ("opaque") areas of greater density ("sclerotic") than the surrounding cortex in some of the cerebral circumvolutions" and "white nodular tumours embedded in the corpus striatum and protruding into the lateral ventricles". Bourneville coined the term *tuberous sclerosis* (because of the "potato-like consistency" of the sclerotic areas in many convolutions) *of the cerebral convolutions*. Notably, he also found small yellowish white tumours in the kidney which he thought were unrelated to the cerebral pathology.

A year later (1881) **Bourneville** and **Brissaud** reported on a second child who died at *La Biçétre* of status epilepticus at age 4 years with similar cerebral (and kidneys) pathological findings. Between the years 1880 and 1900 the same authors (Bourneville and Brissaud 1881, 1900) reported on a total of ten patients and emphasised the association of cerebral TSC with renal tumours.

Désiré-Magloire Bourneville was the son of a small Normandy landowner born on 20 October 1840 in the little village of Garanciéres (Eure) (Jansen et al 2004; Poirier and Signoret 1991). He studied in Paris and became interne des hôpitaux at the Bicêtre, the Salpêtrière, the Hôpital St. Louis and the Pitié. During the Franco-Prussian War he was surgeon to the 160th Battalion of the Garde Nationale. Later he became assistant medical officer at the field hospital of the Jardin des Plantes. Finally, even though he was a well-established physician, he resumed his internship at the Pitié, which was then covered by fire from German artillery. During the Paris Commune in 1871, when the violent revolutionaries wanted to execute their wounded political enemies, Bourneville personally intervened and saved several of his patients. He received his doctorate in 1870 in Paris. He was physician at the paediatric service at Bicêtre with the title of Médecin des services d'alienés from 1879 to 1905, and upon his retirement still held the directorship of the Foundation Vallée at the Bicêtre. In 1873 Bourneville founded the journal "Progrés Médical"; in 1880 the "Archives de neurologie"; he also established the "Revue photographique des hôpitaux de Paris". Besides his own works he arranged for the publication of an edition of the works of Jean Martin Charcot (1825-1893). He was the founder of the first school for mentally retarded children (Reyre 1989). In addition to his description of tuberous sclerosis, he made observations on myxoedema, cretinism, and mongolism. He retired as physician at the Bicêtre in 1905, and then was entrusted the directorship of the Fondation Vallée, concentrating his efforts on the

treatment of mentally retarded children. He founded the first day school for special instruction of defective children in Paris, a movement that later took hold in many countries (Who named it? 2007). On Saturdays he held open-house at the Bicêtre in which his charges performed exercises and dances to the accompaniment of a band composed of idiots, epileptics, and spastics; the thrombonist had wooden legs. From 1876 he was a member of the Paris city council and in 1873 became a member of parliament, both positions an enthusiastic advocate of reforms of the health system (Brais 1993; Gateaux-Mennecier 2002). Paris owes him for the expansion of its hospitals. He championed the worldliness of the care of the sick and created public school for the education of nurses, he founded isolatory departments for contagious diseases, special wards for sick children (Gateaux-Mennecier 2003; Who named it? 2007). He died on 29th May 1909 at his home, 14 rue des Carmes in Paris (Poirier and Signoret 1991).

In the same period of these first descriptions *Hardegen* (1881) described the brain cutting findings of a 2-day old infant who died in status epilepticus: his "areas of sclerosis throughout the cerebral cortex" and the "small tumours protruding into the lateral ventricles" contained giant ganglionic cells and giant hyperplasia that Hartdegen supposed to be a "congenital gangliocellular glioma" offering a tumour aetiology hypothesis later supported by Vogt (1908) and Bielschkowsky (1914).

During the remainder of the 19th century, dermatologists led by *Balzer* and *Ménétrier* (1885) and *Hallopeau* and *Lerede* (Gomez 1999) in France and by **Pringle** (1890) in Great Britain recognised and named "*adenoma sebaceum*" a characteristic facial lesion found in some individuals (and also running in some families) with seizures and mental handicap.

Clinical, pathological and early genetics developments of the 20th century

Histopathological studies of the cerebral lesions began with *Pellizzi* (1901) in the 20th century who emphasised the dysplastic nature of the cerebral lesions (disordered cortical architecture, heterotopias and defective myelination) and went on with *Perusini* (1905) who drew similar conclusions and also observed the association of cerebral, renal and cardiac lesions with facial angiofibromas ("adenoma sebaceum") in TSC patients.

In 1905 *Campbell* described the TSC-associated ocular findings and in 1908 *Heinrich Vogt* diagnosed TSC apparently for the first time on a living patient who had seizures, mental handicap and "adenoma sebaceum" and thus this "triad" was named after him. He also noted that heart and kidney tumours were part of the disease.

Kirpicznik (1910) and *Berg* (1913) first noted and emphasised the hereditary nature of TSC by studying multiple generation TSC families (Berg 1913, Kirpicznik 1910) and describing the condition in identical (and fraternal) twins (Kirpicznik 1910). *Schuster* (1914) reported on a unique case of a TSC individual with only the "adenoma sebaceum" component of the classic Vogt triad (i.e., without intellectual impairment and seizures) and coined the term *forme fruste* (from the French *fruste* = defaced). *Nieuwenhuise* in 1912 first drew attention to the long life span of TSC patients. At the same time the British physician *Sherlock* (1911) coined the unfortunate term (used mainly in the UK for the severe TSC phenotypes) of "*epiloia*" (reflecting the combination of *epilepsy* and *anoia* or mindlessness (Critchley 1988)).

Van der Hoeve, in 1920, called attention to the retinal astrocytic hamartomas and other well-circumscribed organ lesions in TSC patients listing the varieties of these lesions in TSC. Noting the similarities between TSC, neurofibromatosis and von Hippel-Lindau disease in the spotty distribution of these lesions and their tendency to grow as benign tumours, he introduced the term *phakoma* and the concept of *phakomatosis*. In the first decades of the 20th century it was soon realised that TSC was not as rare disease as previously thought the majority of reported patients however were inmates of hospitals, asylums or (similar) homes for mentally handicapped or epileptic individuals. By counting these inmates the first TSC population-based studies recorded prevalence figures of 1 in 30,000 or 1 in 100,000 inhabitants (Nevin and Pearce 1968).

In 1932 *Critchley* and *Earl* published a thoroughly description of 29 TSC cases emphasising for the first time the clinical value of white spots (hypomelanotic macules) in diagnosing the disease, a feature that was subsequently emphasised by *Gold* and *Freeman* (1965), *Harris* and *Moynahan* (1966) and *Fitzpatrick et al.* (1968). Critchley also noted (years before the first description of "infantile autism" by Kanner) the association of autistic behaviour with TSC.

It was however earlier, in 1924, and then in 1935 that *Marcus* (1924) and *Dalsgaard-Nielsen* (1935) described intracranial calcifications by means of X-rays. In the meantime *Berkowitz et al.* (1934) demonstrated intraventricular subependimal nodules by pneumoencephalography in a living patient (the so-called "candle-guttering" sign taken from the resemblance of nodules to the drippings of a burning candle). As a consequence of both these discoveries, the number of patients diagnosed increased dramatically.

The landmark for the understanding of natural history of TSC was however achieved by the study of **Lagos** and **Gomez** in 1967: these authors demonstrated, in a series of 71 TSC patients from the Mayo clinic, that only 62% had intellectual handicap while 38% had normal or near normal intelligence. More interestingly was the finding that all the mentally retarded TSC patients had had seizures but among those with average cognitive capacities, some had had seizures and some had not.

Impact of the new technologies

The introduction and progressive improvement of imaging methods which began in the mid-1970s with computed tomography followed by echocardiography and abdominal ultrasound and in the 80s by magnetic resonance imaging provided reliable non-invasive methods of diagnosis that aided in establishing new and more extensive criteria for diagnosis of TSC. The number of TSC new diagnoses increased and new prevalence estimates in the general population varied between 1 in 6,000 to 1 in 10,000 (Sampson et al. 1989, Osborne et al. 1991). Milder phenotypes and patients lacking neurologic symptoms, mostly relatives of index cases, were increasingly recognised. Then came the first linkage studies localising one *TSC* gene at chromosome 9q34 (called *TSC1* gene) (Fryer et al. 1987) and a second *TSC* gene on chromosome 16p13.3 (called *TSC2* gene) (Kandt et al. 1992).

In 1993 the ***TSC2* gene** (42 exons) was cloned and its product (a 1807 aminoacid protein called tuberin) identified by the European Chromosome 16 consortium (1993) and a few years later, the ***TSC1* gene** (23 exons) and its protein product (hamartin, 1164 aminoacids) (van Slegtenhorst 1997). In the very last few years, the first comprehensive genotype-phenotype studies have been published (Dabora et al. 2001).

To underline the complexity of the clinical presentation the term *tuberous sclerosis complex* obtained a general favour, so that its acronim became the official name of the two genes. Presently, both terms *tuberous sclerosis* and *tuberous sclerosis complex* are equally used to define the disease.

Incidence and prevalence

The incidence of TSC at birth cannot be assessed since a still undefined fraction of mutation carriers are either free of neurologic symptoms (seizures and intellectual/behavioural deficit), or show mild phenotypes that do not prompt them to ask for medical assistance. The generally reported prevalence of 1 in 6000 has been estimated from the screening of children attending the primary schools. Three-quarters of patients are sporadic (Dabora et al. 2001, Jóźwiak et al. 2000), the remaining have one or more affected relatives.

Among familial cases, about half are due to a defect of the *TSC1* and half to the *TSC2* gene. On the other hand, sporadic cases, thought to represent new mutations, are five times more commonly caused by *TSC2* than to *TSC1* gene mutations (Dabora et al. 2001).

Clinical manifestations

The clinical features of TSC involve several body systems (Table 1) and most importantly develop at

different ages. In addition, not all clinical signs and symptoms appear in every patient. This means that, as in NF1 (see Chapter 3), the assessments should vary according to the age of affected individuals. For many years it was believed that the classic triad of features identified by Vogt (1908) of mental retardation, epilepsy, and "adenoma sebaceum" (now called "facial angiofibroma") had to be present for the diagnosis of TSC. However as this triad is only present in less than 30% of cases many patients were undiagnosed. We now know that milder phenotypes exist and cases with single (or few) lesions harbour TSC mutations; in addition, clinical expression and severity are variable between families and even within the same family. This led to a dramatic revision of diagnostic criteria (see Roach et al. 1992, 1998; Gomez et al. 1999) and to reconsideration of previously established diagnostic workups. Even with current imaging techniques however the diagnosis of TSC can be difficult in individuals with subtle findings.

Table 1. Revised diagnostic criteria for tuberous sclerosis complex

Major features
Facial angiofibromas or forehead plaque
Nontraumatic ungual or periungual fibroma
Hypomelanotic macule (three or more)
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 [§]
Definite tuberous sclerosis complex:
Either two major features or one major feature plus two minor features.
Probable tuberous sclerosis complex:
One major plus one minor feature.
Possible tuberous sclerosis complex:
Either one major feature or two or more minor features.

[#]When cerebral cortical dysplasia and cerebral white matter migration tracts occur together, they should be counted as one rather than two features of tuberous sclerosis.
*When both lymphangiomyomatosis and renal angiomyolipomas are present, other features of tuberous sclerosis should be present before a definite diagnosis is assigned.
[§]Histologic confirmation is suggested.
[&]Radiographic confirmation is suggested.

Skin manifestations

Due to their long list and clinical accessibility dermatological manifestations belong to the most important diagnostic markers of TSC. A careful skin examination of patients at risk for TSC continues to be the easiest method of establishing the diagnosis. Some of them are pathognomonic, but some are not and may be seen in healthy persons. The careful examiner may reveal some skin manifestations of TSC even in the neonatal period. As the child grows additional cutaneous lesions may appear and the diagnosis becomes frequently evident. Because about 30% of cases are familial, and some skin manifestations are better seen in adults, it should be stressed that in all suspected paediatric cases careful skin examination should be done not only in the child, but also in the parents (Jóźwiak et al. 1998a, Jóźwiak and Schwartz 2003). The knowledge about the incidence of the lesions, specificity for TSC and typical age of presentation may be crucial for the proper diagnosis.

Hypomelanotic macules

The most characteristic type of hypomelanotic macule is leaf-shaped or lance ovate, resembling the leaf of the European mountain ash tree (Fig. 1). However, other shapes such as round macules are also observed in TSC patients (Fig. 1A, B). Their margins are usually

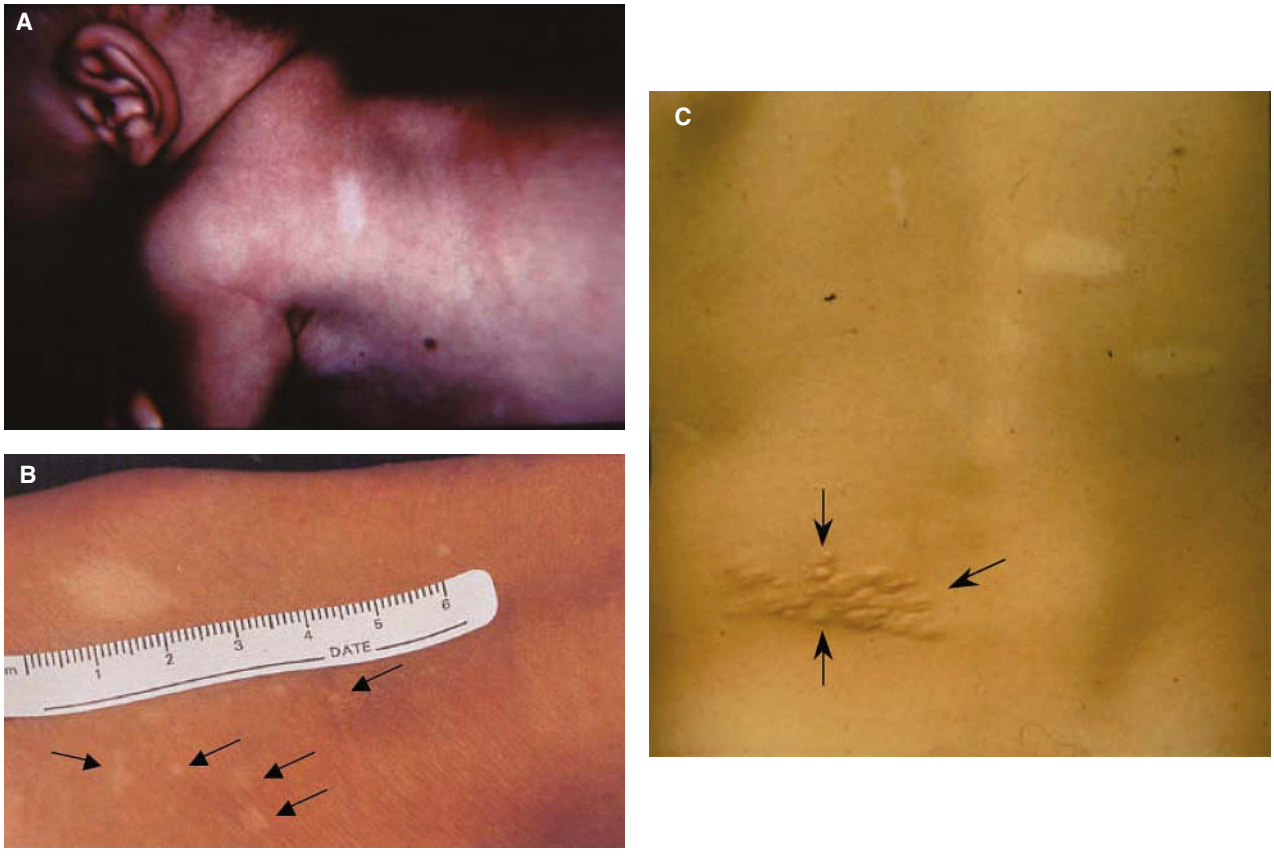


Fig. 1. Different aspects of hypomelanotic macules in the skin in TSC patients: (**A**) large, leaf-shaped macules in the trunk of an infant; (**B**) a large (2 cm across) rounded to ovalar macule associated to multiple "confetti-like" lesions (black arrows) in a toddler; (**C**) multiple, large, leaf-shaped macules in the posterior trunk associated to a shagreen patch (black arrows) in a 10-year-old boy.

well demarcated; their size can range from a few millimeters to several centimeters and their number can range from 2 or 3 to over 40. They are asymmetrically distributed over the body, especially over the trunk and buttocks and are rarely evident on the face. The involvement of the scalp may produce areas of poliosis.

Hypomelanotic spots are frequently seen in newborns and infants with TSC and thus are regarded as the earliest visible sign of the disease. Hypomelanotic macules may be the only skin finding in infants and, if coupled with the presence of infantile spasms, they strongly allude to the diagnosis of TSC. Overall, hypomelanotic macules are observed in approximately 90% of children below 2 years of age and in about 95–97% of older patients (Jóźwiak et al. 1998a).

The hypomelanotic macules may be found in 4.7% of the general population, so the presence of less then 3 macules does not indicate the necessity of an extensive evaluation to confirm TSC. The use of ultraviolet light (Wood's lamp, 365 nm) can reveal lesions that are invisible on skin examination under the normal light.

Skin biopsy specimens taken from hypomelanotic macules of patients with TSC usually demonstrate a normal number of melanocytes with reduction in intensity of histochemical reaction as compared to the surrounding normal skin. Electron microscopic studies, showing reduced number, diameter and melanization of melanosomes in melanocytes in TSC patients, may be necessary to differentiate a hypomelanotic macule from vitiligo, nevus anemicus, nevus depig-

mentosus, piebaldism or Vogt-Koyanagi-Harada syndrome (Schwartz and Janniger 1997).

Confetti-like lesions

These small lesions are the second type of hypomelanocytic macules associated with TSC (Fig. 1B). They are regarded as a separate diagnostic feature from the other types hypomelanocytic macules in the clinical criteria of tuberous sclerosis (Roach et al. 1998). Webb et al. (1996) reported them in 28% of patients with TSC. In our experience they are more common in the second decade of life and adulthood and in cases with a more severe neurocutaneous phenotype. Confetti-like macules present as multiple, 1-2 mm white spots symmetrically distributed over the extremities. Their histopathology is similar to that of the hypomelanotic macules.

