

## TUBEROUS SCLEROSIS COMPLEX: FROM GENES TO BEHAVIOURAL AND COGNITIVE PHENOTYPES

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### ABSTRACT

*Tuberous sclerosis complex (TSC) is a disorder of cell migration, proliferation, and differentiation, resulting from mutations in TSC1, the gene on chromosome 9q34, and in TSC2, the gene on chromosome 16p13. Cortical tubers constitute the hallmark of the disease and are pathognomonic of cerebral TSC. The number and the localisation of cortical tubers may account for the variability in the neurobehavioural phenotype observed in affected individuals. Symptoms of cortical tubers include seizures, mental retardation, learning disabilities, attention deficit disorder with hyperactivity, and autism. Patients with TSC range from intellectually normal to severely retarded. The prevalence of learning disabilities ranges from 38 to 80 per cent. Children with normal intelligence may have different specific neuropsychological deficits related to the strategic location of small, isolated cortical tubers, even when they are seizure-free. Studies of TSC children have identified rates of autism ranging from 17 to 61 per cent. The cause of this association remains unknown. Various mechanisms may be implicated in the genesis of behavioural and cognitive phenotypes in TSC patients. Recently, several genome-wide scans have suggested that a region on chromosome 16p13 shows a significant linkage to autism. New advances in molecular genetics and functional neuroimaging techniques will provide new insights into the neurobiological bases of the behavioural and cognitive phenotypes associated with this disease. (Int. J. Ch. Neuropsychiatry, 2005, 2(1): ??-??)*

### INTRODUCTION

Tuberous sclerosis complex (TSC) is a multisystem, autosomal dominant genetic disorder which primarily affects the brain, skin, kidneys, eyes, and heart. The unpredictable distribution of the lesions results in a broad range of clinical phenotypes, with variable expression even in the same family <sup>(1)</sup>. The disease affects

1/10,000 births. About two thirds of all cases of TSC are sporadic, indicating the occurrence of new mutations.

### GENETICS

TSC results from mutations in TSC1, the gene on chromosome 9q34, and in TSC2, the gene on chromosome 16p13 <sup>(2)</sup> <sup>(3)</sup>.

The TSC1 gene consists of 23 exons, of which the last 21 contain the coding sequence. The TSC product, which is called hamartin, consists of 1164 amino acids with a calculated mass of 130 kilodaltons (kd). The protein is generally hydrophilic and has a single transmembrane domain at amino acids 127–144, as well as a probable 266 amino acid coiled-coil region beginning at position 730<sup>(3)(4)</sup>.

The mutations observed in TSC1 consist of small deletions, small insertions, and point mutations. The majority of mutations are likely to inactivate the protein function, and this finding supports the hypothesis that TSC acts as tumour suppressor gene.

TSC2 gene has a complex genomic structure containing 41 coding exons and non-coding leader exons which are distributed over 44 kb of the genome. The sequence predicts a protein product of 1807 amino acids called tuberin. Near the COOH terminus, approximately 160 amino acids are homologous to the catalytic domain of a guanosine triphosphatase (GTPase) activating protein GAP3 (rap1GAP). GAPs are regulators of the GTP binding and hydrolysing activity of the Ras superfamily of proteins that help to regulate cell growth, proliferation, and differentiation. Recent evidence suggests that hamartin and tuberin directly interact, forming a cytosolic complex; this interaction is abolished by some TSC associated mutations<sup>(5)(1)</sup>.

At present, there are more than 500 different reported mutations. There is an equal distribution of mutations between TSC1 and TSC2 among familial cases, while TSC2 mutations are about five times more common than TSC1

mutations in sporadic cases. These observations suggest that patients with TSC1 mutations may be less severely affected than those with TSC2 mutations. In a recent analysis of unselected patients with TSC, it was reported that TSC1 subjects had a lower seizure frequency, a lower percentage of severe mental retardation, a smaller number of subependymal nodules and cortical tubers, less severe kidney involvement, and a lower frequency of facial angiofibromas than TSC2 patients. Interestingly, TSC2 mutations are associated with a significantly earlier presentation of epilepsy, mainly infantile spasms<sup>(6)</sup> (Table 1). Parents with more than one child affected by TSC and no clinical manifestations of the disease, are more likely to have germline mosaicism than non-expression of the mutation.

## NEUROPATHOLOGY

Pathologically, this disease is characterized by the widespread development of hamartias, or non-growing lesions, and hamartomas – benign tumours that rarely progress to malignancy. Abnormalities of neuronal migration and cellular differentiation along with excessive cell proliferation contribute to the formation of the various brain lesions observed in TSC<sup>(7)</sup>. Cerebral lesions include cortical tubers, subependymal nodules, radial hypomyelinated tracts extending from subependymal area to the cortex, and giant cell astrocytomas.

Cortical and subcortical tubers are hamartias that are located mainly in the cerebral cortex and in the underlying white matter. Subependymal nodules

(SEN) are hamartomas that are typically seen in the subependymal wall of the lateral ventricles, mainly at the foramina of Monro.

demonstration of the various pathological lesions. Cortical tubers detected by T2 weighted MRI as high intensity signal areas constitute the hallmark of the disease and are pathognomonic of cerebral TSC. Tubers can be detected in the foetal brain as

Magnetic resonance imaging (MRI) studies provide excellent *in vivo*

early as 20 weeks of gestational <sup>(8)</sup>. The number and localisation of cortical tubers may account for the variability of the neurological phenotypes including seizures, mental retardation, learning disabilities, attention deficit disorder with hyperactivity, and autism.

