Advances in the understanding of tuberous sclerosis

Finbar J O’Callaghan, John P Osborne

Medical textbooks still define tuberous sclerosis (TSC) as a triad of clinical features, mental retardation, epilepsy and “adenoma sebaceum”, first described by Vogt in 1908. Not surprisingly therefore many clinicians still do not realise that half of TSC sufferers will have normal intellect, a quarter will not have epilepsy, and almost any organ in the body can be affected. Recent advances in molecular genetics and imaging have begun to unravel the complexity of the disease, explaining the mechanisms behind the clinical features and providing insights into how these patients should be managed.

Genetic heterogeneity of TSC

Gunther and Penrose described the autosomal dominant pattern of inheritance of TSC in 1935. We now know that there are two genes (and probably only two) causing the TSC clinical phenotype. TSC1 is found on the long arm of chromosome 9, and its protein product is called hamartin. The TSC2 gene’s protein product is named tuberin and it is situated on the short arm of chromosome 16, only 48 base pairs of DNA from the gene for adult onset polycystic kidney disease (PKD1). This genetic heterogeneity in TSC raises the question of whether the clinical syndrome produced by the two different genes is the same. In some conditions, neurofibromatosis for example, genetic heterogeneity helps explain clinically distinct forms of the disease. However, all the complications of TSC have been seen in both TSC1 and TSC2 except for the contiguous gene deletion syndrome (see below). There may, however, be subtle differences in the phenotype produced by the two genes or by specific mutations. There is already some evidence from case series that mutations in TSC2 tend to produce more severe disease than TSC, but this needs to be confirmed in larger less biased studies. We may then see no significant differences in the phenotypes because hamartin and tuberin are thought to function as part of, or at adjacent steps within, the same intracellular pathway.

Function of the genes

We know that both tuberous sclerosis genes are tumour suppressor genes. Their function is to help regulate cell growth and differentiation. When altered, by mutation, control of cell growth is disturbed and tumours form throughout the body. The strongest evidence for the tumour suppressor hypothesis was provided in 1994 when it was reported that some of the hamartomas of tuberous sclerosis patients showed loss of heterozygosity, either in the chromosomal region 9q34 or in 16p13. Markers on 9q34 or 16p13 that were heterozygous in the patient were homozygous in the tumour. The loss of heterozygosity implies that an individual with tuberous sclerosis inherits or acquires through an early embryonic mutation, a deletion in one copy of the gene but only develops lesions when there is a somatic mutation in the other previously normal copy. This two hit mechanism was first proposed by Knudson to explain the pathogenesis of retinoblastoma.

The tumour suppressor hypothesis is further strengthened both by recent research in the Eker rat (an animal model of tuberous sclerosis), and by studies of the possible intracellular function of tuberin. The Eker rat, which has a mutation in the rat homologue of the TSC2 gene, suffers from dominantly inherited renal cell carcinoma and subependymal and subcortical hamartomas. Reintroduction of a wild type TSC2 gene suppresses the development of renal tumours in this model. A search for sequence homologies at the protein level has revealed a region of similarity between tuberin and the GTPase activating protein GAP3. The GTPases are known to be involved in the regulation of cell proliferation and differentiation and it is thought that tuberin may have a role in mediating this activity.

Contiguous gene deletions in TSC

Renal cysts are common in TSC. They occur in a substantial minority of patients and have been associated with both TSC1 and TSC2 disease. A particularly severe phenotype has been observed in some patients (very early onset polycystic kidney disease). This is caused by a contiguous deletion that affects both TSC2 and PKD1 genes. It is sensible to perform renal ultrasonography on all newly diagnosed children with TSC to identify this rare problem early. An alternative is to monitor blood pressure and serum creatinine.

Mosaicism in TSC

Some of the patients with contiguous deletions of the TSC2 and PKD1 genes have a milder
clinical course than others and this is, in some cases, caused by the fact that these patients are somatic mosaics for the contiguous gene deletion. Somatic mosaics have two or more cell lines, and only one cell line possesses the disease causing mutation. Somatic mosaicism has been described in both TSC1 and TSC2 patients with and without the contiguous TSC2-PKD1 syndrome. The severity of disease in mosaics is variable but can be severe.