Forehead fibrous plaques

Forehead fibrous plaques are yellowish-brown or flesh-colored patches of raised skin of variable size and shape from a few millimeters to several centimeters in diameter (Fig. 2). The lesion is usually located on the forehead or scalp, is soft, medium or hard in consistency, and may have a smooth or rough surface. Single large or sometimes multiple lesions can be seen. Because of a lesser vascular component as compared to facial angiofibromas, forehead fibrous plaque is not altered by warm weather or when the child cries. Contrary to facial angiofibromas, forehead plaque may become evident at any age (Jóźwiak et al. 1998a). In some patients they can be seen at birth. In newborns and infants they are hyperpigmented, flat and soft in consistency and gradually grow becoming raised and solid after many years (Fig. 2B).

As forehead fibrous plaques and facial angiofibromas share a similar histological appearance, Roach et al. (1998) suggested that these two lesions should be regarded as a single entity for the diagnostic criteria of TSC.

Forehead fibrous plaques may be noted in about 20% of children and more than 40% of adult patients (Jóźwiak et al. 1998a, Webb et al. 1996).

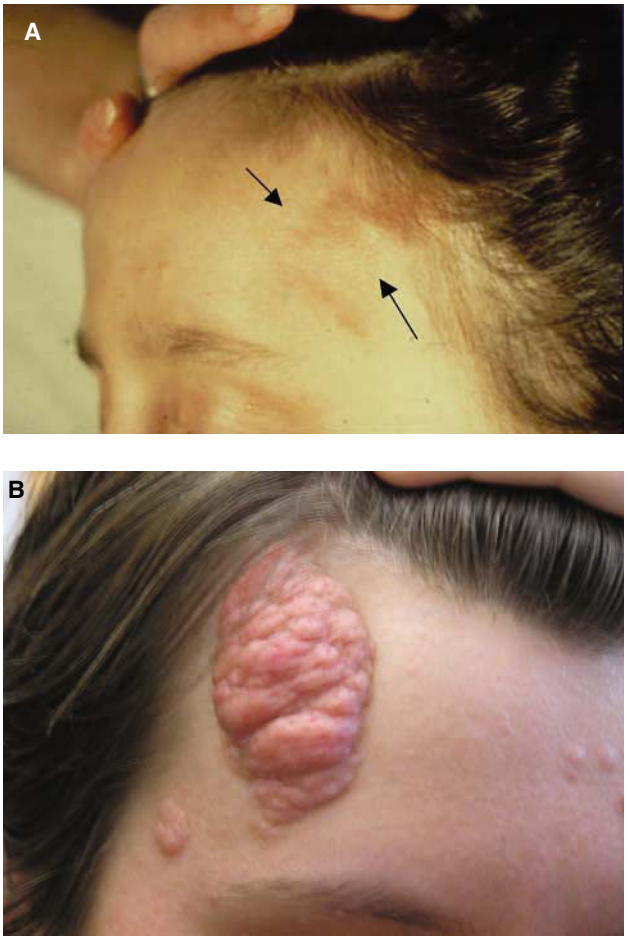


Fig. 2. (**A**, **B**) Forehead plaques in two children with TSC (black arrows).

There is a higher incidence of forehead plaques in patients harbouring TSC2 versus TSC1 mutations (Dabora et al. 2001).

Laser treatment of these lesions may be recommended. Removal of large disfiguring forehead fibrous plaques may be necessary especially in adolescents and adults with fair mental development.

Shagreen patches

They represent the third most common skin feature of TSC, after hypomelanotic macules and angiofibromas (see below). They are firm yellowish-red or

pink nodules slightly elevated above the surrounding skin, with their surface resembling in texture the skin of the orange (Figs. 1C and 3). These lesions are usually found on the dorsal body surfaces, especially the lumbosacral area. In the majority of patients the lesions are multiple and small, from few millimeters to 1 centimeter in size and might be easily overlooked in younger children. Usually appearing in clusters in a few patients they become large lesions (more then 10 centimeters in diameter). Their first appearance usually takes place soon before or around puberty, but we observed several patients with shagreen patches being present from early infancy. Their incidence increases with age reaching about 50% in adult patients (Jóźwiak et al. 2000). Multiple, small lesions may be observed and may easily be overlooked in early childhood.

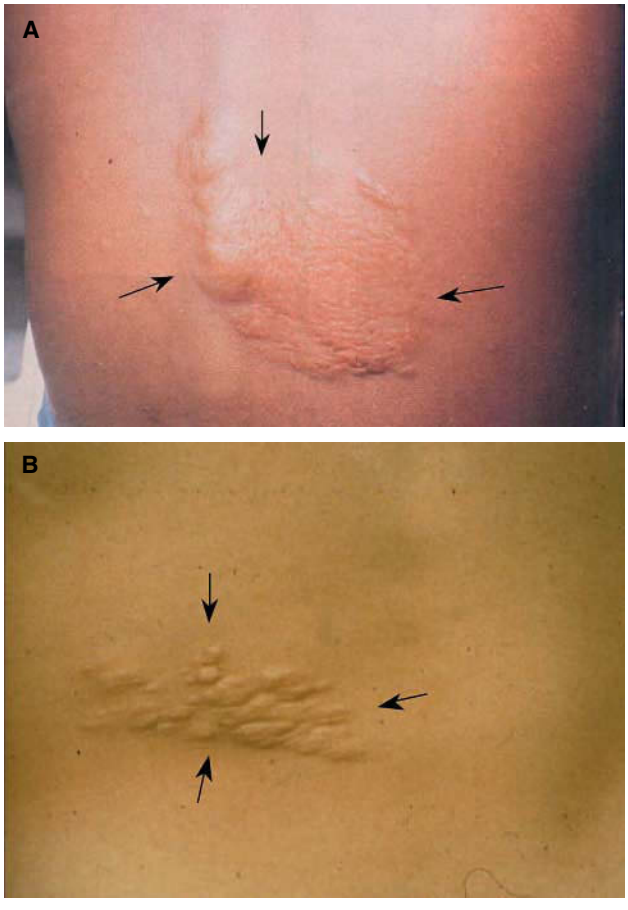


Fig. 3. (**A**, **B**) Different aspects of the shagreen patches (black arrows) in the posterior trunk: note the size and more homogeneous tissue texture of one plaque (**A**) as compared to the more irregular aspect of the other plaque (**B**).

The shagreen patch is a connective tissue hamartoma composed of excess collagen and elastic tissue. Rogers (1988) delineates two main types of shagreen patches. In the first, more common type, a band of superficial dermis is normal but its deeper layers are composed of a haphazard arrangement of collagen fibers. In the second type a uniform hamartomatous proliferation of collagen throughout the whole section of dermis is seen. The general appearance of both types is that of excess collagen and elastic tissue in disproportion to the amount of muscle, adipose tissue, appendages, and vascular structures (Rogers 1988).

The shagreen patch is difficult to differentiate both clinically and histopathologically from other connective tissue nevi. The differential diagnosis should include connective tissue nevi with osteopoikilosis (Buschke-Ollendorf syndrome). Large shagreen patches may require cosmetic treatment.

Facial angiofibromas

The earliest illustration of the lesion was displayed in the color atlas of skin diseases of Rayer in 1835 (Rayer 1835). The author described and illustrated a man with facial erythematous papules: "vascular vegetations … a rare and little known condition … characterized by little red vascular persistent papules, single or in groups … occurring most often on the face". In 1880 Bourneville (1880) described similar lesions in his patients with TSC but considered them as coincidental and not related with cerebral and renal pathology. The name of the lesions is usually linked with the name of Pringle, as "Pringle's sign", who reported: "indolent, firm, whitish, or yellowish, sago-grain like, solid papules or little tumours 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 telangiectases" (Pringle 1890). Pringle and other authors of that

times inaccurately labelled the lesions "adenoma sebaceum". This term is sometimes erroneously used even in nowadays publications.

The typical facial angiofibromas are red to pink papules or nodules with a smooth, glistening surface imbedded in the skin at different depths (Fig. 4). They are symmetrically and bilaterally distributed over the centrofacial areas, especially over the nasolabial folds, cheeks and chin. Interestingly, these lesions tend to spare the upper lip. Angiofibromas with a prominent vascular component are more obvious when the child is irritated or in warm weather. Early angiofibromas are red due to an excessive vascular proliferation.

It is a peculiar skin lesion in TSC with a clearly defined age of presentation. Angiofibromas usually become apparent between the second and fifth year of life and become more prominent with age. We found them in 74.5% (79 out of 106) of paediatric patients (Jóźwiak et al. 1998a). Webb et al. (1996) recorded facial angiofibromas in 88% of patients aged more than 30 years. In pubertal children the lesions should be differentiated with *acne vulgaris*.

Some reports have shown an association between facial angiofibromas and MEN 1, suggesting that facial angiofibromas are not characteristic for TSC (Darling et al. 1997). However, these studies did not mention sufficiently results of examinations to exclude the diagnosis of TSC. As it is known that some patients with TSC present MEN1 manifestations we believe that the aforementioned studies described rather patients with TSC and MEN1 features, rather then patients with MEN1 and isolated angiofibromas.

Histopathological studies revealed that the term *angiofibroma* seems to be more proper and acceptable, than *adenoma sebaceum*, as there is hyperplasia of both connective tissue and vascular elements of the dermis (Fig. 4D). The multitude of vessels results in some patients in a red colouring of the lesions. Large angiofibromas may be polypoid and are characterised by the presence of dense fibrous tissue with collagen bundles often arranged in layers around adnexal structures. In perivascular areas can be found multinucleated giant cells. With increasing age the collagen becomes sclerotic and layered.

The diagnostic significance of facial angiofibromas has never been questioned. Since 1908 the facial angiofibromas (as "adenoma sebaceum") were included by Vogt (1908) in the diagnostic triad of TSC (with mental retardation and epilepsy). In the recent classifications of diagnostic criteria from 1992 (Roach et al. 1992) and 1998 (Roach et al. 1998) multiple, bilateral lesions in characteristic distribution are regarded as primary or characteristic of TSC and do not require histopathological confirmation. In adults, facial angiofibromas are often misdiagnosed as acne rosacea.

Various modalities have been used in the treatment of facial angiofibromas including shave excision, cryosurgery, dessication, dermabrasion, carbon and argon laser (Papadavid et al. 2002, Bittencourt et al. 2001).

There is no consensus about the most suitable time of the treatment. Some authors suggest removal of early angiofibromas, being convinced that such approach should prevent the development of full-fledged, fibrous angiofibromas. Still, as the lesions may continue to grow until adulthood and faster growth during puberty may be noted, the postponement of the treatment until then may be justified. The decision must be balanced by the serious psychosocial problems seen in adolescents with fair mental development and extensive angiofibromas. Especially in these group of children cosmetic treatment of the lesions is strongly recommended.

Ungual or periungual fibromas

Ungual or periungual fibromas are regarded as very characteristic or pathognomonic for TSC. According to the last diagnostic criteria of TSC by Roach et al. (1998) periungual fibromas are regarded as a major sign of TSC. The lesions are known also as Koenen's tumours since their description by Koenen (1932) in members of a Dutch TSC family. These fibromas are skin colored or reddish nodules usually arising from the finger or toe nail bed, appearing clinically over the lateral nail groove, nail plate or along the proximal nail folds (Fig. 5). Their size

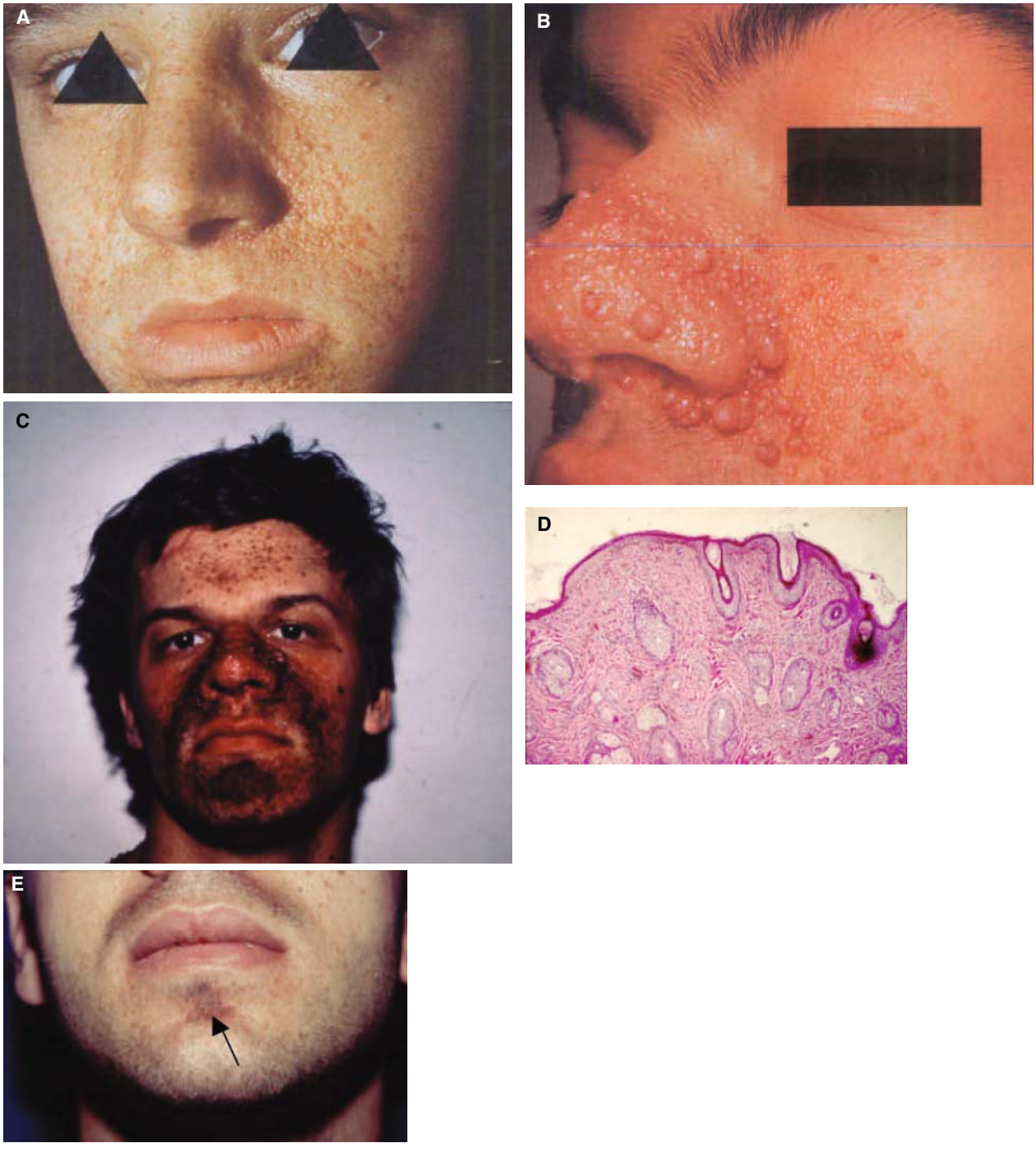


Fig. 4. Facial angiofibromas in TSC patients at different ages and with variable phenotype severity: (**A**) the angiofibroma is more diffuse and (relatively) milder at age 8 years (**A**); the lesions are more pronounced (**B**) and much more diffuse (**C**) in patients with more severe neurocutaneous phenotypes; (**D**) histopathological aspect of an angiofibroma (see text for explanation); (**E**) a solitary facial angiofibroma of the chin (black arrow) in a 30-year-old TSC patient who had (otherwise) normal skin appearance, normal intellect and no other TSC lesions besides few cortical tubers in the brain: this proband was referred because he asked to remove the chin lesions which in turn (at histology, see **D**) turned out to be an angiofibroma and prompted investigation in the proband and in his family (16 asymptomatic members of this family harbouring a TSC1 mutation were diagnosed with TSC).

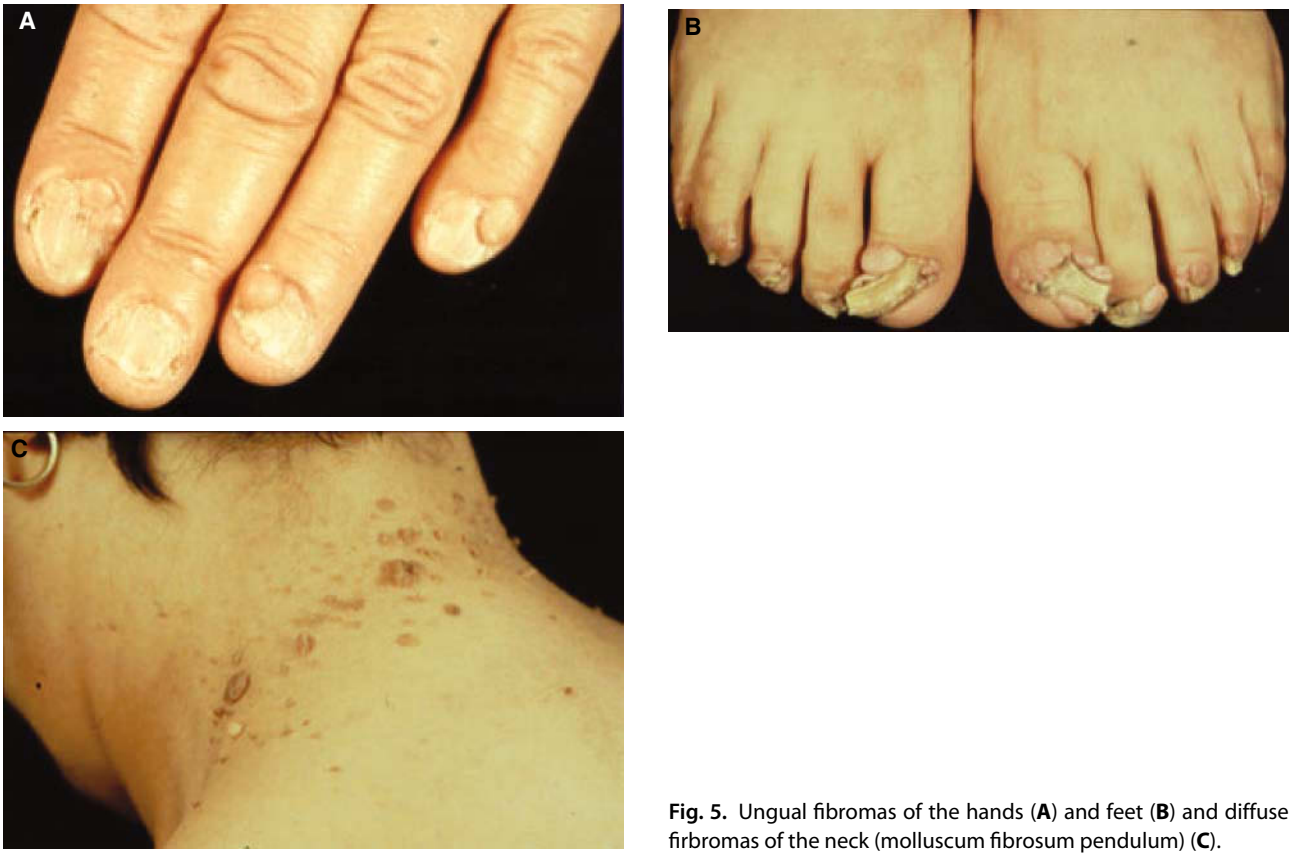


Fig. 5. Ungual fibromas of the hands (**A**) and feet (**B**) and diffuse firbromas of the neck (molluscum fibrosum pendulum) (**C**).

ranges from several millimeters to about 1 centimeter. They are more commonly found on the toes than on the fingers.

These lesions usually present at puberty or soon after and become more common with increasing age. They are usually absent in younger patients with TSC. Webb et al. (1996) found them in none of their TSC children before the age of 5 years, but in 68% between the ages of 15 and 29 years. In our paediatric population of TSC patients we have seen them in 16 out of 106 children (15.1%) – in one child aged 2 to 5 years, in 3 patients aged 5 to 9, in 8 children aged 9 to 14 years and in 4 children over 14 years (Jóźwiak et al. 1998a).

It has been suggested that tight shoes may stimulate fibroma growth, especially on the lateral aspect of the fifth toe. Usually, these fibromas tend to regrow after their removal. Special attention should be paid to single lesions which may arise spontaneously or after trauma, and may not be related to TSC. Practically, in our opinion, multiple lesions without any history of trauma can be regarded as pathognomonic to TSC. We found them also helpful in making the diagnosis in young children, when ungual fibromas are demonstrated in their apparently non-affected parents.

Histologically, these lesions are fibromas or angiofibromas, similar to the fibrous forehead plaques and the facial angiofibromas.

Excision of large or symptomatic ungual fibromas is the choice method of treatment, although recurrences are common (Berlin and Billick 2002).

Other skin lesions

In TSC many other non specific skin lesions have been reported, among them – cafè au lait spots and

molluscum fibrosum pendulum (see Fig. 5C). Because of their high prevalence in the general population and uncertain frequency in the TSC population, it is difficult to judge whether these lesions represent a coincidental finding or are the result of the hyperproliferative nature of TSC. So far these cutaneous findings are not included in the clinical diagnostic criteria for TSC.

Oral manifestations

Gingival fibromas and dental pitting (Fig. 6) are included in the diagnostic criteria for TSC (Sparling et al. 2007). Oral fibromas are common in adults with TSC: these are usually gingival (>50%) or at other oral mucosal sites (40%) including the buccal mucosa (inside the angular commissure), the labial mucosa, the superior labial frenulum, and palate and tongue. Oral fibromas also can occur sporadically in the general population but at a much lower frequency (e.g., 12/1000).

Another common (drug induced) oral complication of TSC is gingival overgrowth (hyperplasia or hypertrophy) usually secondary to the use of phenytoin.

Dental enamel pitting (Fig. 6) is observed in up to 100% of patients with TSC. Dental pits can also be observed in the general population but at lower frequency and with fewer lesions than in TSC.

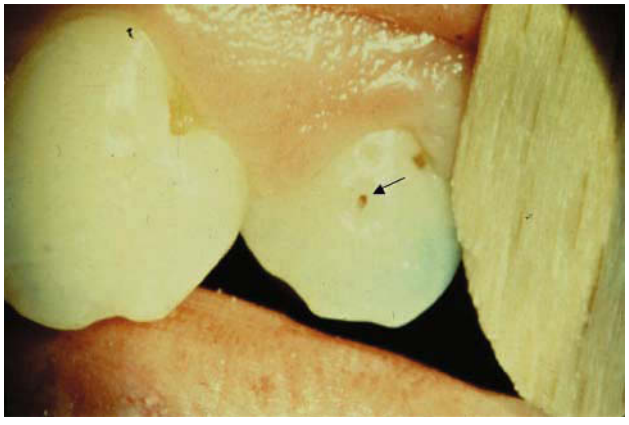


Fig. 6. Dental pitting (black arrow) in a TSC child. Courtesy of Dr. Rudolf Happle, Marburg, Germany. From: Vakilzadeh F, Happle R (1980) Schmelzdefekte bei tuberöser sklerose. Hautarzt 31: 336–337.

Neurological manifestations

There is considerable heterogeneity in the neurological manifestations. The spectrum includes patients with normal intellect and no seizures and extends to those with severe mental retardation and incapacitating seizures (Leung et al. 2007). However, when present, neurological complications are the most common causes of mortality and morbidity and the most likely to affect the quality of life.

Epilepsy

The most frequent neurological feature, epilepsy, is diagnosed in 60–90% of TSC patients during their lifetime (Curatolo et al. 2005, Holmes et al. 2007). Seizures occurred in 96% of patients aged 9–14 years referred to a child neurology clinic (Jóźwiak et al. 2000). In a recent retrospective epidemiological study on the prevalence of TSC in Northern Ireland, it was noted that 93.2% had epilepsy (Devlin et al. 2006). However, in an unbiased genetic linkage study only 62% of the patients developed seizures (Webb and Osborne 1991): it must be noted that these patients were not followed throughout their lifespan and therefore the incidence figures may be low (Holmes et al. 2007).

Seizures are the presenting sign in 67% of patients and in most patients the onset of epilepsy is between the 4^{th} and 6^{th} month of life. The most common type of seizures in the first months of life are infantile spasms: the other commonest seizures types are complex partial, generalized tonic-clonic and myoclonic (Holmes et al. 2007). There is a relationship between epilepsy and mental retardation (Jóźwiak et al. 1998b). Patients with early onset of epilepsy ($<6^{th}$ month of life) and unremitting seizures are more prone to develop severe mental retardation. Patients with a sustained remission are more likely to have normal intelligence. Overall poor prognostic factors include multiple seizure types, seizures onset before one year of age, and multifocal EEG abnormalities. The clinical challenge is to predict seizure intractability and inter-

vene before it occurs (Holmes et al. 2007). There is an increasing body of evidence that some in same infants with confirmed diagnosis of TSC and epileptic discharges in EEG, the introduction of antiepileptic treatment even before the appearance of clinical seizures may prevent from epilepsy and subsequent mental retardation (Jozwiak et al. 2007, Stafstrom et al. 2007).

Infantile spasms are particularly prevalent among children with TSC (Curatolo and Cusmai 1987, O'Callaghan et al. 2004, Thiele 2004), and TSC accounts for up to 25% of infantile spasms cases (Young 2002). Data is accumulating that infantile spasms and associated EEG findings in TSC are somewhat different than those seen in classic West syndrome. In TSC, focal seizures can precede, coexist with, or evolve into infantile spasms (Curatolo et al. 2005). EEG features of focal or multifocal spikes are most common when seizures are first identified, with hypsarrhytmia (often with focal features) evolving later (Holmes et al. 2007).

Epilepsy in children with TSC tends to be progressive, with increasing seizure frequency and pharmacological intractability over time. Despite the multifocal occurrence of tubers and hence multifocal nature of epileptic foci in TSC, many children are considered for epilepsy surgery, especially if a single tuber acts as a predominant focus. The success of tuber resection is encouraging enough to warrant an aggressive approach (Bebin et al. 1993; Guerreiro et al. 1998; Koh et al. 2000; Weiner et al. 2004, 2006). In addition to EEG, epileptogenic areas can be identified using magnetic resonance imaging (MRI) and positron emission tomography (PET) scans (Asano et al. 2000, Kagawa et al. 2005, Jansen et al. 2006). A multimodality approach is most helpful in identifying the epileptic focus (Lachhwani et al. 2005).

The surgical outcome varies: recent studies indicated 60% surgically treated children seizure-free at a median follow-up of 15 months (Kagawa et al. 2005). However, seizures can recur after removal of an offending tuber. Similarly, the effect of surgical resection on cognitive and behavioural outcome is unclear (Holmes et al. 2007).

Learning disabilities and mental retardation

These are very common in TSC, affecting from 40% to 80% of patients (Jambaque et al. 1991, Osborne and Webb 1993, O'Callaghan et al. 2004). Cognitive disabilities tend to be moderate or severe in degree. Children with a TSC2 mutation generally have a greater cognitive disability. A higher number of tubers correlated with a poorer cognitive outcome in some studies (Jambaque et al. 1991, O'Callaghan et al. 2004), but not in others (Doherty et al. 2005). There is no direct association between cognitive impairment, brain tuber localization, infantile spasms or focal EEG abnormalities and autism in TSC. However, the presence of cortical tubers in frontal and temporal lobes as opposed to a history of infantile spasms was associated with TSC in a recent study (Raznahan et al. 2007).

Attention deficit, hyperactivity, and sleep problems are the most frequent behavioural disorders. Cognitive disabilities of various degrees of severity are recorded in adulthood (Pulsifer et al. 2007, Winterkorn 2007). De Vries et al. (2007) in a postal survey of physical and behavioural abnormalities in children and adolescents with TSC in UK reported that patients with mental retardation were significantly more likely to have an autism spectrum disorder, attention deficit-related symptoms and speech and language difficulties. They were more likely to have a history of epilepsy, facial angiofibromas and shagreen patches and tended to have a greater number of physical features of the disorder. However, about one third of the children without mental retardation had features suggestive of a developmental disorder. Anxiety symptoms, depressed mood and aggressive outbursts occurred at equally high rates in those with and without mental retardation and were often not recognised (de Vries et al 2007). A consensus panel for the evaluation of cognitive and behavioural profiles has been recently proposed (de Vries et al 2005) for being incorporated in the overall formulation of the needs of the persons with TSC to plan educational, social and clinical management strategies. Assessments should be documented so that individual regular longitudinal progress can be monitored.

Autism

The exact proportion of autistic patients suffering from TSC is not well established, however, the risk of autism in TSC is much higher than in general population. Several studies tried to establish the prevalence of TSC in the autism spectrum disorder population and estimates varied from 0.9 to 1.1% (Frombonne et al. 1997; Wong 2006) to 9% (Gillberg et al 1994) whereas features of autism were present in 5% (Webb et al. 1996) to 61% (Gillberg et al. 1994) of patients with TSC (see also the studies by Baker et al. 1998, Bolton and Griffiths 1997, Calderon Gonzalez et al. 1994, Curatolo et al. 1991, Gutierrez et al. 1998, Hunt and Dennis 1987, Hunt and Shepherd 1993, Riikonen and Simell 1990, Smalley et al. 1992, Wiznitzer et al. 2004).

Despite considerable progresses in the last few years, the neurobiological basis of autism in TSC is still largely unknown and its clinical management represents a major challenge for the physicians involved in taking care of TSC patients. Recent evidence suggests that early-onset refractory epilepsy (Deonna et al. 2007, Humphrey et al. 2006) and/or functional deficits associated with the anatomical lesions in the temporal lobes (Chou et al. 2007) or in the cerebellum (Eluvathingal et al 2006) or the overall load of the cerebral lesions (Chou et al. 2007, Wong and Khong 2006) may be associated with autism. The emerging evidence is consistent with the notion that early onset electrophysiological disturbances within the temporal lobes (and perhaps other locations) (Deonna et al. 2006, Waltz et al. 2002) has a deleterious effect on the development and establishment of key social cognitive representations concerned with processing social information, perhaps especially from faces (Bolton 2004). No one factor alone (cognitive impairment, tuber localization, occurrence of infantile spasms, focal EEG abnormalities), can be causally linked with the abnormal behaviour. Autism may also reflect a direct effect of the abnormal genetic program (Au et al. 2007, Holmes et al. 2007). In this respect, the likelihood of a child with TSC developing autism is greater if the child harbours a mutation in the TSC2 gene, although autism also develops in children with TSC1 mutations (Bolton and Griffiths 1997, Holmes et al. 2007).

Neuroimaging

There are several types of intracranial lesions found on imaging studies in TSC patients: cortical tubers, periventricular (subependymal) nodules and subependymal giant cell astrocytomas, white matter abnormalities, parenchymal cysts and vascular lesions (Barkovich 2005, Luat et al. 2007, Tortori-Donati et al. 2005).

Cortical tubers (cerebral hamartomas)

These are the most pathologically characteristic lesions in TSC. Macroscopically they are smooth, whitish, slightly raised nodules that appears as enlarged, atypically shaped gyri; they may be either round or polygonal. Histologically, they consist of bizarre giant cells, dense fibrillary gliosis, and diminished, disordered myelin sheaths; ballon cells may be seen, making these lesions indistinguishable from focal cortical dysplasia with balloon cells. Any single patient may have as few as one to two or as many as 20 to 30 or more tubers. They are most commonly supratentorial, although 8% to 15% of affected patients have cerebellar tubers. The proportion that calcifies has not been reliably determined. The number of calcified cortical tubers seen on CT increases with age (by age 10 years, calcified cortical tubers are present in up to 50% of TSC patients). The cortical calcifications may be gyriform, simulating the appearance of Sturge-Weber syndrome on CT.

In infants, cortical hamartomas can be seen on transfontanelle ultrasound where they appear as focal hyperechogenicity. Neonatal and infantile tubers appear on CT as lucencies within broadened cortical gyri. The lucency diminishes with age, making the noncalcified cortical hamartomas difficult to identify in older children and adults. The MRI appearance of cortical tubers also changes with age: in neonates, they appear as gyri that are hyperintense as compared to the surrounding unmyelinated white matter on T1-weighted images and

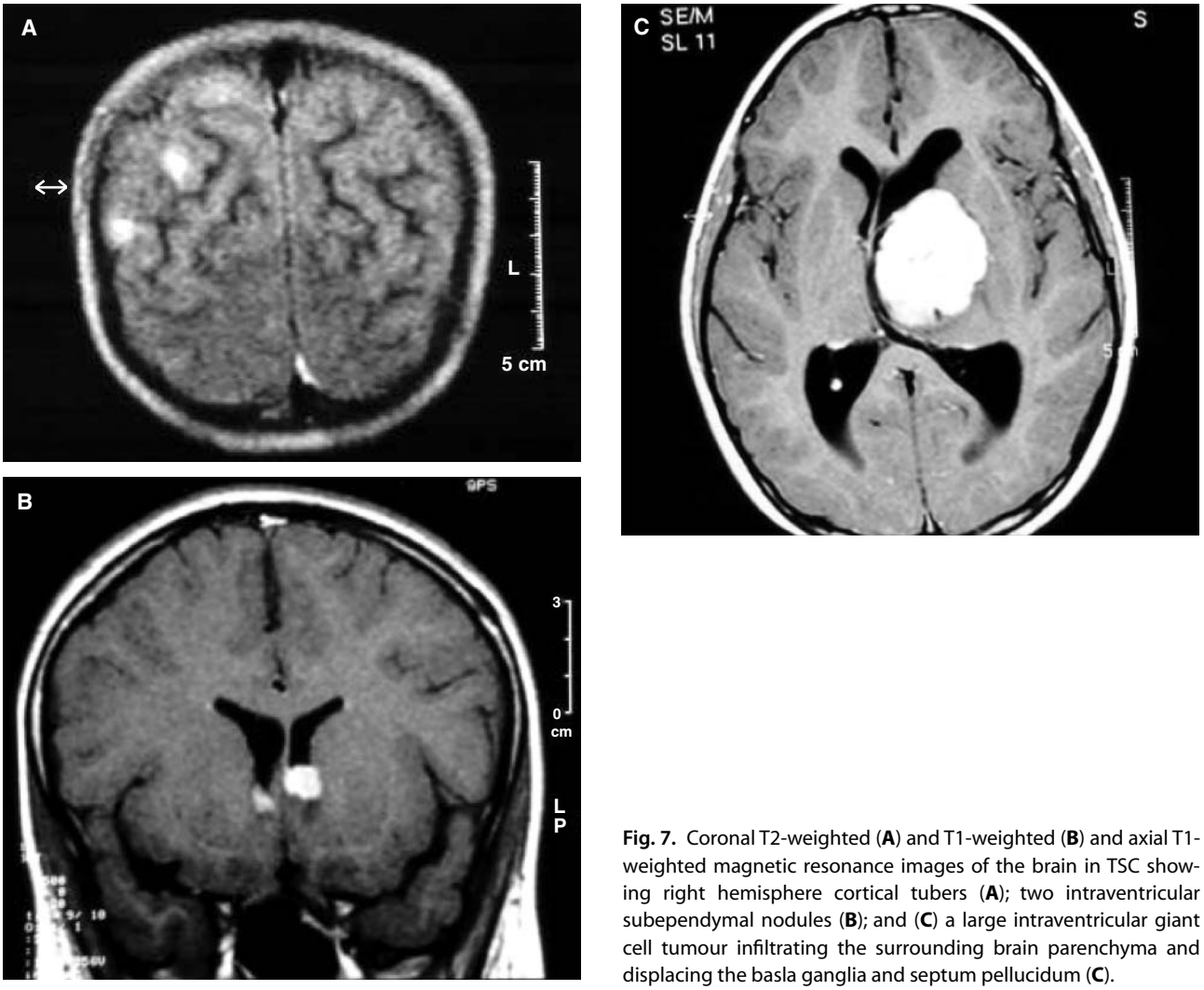


Fig. 7. Coronal T2-weighted (**A**) and T1-weighted (**B**) and axial T1-weighted magnetic resonance images of the brain in TSC showing right hemisphere cortical tubers (**A**); two intraventricular subependymal nodules (**B**); and (**C**) a large intraventricular giant cell tumour infiltrating the surrounding brain parenchyma and displacing the basla ganglia and septum pellucidum (**C**).

hypointense on T2-weighted images (Fig. 7A); about 20% of affected gyri are enlarged and T1 or T2 shortening may extent through the cerebral mantle to the ventricle from the tuber. The appearance changes as the white matter myelinates: the signal of the lesion slowly becomes isointense. In older infants tubers have a low-intensity center on T1-weighted images and high signal intensity on T2-weighted and FLAIR images (see Barkovich 2005 and Tortori-Donati et al. 2005 for review of imaging features).