If the abnormal cell line is confined to the gonad (gonadal mosaicism) then a phenotypically normal parent has a high risk of producing affected offspring (after full investigation) having further affected offspring after the birth of one child with TSC.2

**Cranial imaging and TSC**

The advent of magnetic resonance imaging (MRI) has allowed clinicians to image many of the lesions of TSC with greater clarity. MRI has allowed clinicians to image many of the lesions of TSC with greater clarity. MRI also has the advantage of not exposing the individual to x-rays. Theoretically radiation could cause a “second hit” in the second copy of the gene. Cerebral tubers are shown on T2 weighted images but may be more easily visualised on a fluid attenuation inversion recovery sequence (FLAIR). The number of tubers is related to degree of learning difficulty but variation in the relationship does not allow prediction for the individual. Lesions in the temporal lobe have been shown to increase the risk of autism in TSC. However, MRI does not show the subependymal lesions that are pathognomonic of the condition as clearly as computerised tomography (CT). Therefore it is important that cranial CT is used when an individual is being investigated for a possible diagnosis of TSC.

**Screening in TSC**

There are common hamartomas that can develop potentially life threatening consequences. In the kidney, renal angiomyolipomas are common in affected individuals but infrequently cause serious problems (haemorrhage into renal substance, collecting system, or retroperitoneally). They are usually easily seen as hyperechoic lesions on renal ultrasound, but CT is required to confirm the fat content of any hyperechoic lesions which might otherwise be a renal cell carcinoma (a very rare but serious complication of TSC: such a lesion must be shown to be HMB 45 negative on biopsy before nephrectomy is performed or an angiomyolipoma may inadvertently be removed). In the brain, the giant cell astrocytomas occur in approximately 5% of patients. They arise in the lateral ventricles close to the Foramen of Monro (or rarely in the fourth ventricle), giving rise to signs and symptoms of raised intracranial pressure. Untreated this will cause hydrocephalus, blindness, and ultimately death. No feature of a giant cell astrocytoma on imaging has yet been shown to differentiate it reliably from a subependymal nodule—it is obstruction of CSF flow which causes symptoms and which must be treated.

Diagnosis of TSC

Mutanational analysis is expensive and many mutations cannot yet be detected as it is usual for mutations to be unique to each family or individual. For prenatal diagnosis of offspring of affected individuals, it is now possible to request this. But for diagnosis, clinical skill is still often required. The old method of minor and major criteria is more logically replaced by examining and investigating for hamartomas. These are individually rare, so the finding of two or more “independent” hamartomas (table 1) strongly suggests the diagnosis. Individuals with a single hamartoma might have developed this through the random occurrence of two somatic mutations in a cell in that organ—but that is unlikely to occur twice in different

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<thead>
<tr>
<th>Table 1</th>
<th>Lesions which are individually rare</th>
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<tr>
<td>Facial angiofibromas or forehead plaque</td>
<td>Periungual fibromas</td>
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<tr>
<td>Shagreen patch</td>
<td>Retinal hamartoma</td>
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<tr>
<td>Cortical hamartoma</td>
<td>Subependymal nodule</td>
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<tr>
<td>Giant cell astrocytoma</td>
<td>Cardiac rhabdomyoma</td>
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<td>Renal angiomyolipoma or pulmonary lymphangiomyomatosis</td>
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When two of these lesions are found in one individual, the diagnosis of TSC is beyond reasonable doubt. In most affected individuals it is possible to find evidence of two such lesions.
organs in the same individual without an inherited or early embryonic mutation. In practice, it is usually easy to find two hamartomas in affected individuals. For this purpose, angiomyolipomas and pulmonary lymphangiomyomatosis have to be considered dependent hamartomas as these two lesions often occur together in the absence of other proof of TSC (see table 1). To exclude the disease in an individual, a full clinical examination including fundoscopy and cranial imaging must be undertaken: renal imaging probably confuses the diagnosis of the tuberous sclerosis gene TSC1 on chromosome 16p13.3 in hamartomas from tuberous sclerosis patients. Nat Genet 1994;6:193-6.