Cortical tubers are regarded as highly epileptogenic, and thus their large number is considered a poor prognostic factor for drug-resistant epilepsy (Curatolo et al. 2006, Wu et al. 2006). Cyst-like cortical tubers can be found in the majority of patients below 7 years of age on FLAIR MRI images (Jurkiewicz et al. 2006). Sometimes, the tubers can be found in fetuses on MRI examination. Recent molecular studies revealed that some patients with focal cortical dysplasia type IIb and absence of other manifestations of TSC may represent a focal form of TSC restricted only to the brain (Jóźwiak et al. 2006a).

Chandra et al. (2007) in a study of children with and without infantile spasms, determined brain

volumes and cell densities in epilepsy surgery patients with TSC and cortical dysplasia with balloon cells. Patients with tuberous sclerosis without spasms showed microencephaly associated with decreased cortical neuronal densities. In contrast, cortical dysplasia patients without spasms were normocephalic with increased cell densities. The authors inferred that their findings supported the concept that TSC and cortical dysplasia have different pathogenetic mechanisms despite similarities in refractory epilepsy and postnatal histopathology. Furthermore, a history of infantile spasms was associated with reduced cerebral volumes in both cortical dysplasia and TSC patients, suggesting that spasms or their treatment may contribute to microencephaly independent of aetiology.

Subependymal hamartomas (nodules)

These tend to be located along the ventricular surface of the caudate nucleus, most often on the lamina of the sulcus thalamostriatus immediately posterior to the foramen of Monro. Less commonly, the nodules may be detected along the frontal and temporal horns, the lateral ventricular bodies, the third venricle, or the fourth ventricle.

In neonates, subependymal nodules can be detected by transfontanelle sonography, on which they appear as echogenic subependymal masses. They cannot be differentiated from germinal matrix haemorrhages or gray matter heterotopia by cranial sonography alone. The imaging appearance on CT and MRI changes with age: they are rarely calcified in the first year of life; the number of calcification typically increases with age. On MRI they typically appear as irregular subependymal nodules that protrude into the adjacent ventricle (Fig. 7B). In infants (who have unmyelinated white matter), the hamartomas are relatively hyperintense on T1-weighted images and hypointense on T2-weighted images; in premature babies they can be mistaken for subependymal haemorrhages (Barkovich 2005). They are detectable in 95–98% of patients. There is no direct correlation between the number of the nodules and severity of disease.

Giant cell tumours

In 5–12% of patients, prevalently in the first and second decade of life, intraventricular tumours may develop: the term giant cell tumour is given to the enlarging subependymal nodules that are usually situated near the foramen of Monro (Fig. 7C). Anatomically, they differ from the subependymal nodule by their size and their rendency to enlarge. Histopathologically are subependymal giant cell astrocytoma (SEGA). On imaging studies they are identified by the demonstration of tumour growth on serial studies (Fig. 8). Most giant cell tumours are located near the foramen of Monro; however, they can occur anywhere along the ependymal surface. Progressive enlargement of a nodule is the more reliable criterion for diagnosis. They tend to grow into the ventricle and only rarely invade the parenchyma. Occasionally, degeneration into higher grade, or infiltrating neoplasms can occur. The tumours frequently obstruct flow of the CSF and produce symptoms of intracranial hypertension.

Neonatal subependymal giant cell astrocytomas may also occur (Hussain et al. 2006, Medkhour et al. 2002, Mirkin et al. 1999, Raju et al. 2007, Ramenghi et al. 1996) – their natural history and prognosis are poorly understood (Raju et al. 2007).

The mainstay treatment strategy for subependymal giant cell tumours in TSC is still surgery. In the series of Torres et al. (1998) surgical criteria included: (1) presence of hydrocephalus; (2) interval increase in tumour size; (3) new focal neurological deficit attributable to the tumour; and/or (4) symptoms of increased intracranial pressure. According to de Ribaupierre et al. (2007) any lesion fulfilling the criteria for a subependymal giant-cell astrocytoma as previously described in the literature (i.e., lesion around the foramen of Monro, greater than 5 mm, with incomplete calcifications) (Nabbout et al. 1999, O'Callaghan et al. 1999) should be removed as soon as clear evidence of growth has been confirmed. Oral rapamycin therapy has recently proven to induce regression of subependymal giant cell astrocytomas associated with TSC offering an alternative to operative therapy of these lesions

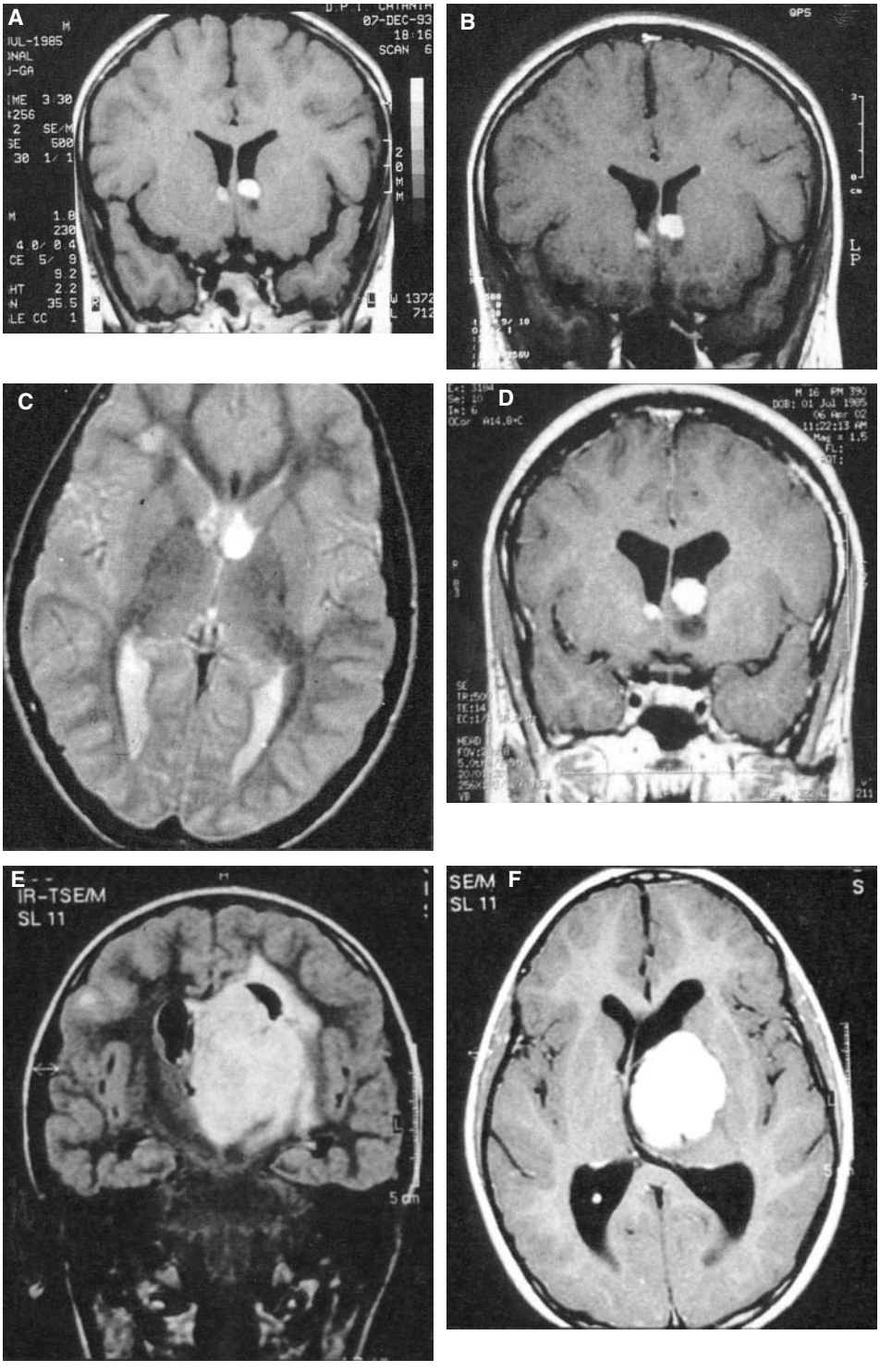


Fig. 8. (**A–G**) Natural history of a growing subependymal nodule transforming into a giant cell tumour. Coronal T1-weighted (**A**, **B**, **D**, **E**), axial T2-weighted (**C**) and T1-weighted (**F**) and sagittal T1-weighted (**G**) magnetic resonance images of the brain show the progression of one subependymal nodule (located near the left foramen of Monro: right aspect of the figures) which starts as a "larger" subependymal nodule with a minor cystic component in the lower aspect of the lesion (**A**, **B**) and grows up (**C**, **D**) to displace the surrounding tissues with cystic lesions within the mass (**E–G**).

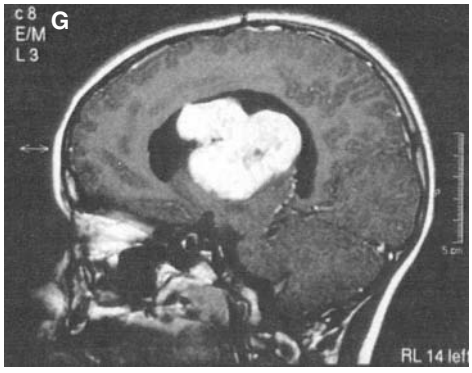


Fig. 8. (Continued)

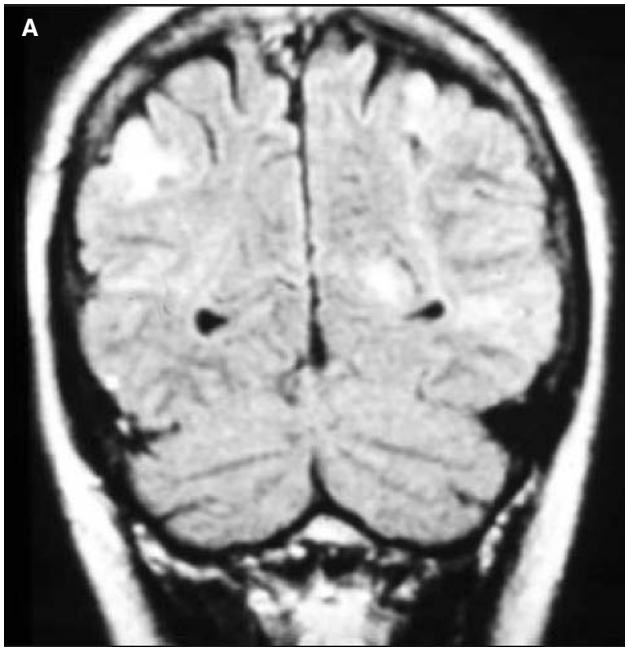
(Franz et al. 2006). In the series of five TSC patients by Franz et al. (2006) all lesions exhibited regression and, in one case, necrosis. Interruption of therapy resulted in regrowth of subependymal giant cell astrocytomas in one patient. Resumption of therapy resulted in further regression. Treatment was well tolerated.

White matter lesions

Islets consisting of grouping of neurons and glial cells are invariably present in the white matter of TSC patients. Microscopically, they contain bizarre cells including neurons and ballon cells (i.e., giant dysplastic cells with intermediate features between neurons and glia). These white matter foci also contain areas of hypomyelination similar to those seen in cortical tubers. Many of these clusters are microscopic and therefore they do not appear on imaging studies. Those large enough have variable imaging characteristics most similar however to cortical tubers (Fig. 9A).

Parenchymal cysts

An unknown percentage of TSC patients have cystic-like structures in the cerebral hemispheric white matter. The cysts are more commonly periventricu-



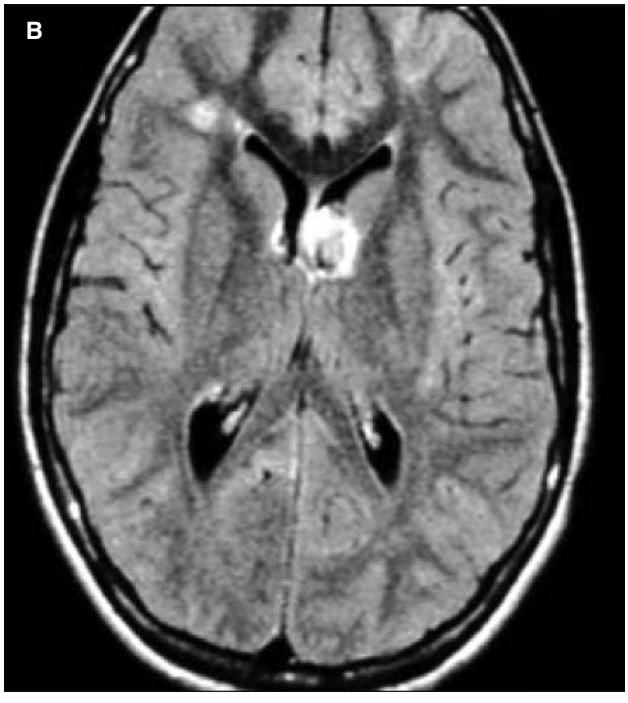


Fig. 9. Coronal (**A**) and axial (**B**) T1-weighted magnetic resonance images of the brain showing (**A**) rounded high signal lesions in the cortical (tubers) and radial (TSC white matter abnormalities) high signal lesions in the subcortical white matter and a cystic lesion (**B**) within a subependymal nodule which is transforming in a giant cell tumour.

lar, but may occur nearly anywhere aven within known brain hamartomas (Fig. 9B). Their clinical significance is uncertain.

Vascular lesions

These are rare in TSC although angiographic studies have demonstrated aneurysms in the kidney, liver, aorta, and distal extremities in affected patients. Involvement of the cerebral vasculare is very rare, but aneurysms have been reported in the internal carotid arteries or in the anterior cerebral arteries.

Spinal cord involvement

Recently, some cases showing spinal cord involvement in tuberous sclerosis have been reported. Hydrosyringomyelia in TSC was found in cervical and dorsal-lumbar part of spinal cord. It can either produce typical symptoms, like pes cavus and scoliosis, or remain clinically silent (Coppola et al. 2006).

Renal involvement

It is estimated that more than 80% of affected individuals with TSC may develop some form of renal manifestation during their lifetime. Renal involvement is second to neural involvement as a cause of morbidity and mortality in TSC patients (Shepherd et al. 1991). Two renal abnormalities are regarded as very characteristic for TSC: angiomyolipomas (AMLs) and renal cysts (Fig. 10).

Bilateral, multiple renal AMLs are found n 80–90% of adult patients and, when present, increase with age. Renal symptoms or signs of angiomyolipomas rarely appear before the third decade of life. Symptoms include flank pain, nausea and vomiting, hypertension, uremia and fever. There are also reports of sudden bleeding into the kidney from ruptured aneurysmatic vessels within the angiomyolipoma followed by bleeding into the retroperitoneal space. However, bleeding or rupture seldom occur in children, and are usually related to larger tumours appearing in adolescents and adults. Large

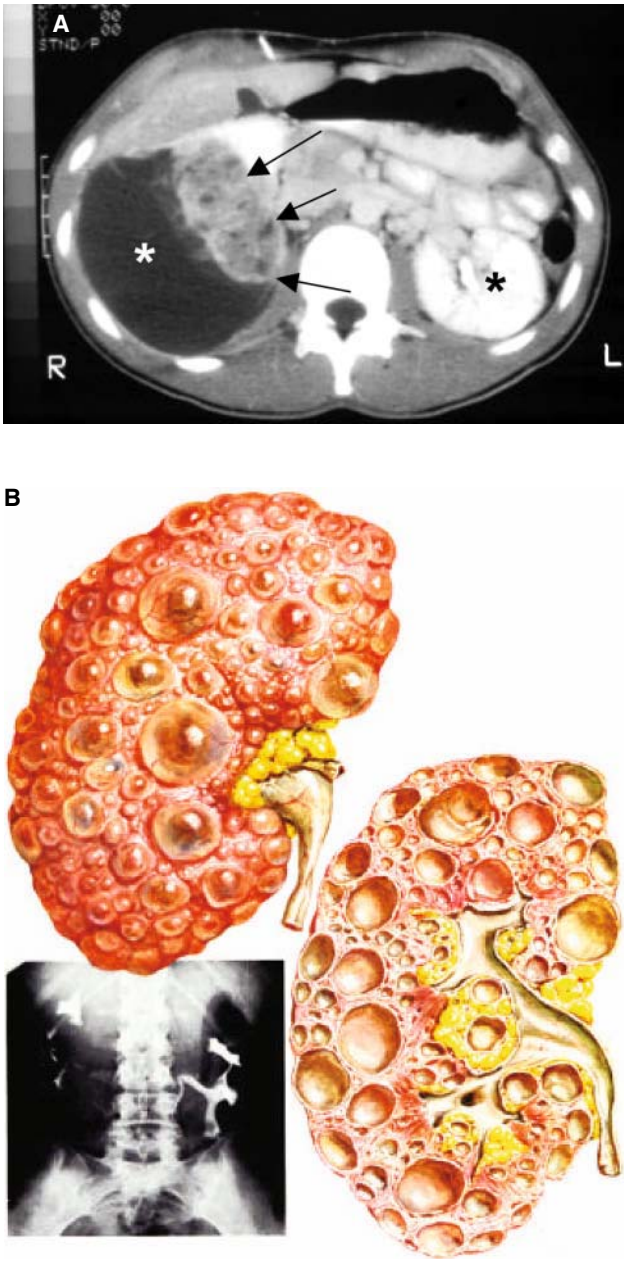


Fig. 10. Kidney lesions in TSC: (**A**) CT scan study of the abdomen showing a large angiomyolipoma located aside the right kidney (shown in the left side of the figure) (white asterix) and multiple cystic lesions of the left kidney (black arrows) (compare with the contralateral normal kidney, black asterix). (**B**) A drawing showing the most severe aspects of multicystic kidney in TSC (in the box is shown the X-ray aspect of the lesions with urinary pathway dilatation).

renal AMLs are much more frequent in patients with TSC2 gene mutations (Dabora et al. 2001). End-stage renal insufficiency caused by replacement of renal parenchyma by tumour masses may be observed in adult patients with multiple and large AMLs and may necessitate renal transplantation. Some of the tumours undergoes malignant transformation into renal clear cell carcinoma (RCC). Therefore the differential diagnosis of renal AMLs includes mostly renal malignancies. In patients with the classic triad of flank pain, painless hematuria and palpable abdominal mass the differentiation of a renal neoplasm from unilateral AML may be particularly difficult. The presence of fever and weight loss, observed in 30% of cases of renal malignancies, are unusual in renal AMLs, unless there is an associated retroperitoneal haemorrhage.

Microscopically, renal AMLs contain varying proportions of blood vessels, adipose tissue and smooth muscle cells, justifying the name "angiomyolipoma". Depending on which tissue predominates, the tumour may also be identified as myolipoma or angiomyoma. There are few descriptions of renal AMLs with retroperitoneal lymph node involvement, suggesting the continuum between AML and renal cell carcinoma. Recent immunohistochemical reports hypothesized that some AMLs may undergo malignant transformation to malignant AMLs. Malignant AMLs histologically may resemble sarcomatoid renal cell carcinomas.

Immunostaining for HMB-45 and cytokeratin may represent a useful staining in distinguishing AML from other tumours of the kidney and liver, especially from renal cell carcinoma and hepatocellular carcinoma (Al-Saleem et al. 1998). Contrary to sporadic RCC, benign AMLs in TSC patients are HMB-45 positive and cytokeratin negative (Koide et al. 1998).

However, TSC-associated RCCs differ distinctly from sporadic RCC. Four of the seven tumours reported by Bjornsson et al. (1999) immunostained positively for a melanocyte-associated marker, HMB-45. None of 10 sporadic RCCs from a control group stained with this marker and all stained with cytokeratin markers, which tended to be negative in TSC-associated tumours.

Patients with TSC require regular periodic sonographies to assess renal AMLs growth. Lesions exceeding 4 cm in diameter may require more frequent examinations, every 6–12 months, because of a high risk of rapid enlargement and bleeding. Such an approach should allow identification of individuals that can be treated with arterial embolisation or nephron-sparing surgery preventing the patients from development of symptoms and life-threatening bleeding (Harabayashi et al. 2004, Hsu et al. 2002, Shiroyanagi et al. 2002, Simmons et al. 2003). Arterial embolisation is regarded by some authors as a treatment of choice in all renal AMLs that are symptomatic and measure more than 4 cm. There is an increasing number of reports about interventional selective embolisation of haemorrhagic AMLs (Williams et al. 2006). Pain and fever lasting for several days after embolisation may be observed in about 90% of patients. To reduce the symptoms associated with the postembolisation syndrome a tapering dose of prednisone over a 2-week period has been administered by some authors (Bissler et al. 2002, Kothary et al. 2005).

Multiple and large renal cysts associated with polycystic kidney disease are found in 2–3% of patient of any age (see Fig. 10B). These patients harbour TSC2 mutation with large deletions involving the PKD gene. Such lesions should be differentiated from very small and solitary cysts found incidentally on control sonography of abdomen. They are much more frequent (up to 30%) in children aged 14 to 18 years and are thought to appear in TSC patients as a result of AMLs formation. They usually do not produce any symptoms.

Hepatic involvement

Until recently hepatic hamartomas had been rarely reported in patients with TSC, probably in part due to their usually asymptomatic course. Their benign nature, coexistence with renal AMLs and angiomyolipomatous appearance in relatively few pathological studies suggest that the vast majority (if not all) of hepatic hamartomas are AMLs. Liver angiomyolipomas are found in 45% of patients over the age

of 10 (Jóźwiak et al. 1992). They are more common in girls than in boys.

The hepatic hamartomas in TSC do not usually cause hepatic dysfunction or other symptoms or signs. The serum levels of liver enzymes are normal. These tumours are found incidentally or during the periodic follow up of TSC patients. Sonographically the liver lesions are highly echogenic, round to ovoid in shape and sharply demarcated from the surrounding normal parenchyma. Contrast enhanced CT is able to provide greater detail by demonstrating low density areas that represent fatty tissue (Fig. 11). In addition, magnetic resonance imaging may demonstrate a hyperintense signal on T2-weighted images indicating better than ultrasonography areas of fat within the tumour. Frequently these tumours are multiple and localised in both liver lobes. The average size of the lesion is 0.5 to 1.0 cm.

There are only two symptomatic hepatic hamartomas reported in the literature presenting with flank pain, spontaneous haemorrhage or rapid growth (Huber et al. 1996, Kristal and Sperber 1989). Both of them proved to be hepatic AMLs. In contrast to renal lesions, hepatic AMLs grow slower and were not mentioned as a possible cause of death in a large study of 355 patients with TSC (Shepherd et al. 1991).

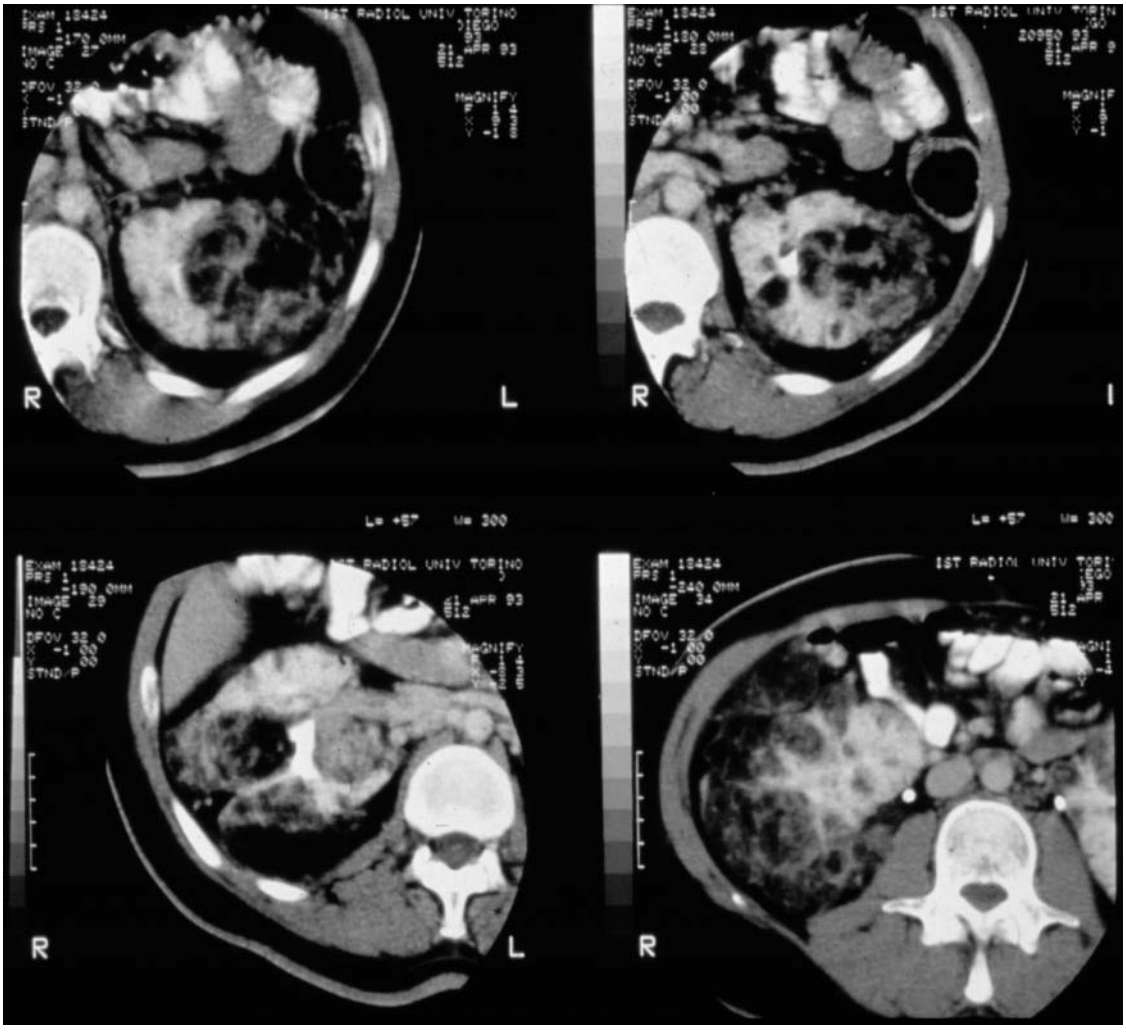


Fig. 11. Multiple cysts in the internal organs (kidney and hepatic parenchyma) in an adult with TSC.

Usually renal AMLs precede the development of hepatic AMLs in TSC patients and are frequent coexisting lesions. They were noted in 9 of 12 children with hepatic AMLs in our series (Jóźwiak et al. 1992) and in all 16 individuals with hepatic lesions reported by Sheffield et al. (1998).

On gross examination the hepatic AMLs are yellow to light tan, depending upon the amount of fat tissue. Histologically, these neoplasms are characterised by a mixture of mature fat cells, blood vessels and smooth muscle cells, with occasional foci of extramedullary hematopoiesis. There are also aneurysmatic dilatations of thick-walled blood vessels, which may facilitate spontaneous bleeding.

Recent immunohistochemical studies demonstrated that hepatic lesions in TSC patients may represent monotypic epithelioid AMLs with pronounced presence of epithelioid PEC (Bonetti et al. 1997). Positive HMB-45 staining has been proposed as a defining criterion of hepatic AML (Tsui et al. 1999).

Cardiac involvement

Cardiac rhabdomyomas represent the earliest detectable hamartoma in TSC and, interestingly, are the only lesion in TSC which may regress with age (Fig. 12). Cardiac rhabdomyomas are found in 47–67% of all patients with TSC (Jóźwiak et al. 2006). There is a higher incidence of cardiac tumours in infants and newborns with TSC (80%) even disclosed prenatally (Bader et al. 2003, Fesslova et al. 2004, Pipitone et al. 2002). The incidence decreases with age to 20% in *2- to 9-year-old* children with TSC and slightly increases again in the pubertal age (Jóźwiak et al. 2000, 2006).

Conversely, the prevalence of TSC in patients with cardiac rhabdomyomas may be close to 100% as very often the tumours are the first manifestation of the disease, being diagnosed in newborns and very young infants, when the majority of symptoms of TSC cannot be noted. We reported a child with multiple cardiac rhabdomyomas diagnosed prenatally, who had not manifested other symptoms of TSC during six years of follow up. Molecular studies confirmed a mutation in the TSC2 gene (Jóźwiak et al. 2005).

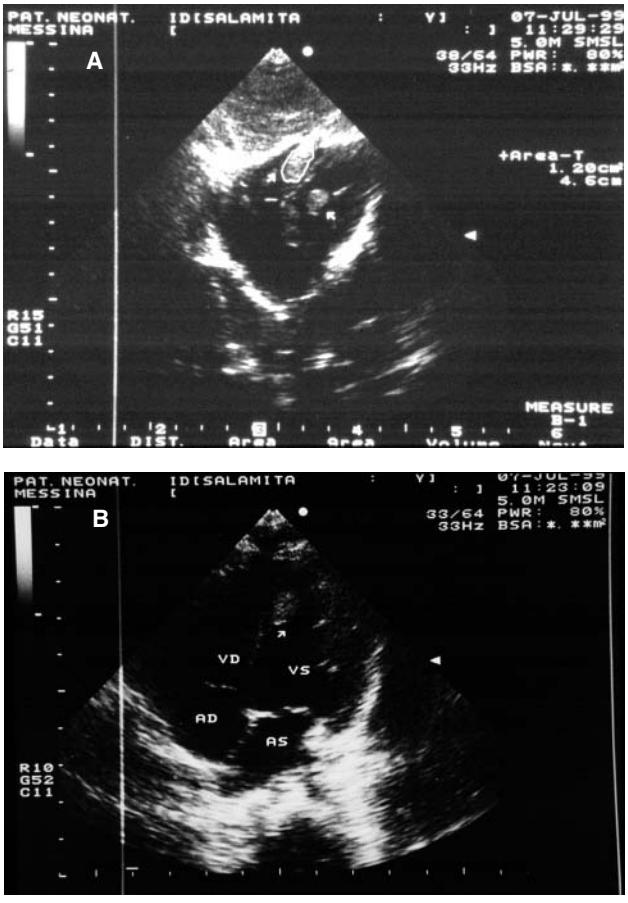


Fig. 12. (**A, B**) Ultrasonographic aspect of multiple cardiac rhabdomyomas (white circles).

Most tumours are asymptomatic. If cardiac symptoms occur, these are largely a consequence of the tumour size or location within the heart. These symptoms and signs may be explained by one or more of the following three mechanisms: obstruction of inflow or outflow tract, secondary to an obstructing intracavitary tumour; myocardial involvement with secondary deterioration of ventricular function; and cardiac rhythm abnormalities.

The tendency of cardiac rhabdomyomas toward spontaneous resolution, particularly in infancy and early childhood, dictates a conservative approach in the majority of patients. According to the latest rec-

ommendations periodic echocardiography of all asymptomatic patients is unnecessary (Roach et al. 1999). Occasionally asymptomatic children may require follow-up echocardiography when the initial study raised specific concerns about the size or location of a rhabdomyoma or in patients considered to be at relative risk of tumour enlargement e.g., patients on ACTH treatment.

In patients with congestive heart failure the treatment with digitalis, diuretics and salt restriction may be utilised. Cardiac rhythm disturbances are usually treated with antidysrhythmic drugs. In children with dysrhythmia refractory to medication a cardiac pacemaker or the division of the abnormal conduction pathway may be considered.

Due to spontaneous regression of the tumours the surgical excision of tumour in infancy is justified only in the presence of a life-threatening hemodynamic condition. These patients, frequently newborns, usually have multiple obstructing masses (Ruggieri et al. 1997). The operative risk depends on the number, size and location of the tumours, but in this group of patients is very high. Still, the prognosis for survival without surgical intervention in these critically ill children is very poor.

An association of arterial aneurysms and TSC has been reported in a number of patients (Jurkiewicz and Jóźwiak 2006). Such a coincidence led some authors to the conclusion that a congenital defect of the arterial wall was part of the condition.

Ocular manifestations

Retinal hamartomas, retinal pigmentary and vascular changes, optic nerve atrophy, glaucoma and coloboma of the iris, lens, choroid and retina have been described in TSC (Fig. 13). However, the most common ophthalmologic sign is the retinal hamartoma (RA) which is often multiple. Three basic morphological forms of retinal hamartomas are recognized: noncalcified, calcified mulberry-like and transitional type, the latter sharing morphologic features of both previously mentioned types. Since all these lesions are mostly located in the periphery and loose early their growth capacity, they are generally asymptomatic. Nevertheless, aggressive hamartomas have been observed (Shields et al. 2005).

The wide range of prevalence (4% to 76%) of retinal hamartomas reported so far in TSC can account in part for the differences in the patient's age distribution (retinal hamartomas can be overlooked in infancy and generally in non-compliant patients) or for different ratios of TSC1 vs TSC2 patients.

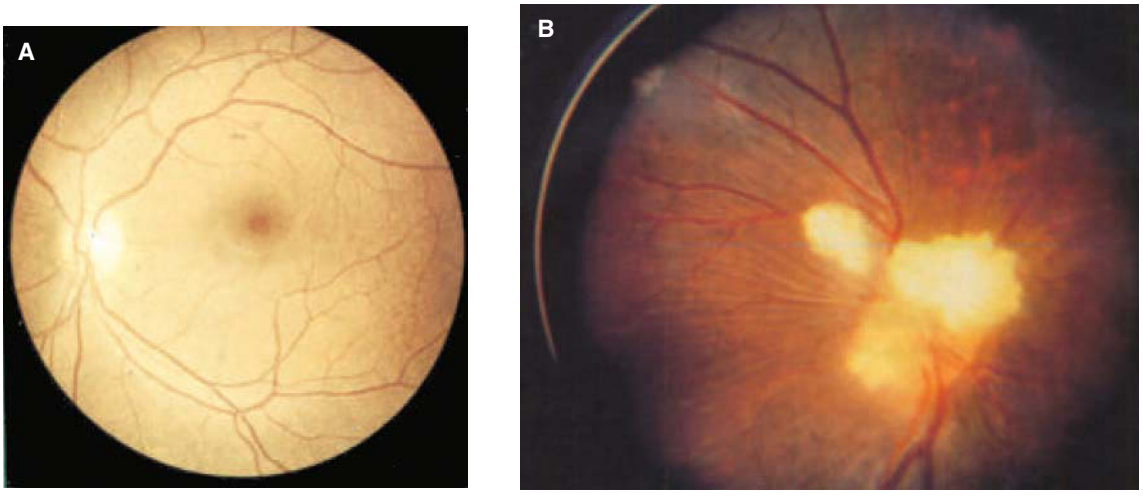


Fig. 13. Retinal hamartomas as demonstrated by fundoscopy (**A**, **B**). Note the red lesion (initial stages of the hamartoma which is not calcified) (**A**) and the yellowish lesions (calcified hamartomas) (**B**) seen at fundoscopy.

Recent data from large series of patients indicate that one third to one half of adult TSC probands have one or more retinal hamartoma (Robertson 1999).

Pulmonary manifestations

Symptomatic lung involvement in TSC patients is rare, the onset is after the second decade of life and is virtually restricted to females, with an estimated incidence between 1% and 6% (Polosa 1996). Two pulmonary lesions with different histopathologic features have been described in TSC: the well known lymphangioleiomyomatosis (LAM) (Fig. 14), and the less common multifocal micronodular hyperplasia of type II pneumocytes (MMPH) (Popper HH et al. 1991, Guinee D et al. 1995, Nagar et al. 2008). The two manifestations, LAM and MMPH, can coexist or appear as a pure, monomorphic lung disease. The major difference is that LAM affects only females, with only rare exceptions, and may progress to cause serious respiratory complications, whereas MMPH occurs in both sexes, is often recognized in subjects without any pulmonary disfunctions and appears to have a more favourable evolution. Both LAM and MMPH have been described as isolated lung diseases in subjects who did not fulfill the TSC diagnostic criteria. A retrospective analysis of CT scans of the chest, abdomen and pelvis in a large series of LAMs with and without TSC has suggested that lymphatic involvement, such as thoracic duct dilatation, chylous pleural effusion, and ascites are less common in LAM with TSC (Avila et al. 2007). The pathogenesis of pulmonary involvement is still not clear. Exacerbation during pregnancy and estrogen supplementation has been reported.

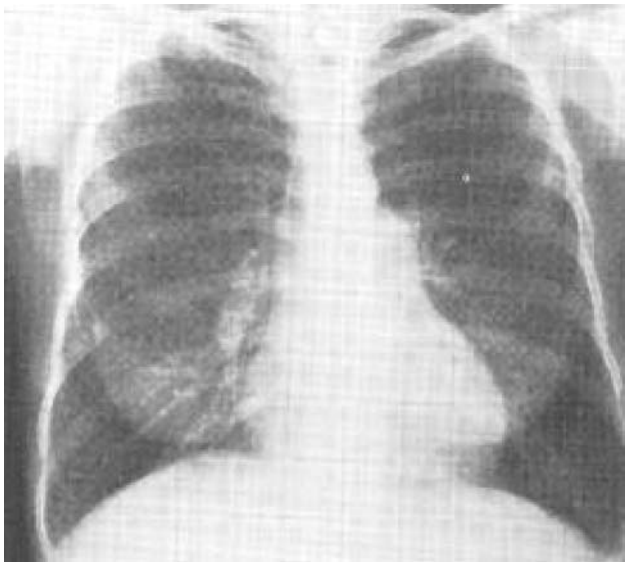


Fig. 14. X-ray appearance of pulmonary limphangioleyomyomatosis.

Pathogenesis/molecular genetics

Molecular genetics

TSC is an autosomal dominant disorder in which sporadic cases account for about two-thirds of all patients, and reflect the occurrence of new mutations (Kwiatkowski et al. 2004). Within families there is a wide variation in the extent and degree of clinical manifestations, indicating that there is no rigid correlation between specific TSC gene mutation and clinical outcome.

TSC1 and *TSC2* genes

Linkage analysis in multigenerational families and positional cloning has been used to map both the *TSC1* and *TSC2* genes (Fig. 15). Linkage of the first TSC locus (*TSC1*) to 9q34, near the AB0 blood groups, was reported in 1987 (Fryer et al. 1987, Northrup et al. 1987). Subsequent studies provided strong evidence for locus heterogeneity (Janssen et al. 1990, 1994; Northrup et al. 1992; Sampson et al. 1989, 1992; Povey et al. 1994) and led to the identification of the second locus, *TSC2*, at 16p13.3, near the autosomal dominant polycystic kidney disease major gene (Kandt et al. 1992). Although the OMIM catalog lists other two putative *TSC* loci, at 12q and 11q, on the basis of the breakpoints of de novo translocations found in two patients with TSC, presently there is no convincing experimental evidence for a third locus. Indeed, all multigeneration TSC families reported so far showed linkage to

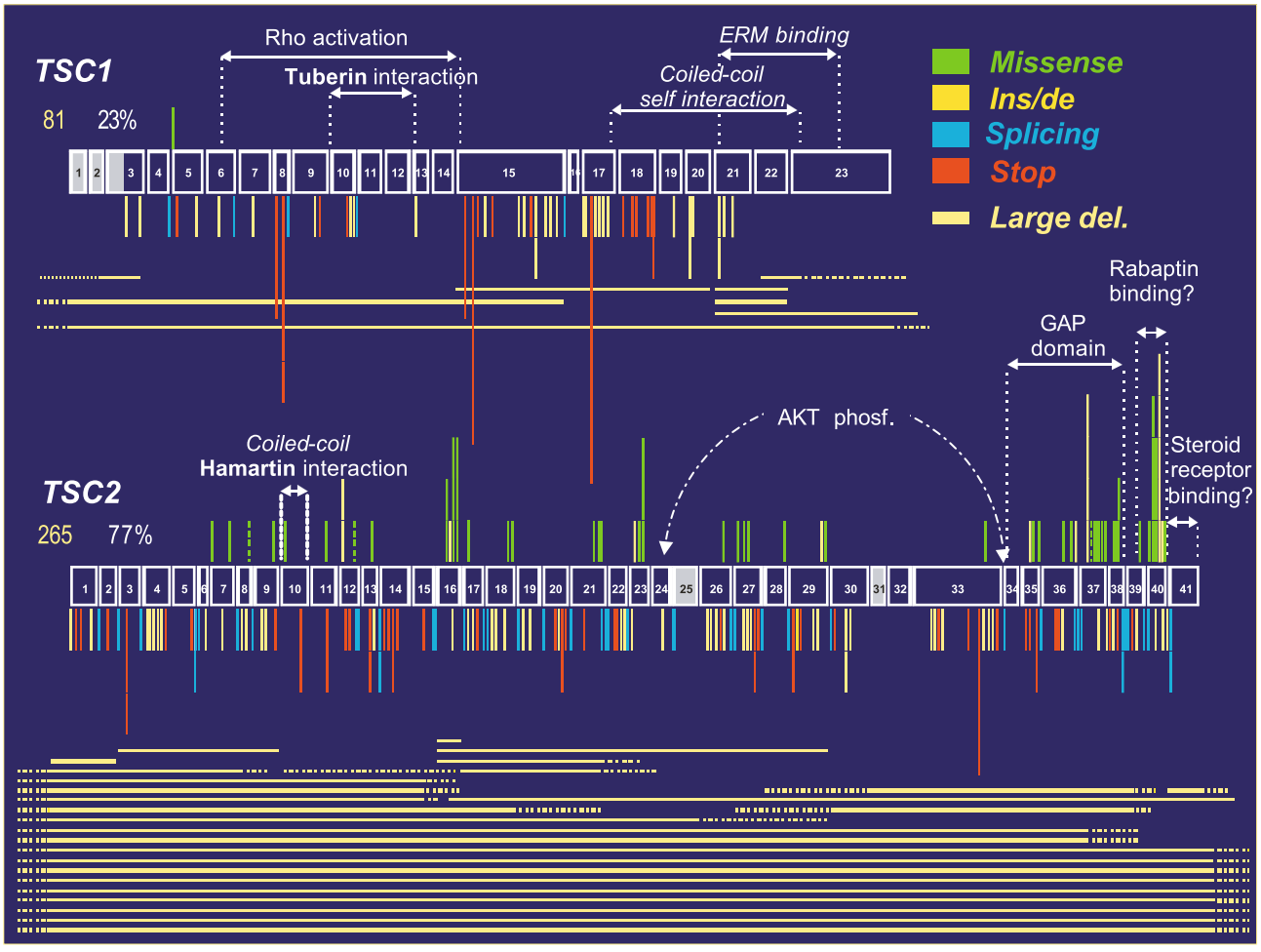


Fig. 15. The *TSC1* and *TSC2* genes.

either *TSC1* or *TSC2*, the few exceptions being eventually solved by the finding of two different *TSC1* and *TSC2* mutations, independently originated in two relatives. Nevertheless, genetic and epigenetic variations in other genes, either related or unrelated to the biochemical pathways of the two known *TSC* genes, are expected to modify or even mimic at least in part the pleiomorphic TSC phenotype.

The *TSC1* transcript is 8.6-kb long and contains 23 exons (Fig. 15), (the first two are non-coding and alternatively spliced), encompassing 55 kb of genomic DNA (van Slegtenhorst et al. 1997). The gene has no known structural homologies to other known gene families.

The *TSC2* transcript is 5.6-kb long, contains 41 coding exons (plus an upstream non-coding exon) (Fig. 15), encompassing 40 kb of genomic DNA (European Chromosome 16 Tuberous Sclerosis Consortium 1993). Exons 25 and 31, the only two *TSC2* exons not showing so far any mutation, are alternatively spliced (Xu et al. 1995); the functional significance of the transcripts showing both, one or neither exons 25 and 31 is not known.

More than 900 different micro-mutations, nearly 100 rare variants of uncertain pathogenicity and 350 polymorphisms have been identified in the two *TSC* genes (http://consortium.liacs.nl/lovd/index.php?-select_db=TSC1 or db=TSC2 and personal data).

Except for the CpG mutational hot spots and short nucleotide repeats, there are no particular regions within the two genes in which mutations occur at a higher rate. Overall, the most recurrent mutation (18-bp deletion in exon 40 of *TSC2*) is found in less than 3% of the patients. If the wide variety of *TSC* mutations can be accounted for the length and structural complexity of the two genes, more intriguing is the dramatic difference between *TSC1* and *TSC2* in the occurrence of in frame mutations (3% vs. 30%). In particular, missense mutations are extremely rare in *TSC1*, whereas 1/4 of *TSC2* mutations are missenses ($P < 10^{-4}$). Large genomic rearrangements, such as partial or full deletions of either *TSC* gene and, rarely, duplications and inversions, have been reported. The prevalence of large deletions is not statistically different between TSC1 and TSC2 patients (9% and 11%, respectively). A subgroup of large genomic deletions or rearrangements affect both *TSC2* and the adjacent *PKD1* gene, frequently mutated in the adult variety of polycystic kidney disease, causing early-onset, multiple renal cysts associated with the TSC phenotype (OMIM # 600273). The *PKD1* and *TSC2 contiguous gene deletion syndrome* may be recognized at birth or shortly thereafter for the enlarged and polycystic kidneys (characterized by a multitude of variably sized cysts closely resembling those more commonly seen in later life in the advanced stages of autosomal dominant polycystic kidney disease, or PKD1; OMIM # 173900) (Brook-Carter et al. 1994, O'Callaghan et al. 1975, Sampson et al. 1997, Stapleton et al. 1980, Wenzel et al. 1970).

In agreement with Knudson's two-hit tumour suppressor gene model (Knudson 1971), inactivation of both alleles of either *TSC1* or *TSC2* gene appears to be required for the development of hamartomas in TSC. Somatic defect of the second allele has been clearly documented in a variety of proliferative TSC lesions, such as renal AML, pulmonary LAM, cardiac rabdomiomas, and brain subependimal giant cell astrocytomas (SEGA). The difficulty of finding a biallelic defect in cortical tubers initially suggested that *TSC1* or *TSC2* haploinsufficiency per se might cause aberrant cortical lamination (Henske et al. 1996, Niida et al. 2001). Later it has been unequivocally shown that a biallelic defect is present, but restricted to a small fraction of the heterogeneous and dysmorphic cells contained in the tubers, i.e., to poligonal or ovoid giant cells with eosinophilic cytoplasm and thickened processes (Crino 2004), identical to the major cell component of the SEGA.

It is reasonable to conclude that the TSC hamartomas prevalent pathogenic mechanism is that already described or that the inactivation of the second allele might be preferentially obtained, at least in the tubers, by epigenetic mechanisms. Indeed, Han et al. (2004) found that in tubers, but not in angiomyolipomas, tuberin is expressed and phosphorylated in an specific residue known to cause its inactivation (see below pathogenesis).

At present, the prevailing opinion is that the mTOR/p70S6K/S6 signal pathway is activated in giant cells, suggesting that the surrounding neurons acquire dismorphic features similar to those of the adjacent dismorphic neurons. Nevertheless, it is reasonable to assume that also epigenetic mechanisms, such as the abnormal tuberin phosphorylation reported in tubers (Han et al. 2004) might interfere with tumour suppressor activity or other critical, yet unknown, TSC functions. Overall, the most reasonable mechanism in classic hamartomas in TSC would be that of second-hit mutations occurring in limited numbers of cells. These mutations are referred to as loss of heterozygosity, since they affect neighbouring heterozygous polymorphic markers. Loss of heterozygosity in *TSC1* or *TSC2* has been consistently observed in the majority of TSC-associated angiomyolipomas, cardiac rhabdomyomas, subependymal giant-cell tumours, and lymphangiomyomatosis cells but has only rarely been found in cerebral cortical tubers (Chan et al. 2004, Henske et al. 1996, Niida et al. 2001, Crino et al. 2006).

Molecular genetic testing

The clinical use of genetic testing in TSC is aimed: (a) to confirm a clinical diagnosis; (b) to offer genetic counselling to probands and family members relatives, including prenatal diagnosis.

The genetic testing for TSC is complicated by the large size of the two genes, the necessity to screen for

both point mutations and large deletions, and the presence of somatic mosaicism (Emmerson et al. 2003, Sampson et al. 1997, Verhoef et al. 1999). As TSC1 mutations are primarily small deletions and insertions and nonsense mutations these are better detected by sequence analysis; *TSC2* mutations also include significant numbers of large deletions and rearrangements that cannot be detected by sequence analysis (Nellist et al. 2005, Northrup and Au 2006). In the major studies so far published the mutation detection rate varied from 62.4% (Au et al. 2004; using SSCA and direct sequencing and including approximately 10% of cases not meeting diagnostic criteria for TSC) to 70% (Sancak et al. 2005; using SSCP, DHPLC, DGGE, direct sequencing, Southern blotting, and FISH analysis), 74% (Dabora et al. 2001), 76% (Hung et al. 2006), 80% (Jones et al. 1999), 83% (Kwiatkowski et al. 2004) and 95.8% (Devlin et al. 2006). Overall, the mutation detection rate by means of sequence analysis varies from 15% (*TSC1* gene) to 60–70% (*TSC2* gene) in sporadic cases and from 30% (*TSC1* gene) to 50% (*TSC2* gene) in familial cases (Jones et al. 2000, Northrup and Au 2006).

Genotype-phenotype correlations

Linkage studies initially suggested that there would be equivalent numbers of families with mutations in each *TSC* gene (Povey et al. 1994). However, the frequency of mutations reported in *TSC2* is consistently higher than in *TSC1*: TSC1 mutations account for only 10 to 30% of the families identified with probands with TSC (Jones et al. 1997, 1999; Kwiatkowska et al. 1998; Niida et al. 1999; Sancak et al. 2005; van Slegtenhorst et al. 1999). In sporadic cases of TSC, there is an even greater excess of mutations in *TSC2* (Crino et al. 2006) whilst identification of *TSC1* mutations appears to be twice as likely in familial cases as in sporadic cases. The disparity in mutational frequency may reflect an increased rate of germ-line and somatic mutations in *TSC2* as compared with *TSC1*, as well as ascertainment bias, since mutations in *TSC2* are associated with more severe disease (Crino et al. 2006; Jones et al. 1997, 1999; Kwiatkowski et al. 2004; Sancak et al. 2005).

Except for the contiguous gene deletion syndrome (PKDTS), the phenotypes caused by mutations in *TSC1* and *TSC2* were initially considered to be identical; however with more genotype/phenotype data available, it appears that *TSC1* mutations produce a less severe phenotype than *TSC2* mutations (Jones et al. 1997, 1998, 1999; Au et al. 1998; Dabora et al. 2001; Lewis et al. 2004; Sancak et al. 2005). The exception is that some missense *TSC2* mutations are associated with milder disease phenotypes (Khare et al. 2001). In individuals harboring *TSC2* mutations: (1) Al-Saleem et al. (1998) reported a greater risk of renal malignancy; (2) Jones et al. (1997, 1998, 1999) found a higher frequency of intellectual disability [however, other series have not replicated this finding (Kwiatkowska et al. 1998, Niida et al. 1999, van Slegtenhorst et al. 1999, Young et al. 1998)]; (3) Dabora et al. (2001) found that 8 out of 16 clinical features investigated occurred at a significantly higher frequency and/or with greater severity including seizures, moderate to severe learning disability, mean number of subependymal nodules, tuber count, kidney angiomyolipomas, mean grade of facial angiofibromas, forehead plaque and retinal hamartomas; (4) Lewis et al. (2004) recorded more commonly autistic disorders, low IQ and infantile spasms; and (5) Devlin et al. (2006) observed a more severe phenotype including epilepsy and learning disabilities; (6) Hung et al. (2006) found a higher incidence of intellectual disability and mental retardation but no significant differences in all the remaining clinical features of TSC. (7) Strizheva et al. (2001) suggested that females with mutations on the carboxy terminus of the *TSC2* gene product (tuberin) may have increased incidence and/or severity of lymphangiomyomatosis. (8) Au et al. (2007) showed that patients with TSC2 mutations have significantly more hypomelanotic macules and learning disability and overall more severe symptoms. In addition they found that male patients have more frequent neurological and eye symptoms, renal and ungual fibromas.

Mosaicism

Among patients meeting the clinical criteria for a diagnosis of TSC, 15 to 20% have no identifiable

mutations (Dabora et al. 2001, Sancak et al. 2005). These persons generally have milder clinical disease (i.e., a lower incidence of mental retardation, seizures, and dermatological manifestations) than patients with identified *TSC1* or *TSC2* mutations (Crino et al. 2006). Mosaicism for a mutation in the first allele of either *TSC1* or *TSC2* has been reported in probands or in the first affected relative of the family, i.e. in a proband's parent or grandparent. It is not known how many de novo TSC mutations of the first allele are generated during the gametogenesis of a healthy parent (germinal mutation) or during the embryonic development of a normal zygote (somatic mutation). In principle, individuals with a post-zygotic mutation should be somatic mosaics, having two cell populations, one with and one without that mutation. It is reasonable to assume that the quantity of "mutated" cells in critical organs such as the brain and kidneys will determine at least in part the severity of the phenotype. Since the sensitivity threshold of the techniques for mutation detection-currently used is low (≥5%), and a single tissue – the peripheral leukocytes – is normally tested, only a fraction is available for screening reasons. In principle, mosaic post-zygotic mutations of the first allele are thought to account for a milder clinical phenotype (Jones et al. 2001; Roberts et al. 2004; Verhoef et al. 1995; 1999; Rose et al. 1999; Emmerson et al. 2003). Germ-line mosaicism has been also confirmed in families with affected siblings and unaffected (Yates et al. 1997) or mildly affected parents. Mosaicism is also a credible explanation for the failure to detect a mutation (Kwiatkowski 2005; Kwiatkowski et al. 1999, 2004). The highest level of mosaicism (7/27 unrelated families, or 26%) was reported in a series of patients with the contiguous gene syndrome due to deletion of both *TSC2* and *PKD1* genes (Sampson et al. 1997).

Cases with solitary, typical TSC lesions which developed from two somatic hit mutations in the TSC genes (in this case *TSC2* gene), rather than being part of a phenotype manifesting with very small fraction of somatic mosaicism have been recorded (e.g., solitary SEGA; Ichikawa et al. 2005).

Penetrance

After careful, detailed evaluation of each individual known to have a *TSC1* or *TSC2* mutation, the penetrance of TSC is now thought to be 100%. Rare cases of seemingly non-penetrance have been reported; however, molecular studies have resolved these cases, revealing two different TSC mutations in the family and the existence of germ-line mosaicism in others (Connor et al. 1986, Webb and Osborne 1991).

There are no other genetically related (allelic) disorders associated with mutations in *TSC1* and *TSC2*. In some cases, DNA extracted from lung tissue in individuals with sporadic pulmonary lymphangioleiomyomatosis (LAM) harbors mutations of *TSC2* or *TSC1* not present in the germ-line (Carsillo et al. 2000, Smolarek et al. 1998): the role of *TSC1* and *TSC2* genes in this process is not yet fully determined. Several lines of evidence support the conclusion that the actions of *TSC1* and *TSC2* are probably limited in the complex process of LAM development (see also below, pathogenesis).

Anticipation

Anticipation has not been observed in TSC.

Pathogenesis (Functions of *TSC1* and *TSC2*)

Animal models

Homologs to the *TSC* genes in model organisms (e.g., rat, mouse, *Drosophila*, *Fugu*, and, more distantly, fission yeast) were identified and found to be highly conserved, suggesting similar, if not identical, evolutionary functions (Au et al. 2004, Piedimonte et al. 2006, Scheidenhelm and Gutmann 2004). Similarly to many evolutionary conserved genes, engineered homozygous *TSC* gene mutants are embryonic lethal in these models (e.g., TSC1 null embryos die at mid-gestation from a failure of liver development; *TSC2* null embryos die at mid-gestation as well displaying dysraphia and papillary overgrowth of the neuroepithelium) (Kobayashi et al.

2001, Kwiatkowski et al. 2002, Rennebeck et al. 1998, Uhlmann et al. 2002).

The *Eker* rat (Eker 1954), a spontaneous mutant predisposed to autosomal dominant renal carcinoma, was the first animal model found to contain an insertion mutation in the *TSC2* gene (Hino et al. 1994, Kobayashi et al. 1995, Kubo et al. 1995, Yeung et al. 1994, 2004). Several engineered *TSC1* and *TSC2* gene disruptions created in the mouse have been shown to have renal pathology similar to that of humans affected with TSC but, interestingly do not have significant brain pathology (Hino et al. 1994; Jin et al. 1996; Kenerson et al. 2005; Kobayashi et al. 1995, 1999; Onda et al. 1999; Takahashi et al. 2004; Wolf et al. 1998). Also, $TSC1^{\pm}$ and $TSC2^{\pm}$ mice have some phenotypic differences [e.g., development of renal cystadenomas, early onset bilateral polycystic kidney disease and extra-renal tumours such as hepatic hemangiomas (Kleymenova et al. 2001, Kobayashi et al. 2001), or anaplastic ganglioglioma (Kwiatkowski et al. 2002, Mizuguchi et al. 2000). A conditional deletion of the *TSC1* gene in heterozygosity (and/or homozygousity) limited/restricted to astrocytes in mice exhibited abnormal neuronal organization in the hippocampus, age-dependent increase of astrocyte proliferation, seizures and death: these findings suggested that the increase in astrocyte proliferation precedes the neuronal abnormalities, causing mass effect changes or disturbance of complex astrocyte-neuron interactions (Uhlmann et al. 2002, Wenzel et al. 2004). $TSC2^{\pm}$ rats exhibits a marked reduction of different forms of hyppocampal synaptic plasticity (e.g., loose of their potential for activity-dependent synaptic modification) (von der Brelie et al. 2006) and enhanced episodic-like memory and kindling epilepsy (Waltereit et al. 2006) and $TSC1^{\pm}$ or $TSC2^{\pm}$ mice and rats exhibit perturbed dendritic spine structures (Tavazoie et al. 2005) and spatial memory impairment (Dash et al. 2006).

Studies on mutations in the *Drosophila TSC1* and *TSC2* (*gigas*) genes revealed an identical *Drosophila* phenotype characterized by enhanced growth and increased cell size with no changes in ploidy (Potter et al. 2001, Tapon et al. 2001). Thus, although the mammalian models have been and will continue to be useful in studying TSC (El-Hashemite et al. 2004, Ess et al. 2005, Hino et al. 2001, Lee et al. 2005, Meikle et al. 2005, Mizuguchi et al. 2004, Momose et al. 2002, Wilson et al. 2005), the studies in *Drosophila* homologs established the role of *TSC1* and *TSC2* genes in regulating cell size, morphology and proliferation (Gao et al. 2001, Potter et al. 2001). This discovery led to localize the function of the *TSC1* and *TSC2* genes in the PI3K-AKT-mTOR (mammalian target of rapamycin) pathway or AKT pathway paving the way to several investigations on potential therapies (Gino et al. 2006) (see below).

TSC products and their functions (hamartin-tuberin structure)

TSC1 encodes TSC1 (*hamartin*) a 140-kDa protein with no homology to TSC2. *TSC2* encodes TSC2 (*tuberin*), a 200-kDa protein with a GAP domain near the carboxy terminal. Tuberin, through its C-terminal GAP domain, is the major regulator of the small G-protein RHEB and downstream protein translation pathway, essential to the cell growth (Inoki et al. 2003). This C-terminal GAP domain is a frequent target of missense mutations in TSC (Sancak et al. 2005).

Hamartin and tuberin interact physically with high affinity to form heterodimers (Fig. 16), suggesting that they may act in concert to regulate cell proliferation (Ess 2006, Plank et al. 1998, van Slegtenhorst et al. 1998) an observation that is consistent with the similar clinical features of patients harboring either *TSC1* or *TSC2* mutations. A shared motif of these proteins is the coiled-coil domain. These domains mediate protein–protein binding and likely permit hamartin and tuberin to interact, although the exact borders of these domains are not known (Hodges et al. 2001). It appears that the hamartin binding stabilizes tuberin, preventing its degradation (Benvenuto et al. 2000). Additional functions assigned to hamartin are the regulation of cytoskeleton-mediated processes through its interaction with the ezrin-radixin-moesin (ERM) family of actin-binding proteins (Lamb et al. 2000) and with

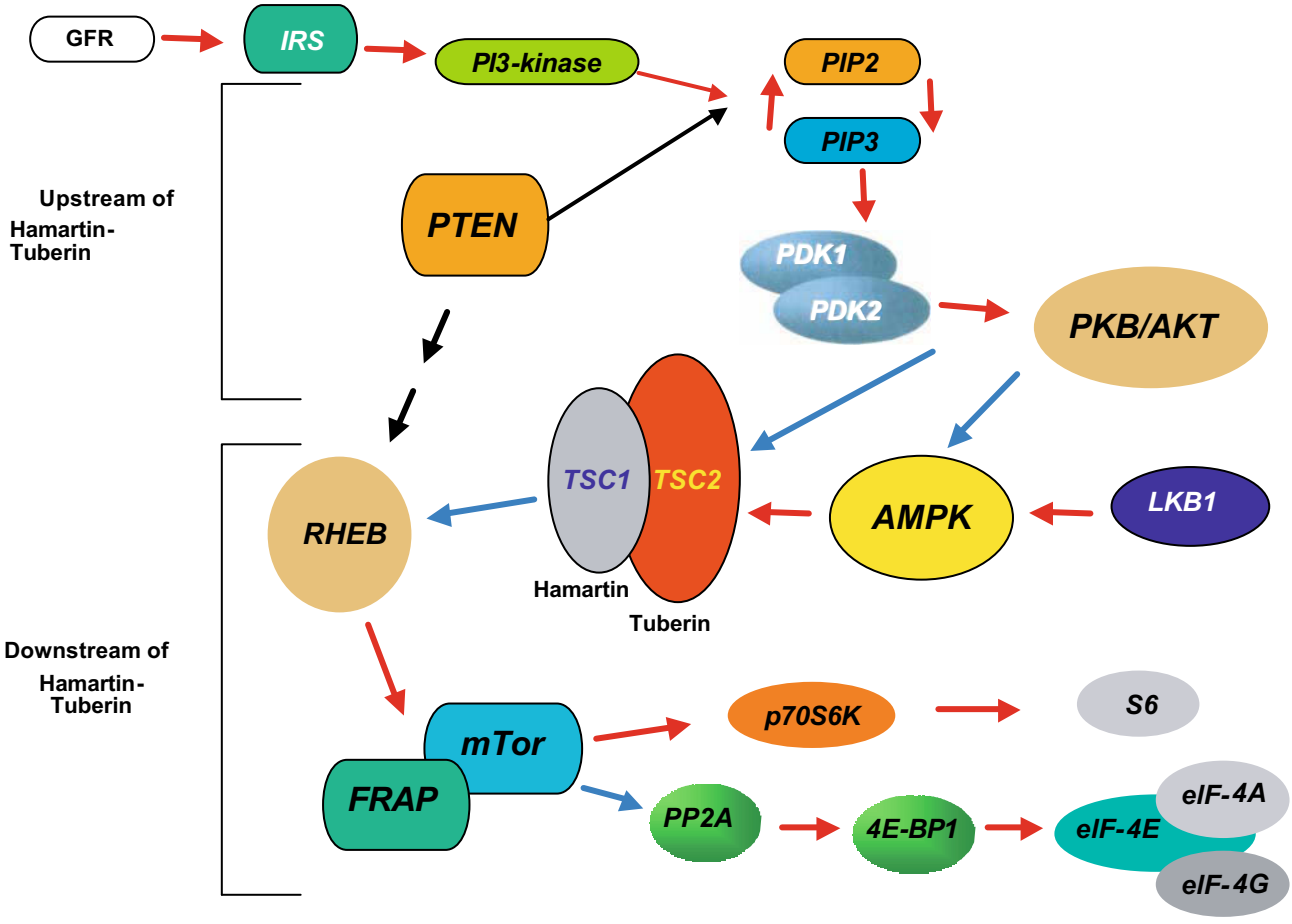


Fig. 16. Upstream and downstream pathways involving hamartin and tuberin functions (see text for explanation). *AMPK* AMP-activated protein kinase; *FRAP* FKBP12-rapamycin associated protein; *GFR* growth factor receptor; *IRS* insulin receptor substrate; *LKB1* serin-threonin protein kinase; *mTOR* mammalian target of rapamycin; *p70S6K* p70 ribosomal S6 subunit-kinase; *PI3-kinase* phosphoinositide 3-kinase; *PIP2* phosphatidytl-inositol (4,5) biphosphate; *PIP3* phosphatidyl-inositol (3,4,5) triphosphate; *PP2A* protein phosphatase 2 A, alpha isoform; *PTEN* phosphatase and tensin homolog; *PDK1* phosphoinositide-dependent kinase 1; *PDK2* phosphoinosititde-dependent kinase 2; *PKB/AKT* protein kinase B/Akt; *RHEB* RAS homolog enriched in brain; *TSC1* tuberous sclerosis complex 1; *TSC2* tuberous sclerosis complex 2; *4E-BP1* eukaryotic initiation factor 4E binding protein 1.

neurofilament-L (Haddad et al. 2002). Moreover, it has been suggested that hamartin regulates the cell cycle through the interaction with CDK (Astrinidis et al. 2003).

Hamartin and tuberin are expressed in all tissues. The intracellular localization of the two proteins is more complex than initially thought (Nellist et al. 1999, Murthy et al. 2000, Yamamoto et al. 2002), i.e., they are not confined to cytosol and nucleus, being detected also in nucleoli and mitochondria (Clements et al. 2006). Moreover, the latter authors, using synchronized cultures of human airways smooth muscle cells, clarified the inconsistent findings on nuclear localization: tuberin and hamartin enter the nucleus at the G1-S phase of the cell cycle (Clements et al. 2006). Tuberin and hamartin have been shown to be key regulators of several cell-signalling pathways including (see Fig. 16): (1) a growth and translation regulatory pathway (PI3K/PKB pathway) involving the "mammalian target of rapamycin" (mTOR) cascade; (2) a cell adhesion/migration/protein transport pathway (glycogen syn-

thase kinase 3 [GSK3]/β-catenin/focal adhesion kinase [FAK]/Ras-related homolog [Rho] pathway); and (3) a cell growth and proliferation pathway (mitogen-activated protein kinase [MAPK] pathway) (Astrinidis et al. 2003, Au et al. 2004, Birchenall-Roberts et al. 2004, Crino et al. 2006, El-Hashemite et al. 2003, Ess 2006, Harris and Lawrence 2003, Kozma and Thomas 2002, Li et al. 2004, Mak and Yeung 2004, Yeung 2003). In most cases the clinical relevance of these TSC1-TSC2 complex interactions is not yet well understood.

Tuberin and Hamartin as growth and translation regulators

The function of tuberin and hamartin in the protein translation cascade involving PI3K/PKB/mTOR is not yet established (Jozwiak et al. 2008).

mTOR

A serine-threonine kinase, mTOR has a central role in the regulation of cell growth and proliferation in response to growth factors, amino acids, and nutrients. First, mTOR phosphorylates and activates the p70S6 kinase 1 to enhance ribosomal protein translation and ribosome biogenesis. Secondly, it phosphorylates and inactivates the eukaryotic initiation translation factor 4E (eIF4E)-binding protein 1 (4E-BP1), the suppressor of protein eIF4E (Fig. 16). Release of eIF4E from phosphorylated 4E-BP1 enables the formation of the eIF4F complex, which is required for cap-dependent translation of mRNAs, such as Cyclin D1 and c-*MYC*, which have extensive secondary structures in their 5´-untranslated region.

AKT, AMPK and PTEN

In normal cells, in presence of insulin or other growth factors, tuberin activity can be suppressed upon via direct phosphorylation (*upstream of hamartin-tuberin*) (see Fig. 16) by AKT protein kinase (also known as PKB). Phosphorylation of tuberin at amino acids serine 939, 981 or threonine 1462 inactivates the tuberin-hamartin complex, likely by disassembling the dimer. Without a functional tuberin-hamartin complex, suppression of S6 kinase 1, mTOR, and translation initiation factor 4E will be released (*downstream of hamartin-tuberin*) (Fig. 16) to facilitate assembly of 40S and 60S ribosomal subunits and other translational initiation factors (A, G and B) on capped messenger RNA to start the protein translation process. Thus, loss of hamartin or tuberin results in increased mTOR-dependent phosphorylation of p70S6 kinase, ribosomal protein S6, and 4E-BP1.

Additional studies revealed that the genetic loss - of the phosphorylated PTEN(a tyrosine phosphatase) in animal models (with biallelic *PTEN* mutations in somatic cells) produced alterations of cell size and proliferation that closely mimicked those seen with loss of the *TSC* genes (Corradetti et al. 2004). These findings support a model in which growth factor binding and receptor activation leads to enhanced PI3-kinase activity that converts phosphatidylinositol 4,5-biphosphate to phosphatidylinositol 3,4,5-triphosphate (*upstream of AKT/hamartin-tuberin*) (Fig. 16). This reaction is reversible and catalysed by PTEN. Loss of PTEN activity then allows the second messenger phosphatidylinositol 3,4,5-triphosphate to increase and activate AKT.

In addition to phosphorylation by AKT, tuberin is also a substrate for the AMPK kinase (see also below) (Inoki et al. 2003). Unlike AKT, tuberin phosphorylation by AMPK potentiates its inhibitory effect on downstream targets. AMPK is inactivated by AKT phosphorylation but is stimulated by the LKB1 kinase (Corradetti et al. 2004). It is worth to note that germline defects of PTEN and LKB1 leads to human diseases that share with TSC features of hamartomatous growth. PTEN defects are associated with Cowden syndrome, and Bannayan-Riley-Ruvalcaba syndrome (Liaw et al. 1997, Nelen et al. 1997, Marsh et al. 1977). A further neurological phenotype caused by a PTEN germline defect is the adult variety of dysplastic gangliocytoma of the cerebellum or Lhermitte-Duclos disease (Liaw et al. 1997). In addition, PTEN is also frequently lost in glioblastoma and

other central nervous system tumours (Bonneau and Longy 2000). Loss of LKB1 causes the autosomal dominant genetic disorder Peutz-Jeghers syndrome (Hemminki et al. 1998). The striking confluence between Cowden/Lhermitte-Duclos syndrome, Bannayan-Riley-Ruvalcaba syndrome, Peutz-Jeghers syndrome and tuberous sclerosis to a common signal transduction pathway suggests that the hamartomatous cell proliferation seen in all these disorders may be due at least in part to the hyper activation of the mTOR pathway. However the severe neurological involvement in TSC suggests that additional roles for the hamartin-tuberin complex remain to be elucidated.

RHEB

So far, most if not all experimentally documented functions of hamartin/tuberin complex of the mTOR pathway appear mediated by RHEB (RAS-homologue expressed in brain) (*downstream of hamartin-tuberin*) (Fig. 16), a member of the RAS-like super-family of GTPases. GTPases cycle between an active GTP-bound state and an inactive GDP-bound state. Tuberin through its GAP domain accelerates the conversion of the active RHEB-GTP to the inactive RHEB-GDP. Loss of tuberin increases Rheb-GTP/GDP and mTOR activation. Since patients with germ-line *TSC1* mutations and those with *TSC2* mutations have similar phenotypes but with significantly different severity, it seems likely that hamartin should have specific roles beyond the before mentioned tuberin stabilization via ethero-dimerization. Tuberin participates in the regulation of tuberin-related GAP activity with respect to Rheb, but its precise role is not yet clear. In addition, other recent findings suggest that the phenotypic findings in TSC might occur secondary to mechanisms other than hyperactivation of S6 kinase 1 through mTOR (Au et al. 2004)

Analysis of surgically resected tubers have revealed cell-specific activation of the mTOR cascade in giant cells, as evidenced by the expression of activated (phosphorylated) components of the mTOR cascade (*downstream of hamartin-tuberin*), including phosphorylated p70S6 kinase and phosphorylated ribosomal protein S6. Since mTOR is a critical regulator of cell size, it is logical to infer that the activation of mTOR is responsible for cytomegaly in tubers and subependymal giant cell tumours. One hypothesis to explain giant cell formation is the second hit mechanism: a neural progenitor cell has one normal *TSC* allele and one existing *TSC* mutation (Fig. 17). A second-hit mutation occurs and there is inactivation of the normal allele so that the cells contain two mutated *TSC* genes. The progenitor cell with two mutations can give rise to a giant cell only or to a mixed population of GC and dysplastic neurons. These cells exhibit abnormal morphology, make aberrant synaptic connections, and migrate to inappropriate cortical layers. In addition, giant cells (or dysplastic neurons) may interfere with migratory pathways of adjacent "normal" neurons containing only one mutated *TSC* gene, and further disrupt the formation of appropriate synaptic connections during brain development (Gino 2004, Gino et al. 2006).

Role of tuberin and hamartin in cell adhesion, migration, and protein trafficking

Loss of heterozygosity at the *TSC1* or *TSC2* locus and hyperphosphorylation of ribosomal protein S6 have been documented in each of the three components of angiomyolipomas (vessels, smooth muscle, and fat), suggesting that all three components arise from a common progenitor and that the tuberin-hamartin complex regulates the differentiation of cells that are derived from mesenchyme (Gino et al. 2006).

Notably, the smooth muscle component of angiomyolipomas is histologically and immunophenotypically identical to the smooth muscle cells of lymphangiomyomatosis. Approximately 60% of women with the sporadic form of lymphangiomyomatosis have renal angiomyolipomas: some of these individuals, in addition, harbor somatic *TSC2* mutations in the abnormal lung and kidney cells but not in the normal cells suggesting that lymphangiomyomatosis and angiomyolipomas are genetically related and most likely arise from a common progenitor cell. These data

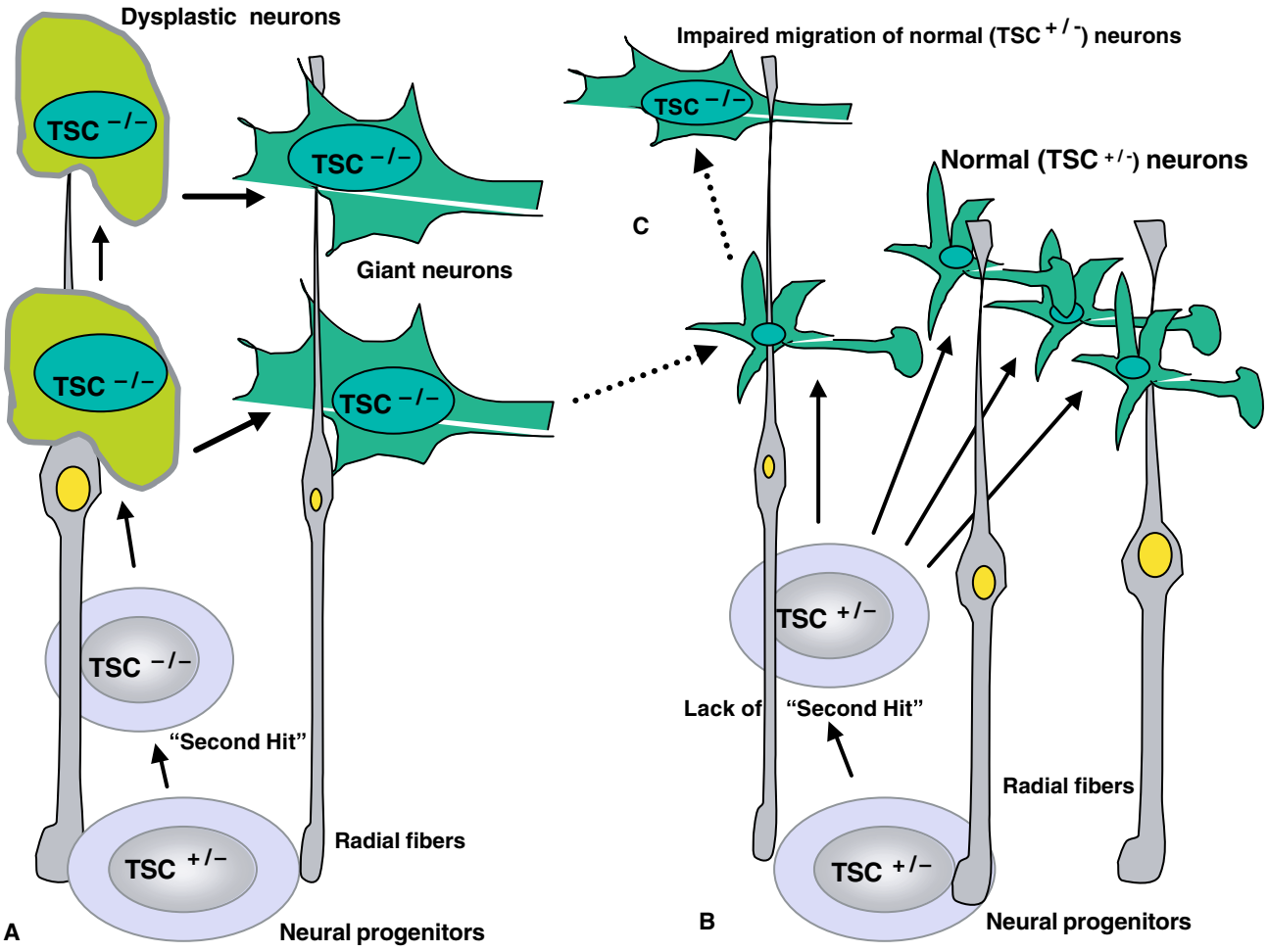


Fig. 17. Diagram showing what occurs to the brain neurons in TSC after the second hit mutations (**A**) and without second hit mutations (**B**) (see text for explanation).

have led to the "*benign metastasis*" hypothesis for the pathogenesis of lymphangiomyomatosis which proposes that histologically benign cells with mutations in *TSC1* or *TSC2* may have the ability to travel to the lungs from angiomyolipomas in the kidney (Gino 2004, Gino et al. 2006). This ability might be linked to an altered (increased in this case) capacity for motility and migration expressed by cells lacking tuberin or hamartin, which is associated with the activation of Rho, a small GTPase that regulates the actin cytoskeleton and focal adhesions. Thus, tuberin and hamartin seems to function to promote cell adhesion and correct migration of precursors cells via the activation of focal adhesion kinase and Rho-guanosine triphosphate. The fact that pulmonary lymphangiomyomatosis occurs only in women has suggested a role for estrogens in regulating TSC signalling and perhaps also cell migration.

Role of tuberin and hamartin in growth and cell proliferation

There is also evidence to indicate that tuberin and hamartin regulate the MAPK signal pathway in controlling cell proliferation. Tuberin phosphorylated at amino acid serine 1210 is mediated by p38 mitogen-activated protein (MAP) kinase, and the

phosphorylated tuberin-hamartin complex is subsequently sequestered from their functional sites by 14-3-3 protein. Notably, the expression of 14-3-3 protein is regulated by tuberin and hamartin, suggesting a tightly interregulated mechanism. On the other hand the tuberin-hamartin complex can function to regulate p42/44 Map kinase activity. Suppressing the phosphorylation of p42/44 MAP kinase results in a decrease in vascular endothelial growth factor production and inhibited growth of TSC2Ang1 tumour cells. Additional roles of tuberin are the upstream regulation of the Erk (Mapk1 and Mapk3) pathway to activate a battery of transcription factors and proto-oncogenes to stimulate cell growth and proliferation (Fig. 17).

Other factors (i.e., platelet-derived growth factor receptor, estrogen receptor, and calmodulin binding) have been suggested to play a role in the function of the tuberin-hamartin complex, suggesting that this complex can be involved in even more cellular functions that have yet to be uncovered.

Natural history

The natural course of the disorder is slow but progressive. All inner organ tumours, except for cardiac rhabdomyomas, enlarge with increasing age. Only cardiac rhabdomyomas usually regress in the first years of life.

Diagnosis

The early recognition of the disease is essential for TSC patients due to: 1) possible devastating effect of visceral lesions requiring frequent control studies, and 2) necessary genetic counselling.

The first diagnostic criteria of TSC were established by the German physician, Vogt, in 1908 (Vogt 1908). This author published the classic diagnostic triad of TSC: seizures, mental retardation and adenoma sebaceum (former term for angiofibromas). However, more recent studies made by M. R. Gomez revealed that all three features of Vogt's triad were found in only 29% of patients, and in 6% of them none of these three findings were observed.

In subsequent years new necessary modifications to the original diagnostic criteria were done. After several editions of proposed sets a consensus conference held at Annapolis in 1998 announced the latest version of diagnostic criteria (Roach et al. 1998). The revised clinical criteria were simplified into two main categories, major and minor, based on the diagnostic importance and degree of specificity for TSC of each clinical and radiographic feature (see Table 1). The definite diagnosis of TSC is established when two major features or one major feature and two minor features are demonstrated.

Despite the long number of clinical features listed in the criteria, the diagnosis of TSC may be extremely difficult early in life. It needs to be understood that the diagnostic criteria in TSC are age dependent. Many features of TSC are absent in infants and become apparent in late childhood or adulthood. Such natural course of TSC limits the value of clinical diagnostic criteria for early diagnosis and prompt management of TSC (Jóźwiak et al. 2000).

In young children or asymptomatic cases genetic testing may now aid in making the diagnosis. Direct sequencing is able to determine the causative mutation in either *TSC1* or *TSC2* gene in approximately 80% of cases (Cheadle et al. 2000). However, universal use of molecular genetics testing for diagnosis of TSC has thus far not been feasible because of genetic variation, limited availability, undetermined sensitivity, and high costs. Therefore, the role of thorough skin, full eye and neurological evaluation coupled with nervous system and systemic imaging will remain essential to the diagnosis of TSC.

Differential diagnosis

Different disorders should be considered in differential diagnosis of TSC. Facial angiofibromas in teenager should be differentiated with acne vulgaris. Depigmented spots should be differentiated with vitiligo or isolated (not-TSC associated) depigmentations. Renal cysts should be differentiated with PKD1.

Treatment, follow-up and management

Presently there is no available causative treatment for TSC. There are two main symptomatic treatment strategies required in the majority of patients with TSC: epilepsy treatment and management of visceral tumours and their complications.

There is no specific treatment of epilepsy in TSC, except for the good effect of vigabatrin in infantile spasms (Curatolo et al. 2005). Due to the increasing number of patients with diagnosed prenatally, one could consider the chance to start antiepileptic treatment very early. Jozwiak et al. (2007) proposed that in order to prevent mental decline in the first years of life, all young infants with active epileptic discharges on EEG should be proposed for an antiepileptic treatment, even before the onset of clinical seizures. Recently, evidence is growing for the high effectiveness of surgical treatment of epilepsy in tuberous sclerosis (Curatolo et al. 2006).

Early identification of visceral lesions in patients with TSC may help their effective management. Due to their possibly life threatening complications renal, cardiac and cerebral lesions are paid particular attention. The Tuberous Sclerosis Consensus Conference held in Annapolis in 1998 developed the latest recommendations for diagnostic follow up of internal lesions (Roach et al. 1999) (Table 2).

Large renal angiomyolipomas (>4.5 cm) may require surgery. Embolization and/or renal sparing surgery are treatment options currently available.

As cardiac tumours regress with age, they usually do not require surgery.

Special attention should be paid to subependymal giant cell astrocytomas (Madhavan et al. 2007, O'Callaghan et al. 2008). They should be early removed as prolonged intracranial hypertension frequently causes optic atrophy and blindness. This is a special problem in mentally handicapped children as in this group of patients the symptoms of growing tumour may be easily overlooked.

Disfiguring dermatological lesions deserve special attention, especially in young patients without mental retardation. The treatment methods currently available for patients with facial angiofibromas include cryosurgery, dermabrasion, chemical peeling, excision and laser. If lesions affect large areas of the face, dermabrasion is a very effective treatment. Surgical excision is a reasonable option only in case where few lesions are present. During the last decade, lasers have become a popular treatment option.

The discovery of the rapamycin effects on growth inhibition of TSC lesions in animal models of the disease raised the possibility of using this drug for treatment of TSC patients (Zeng et al. 2008). Preliminary results of few clinical trials are promising (Bissler et al. 2008, Davies et al. 2008, Paul et al. 2008) showing regression of angiomyolipomas during therapy with tendency of increase of volume after the therapy was stopped and improvement of spirometric measurements and gas trapping that persisted after treatment.

Table 2. Testing recommendations (according to Roach et al. 1999)

Assessment	Initial testing	Repeat testing
Neurodevelopmental testing	at diagnosis and at school entry	as indicated
Ophthalmic examination	at diagnosis	as indicated
EEG	if seizures occur	as indicated for seizures management
ECG	at diagnosis	as indicated
Echocardiography	if cardiac symptoms occur	if cardiac dysfunction occurs
Renal ultrasonography	at diagnosis	every 1–3 years
Chest computed tomography	at adulthood (women only)	if pulmonary dysfunction occurs
Cranial computed tomography*	at diagnosis	children/adolescents: every 1–3 years
Cranial MRI*	at diagnosis	children/adolescents: every 1–3 years

* Either cranial CT or MRI , but usually not both.

Genetic counselling

TSC is inherited in an autosomal dominant manner. About one-third of probands with TSC have an affected parent. Overall, two-thirds of TSC probands carry a de novo mutation in either TSC gene (Northrup and Au 2006). More precisely, a denovo mutation occur in approximately 80% of TSC2 and 50% of TSC1 probands.

Risk to family members: parents of a proband

Recommendations for the evaluation of parents of a child with no apparent family history of TSC include thorough skin examination, retinal examination, brain imaging, renal ultrasound examination, and molecular genetic testing if the disease-causing mutation has been identified in the proband. Molecular genetic testing plays an important role whenever asymptomatic parents of a TSC child, or other family members, desire to plan a pregnancy. In these cases, gene testing is particularly helpful if the parents are healthy and wants to determine their own TSC genotype without undergoing extensive diagnostic work-up. Unaffected parents have a 12% chance of having gonadal mosaicism (Verhoef et al. 1999). A careful clinical investigation of the parents, or genetic testing, may disclose TSC lesions missed by previous controls by health care professionals because of a milder phenotypic presentation. Therefore, an apparently negative family history, or a negative physical examination by physicians with no direct experience on TSC should be confirmed by appropriate evaluations.

Risk to family members: sibs of a proband

The risk to the sibs of the proband depends on the genetic status of the parents: if a parent is affected or has the disease-causing mutation identified in the family, the risk to the sibs is 50%; if neither parent has any findings indicative of TSC or if neither parent has the disease-causing mutation detectable in DNA extracted from leukocytes, sibs of a proband have a 1 to 2% recurrence risk because of the possibility of gonadal mosaicism.

Risk to family members: offspring of a proband

Each child of an individual with TSC has a 50% chance of inheriting the mutation.

Risk to family members: other family members of a proband

The risk to other family members depends upon the genetic status of the proband's parents. If a parent is found to be affected or to have the disease-causing mutation, family members of the parent are at risk.

High-risk pregnancies

Prenatal diagnosis by chorionic villi sampling (CVS) at the 10–12th week of gestation should be offered to high risk couples with an ongoing pregnancy if the disease-causing mutation has been previously identified in one affected relative. The process of *TSC1* and *TSC2* mutation screening usually requires a few months, thus the testing should initiate far in advance of the desired pregnancy. On the other hand, the search for a known mutation can be completed in a week. For families who present too late for a prenatal TSC test high-resolution ultrasound examination for cardiac rhabdomyomas can be performed in a reference center at the 20th week of gestation, followed by a second examination at the 28th week. Even in the best centers using the highest resolution available, cardiac rhabdomyomas are rarely detectable earlier than the 20th week. Moderately large brain displastic areas, particularly if associated to ventricular asymmetries can be revealed by fetal MRI, thus this investigation may be offered to high risk pregnancies. However, the parents must be informed that normal results of the heart and brain fetal examinations do not significantly reduce the a priori probability to be a carrier of a TSC gene defect. Pre-implantation genetic diagnosis is available and has been utilized by families in which the disease-causing mutation had been previously identified.

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