Table 1. Genotype-phenotype correlations in sporadic TSC1 vs TSC2 [from Dabora *et al.*, 2001 (modified)]

	<b>TSC1</b>	<b>TSC2</b>
<b>Seizures</b>	86%	99%
<b>Mental retardation</b>	46%	72%
<b>Moderate to severe mental retardation</b>	14%	46%
<b>SEGA</b>	5%	11%
<b>Mean tuber number</b>	4	13

SEGA, subependymal giant cell astrocytomas; TSC, tuberous sclerosis complex.

Table 2. Behavioral phenotypes in tuberous sclerosis complex

Hyperactivity	61%
Sleep disorders	60%
Peer problems	59%
Autism	50%
Emotional problems	38%
Aggressiveness / conduct disorder	37%
Social withdrawal / language abnormalities	30%

## COGNITIVE PHENOTYPES

Patients with TSC range from intellectually normal to severely retarded. In the past several studies have reported a prevalence of learning disabilities in TSC patients ranging from 38 to 80 per cent. The intellectual impairment, when present, was moderate or severe in degree. However, in recent years it has been postulated that the frequency of cognitive delay was overestimated owing to referral bias. A distinct set of factors may lead to mental retardation in TSC. One possibility concerns the nature of the TSC mutations. Individuals with TSC2 mutations may have

a more severe neurobehavioural and cognitive phenotype than those with TSC1 mutations. The reason for this is still unknown. It is possible that the TSC2 gene is more often involved in second hit somatic mutations or that TSC2 mutations have a dominant negative effect determining more extensive brain involvement, which is likely to lead to more severe intellectual impairment<sup>(9) (6)</sup>. The second hypothesis is that epilepsy may have an adverse effect on intellectual development, especially if seizures are very severe and frequent<sup>(10)</sup>. Children with infantile spasms and hypsarrhythmia are reported to be more severely affected than those with other types of seizure<sup>(11) (1)</sup>. Vigabatrin may reverse infantile spasms,

and the cessation of spasms is associated with significant improvement in cognition and behaviour in children with TSC<sup>(12)</sup>. The question arises as to whether seizures cause mental retardation or whether mental retardation and seizures in children with TSC are two different aspects of the same underlying brain dysfunction.

Another possibility is that cortical tubers and subependymal nodules give rise to severe or profound mental retardation when they are numerous or if they involve certain key brain regions or structures. Several reports have shown that the degree of mental disability is associated with the number of cortical tubers detected by MRI. For this reason, the relation between the presence and the degree of mental retardation and number of cortical tubers has been investigated. Although there was a considerable variation in the mental function of patients with five or fewer cortical lesions, the development of all patients with 10 or more cortical lesions was severely impaired<sup>(13) (14)</sup>. Curatolo *et al.*<sup>(15)</sup> have suggested that both the number and the localisation of cortical tubers may play an important role in mental outcome, and that epilepsy and mental retardation probably reflect the underlying brain dysfunction caused by cortical tubers. TSC patients with normal intelligence often develop specific neuropsychological deficits including dyspraxia, speech delay, visuo-spatial disturbance, memory impairment, and dyscalculia<sup>(15)</sup>. These children had small, isolated cortical tubers, mainly localized in the parietal and rolandic regions, and less severe epilepsy. By contrast, patients with stable mental retardation showed multiple bilateral cortical tubers on MRI and suffered from frequent partial seizures, developing multifocal or secondary generalized epilepsy. The progressive mental deterioration observed in TSC children with intractable seizures may also reflect the intrinsic epileptogenicity of parasagittal frontal tubers. The relation between the localisation of cortical tubers and the degree of intellectual impairment is still unclear. It is possible that the extent of frontal involvement in TSC would be most closely correlated with the degree of cognitive impairment<sup>(16)</sup>. As the number of tubers is determined very early on in the gestational

period, it is likely that extensive brain disruption may predetermine which individuals have a poor mental outcome.

## BEHAVIOURAL PHENOTYPES

In addition to mental retardation, sleep disorders, hyperactivity, attention deficit, aggressiveness, and autism have been found in children with TSC (15) (17) (Table 2). Sleep disorders are considered one of the most common behavioural manifestations in these patients (18). Sleep organisation of TSC patients is characterized by a reduced REM sleep, sleep instability, and fragmentation of the awake/sleep cycle owing to frequent awakenings. Children with seizures show a more disrupted sleep architecture than seizure-free children. Thus sleep disorders seem to be mainly caused by sleep related epileptic events (19). In children with TSC a high rate of hyperactivity with attention deficit disorder has been reported. It is not yet clear if hyperactivity reflects a primitive impairment of the brain or is a consequence of the seizure disorder or its treatment. Smalley *et al.*<sup>(20)</sup> suggested that a susceptibility locus for ADHD is located on chromosome 16p13.3. An association of TSC and autism is based on the joint occurrence of these two relatively rare disorders. Different studies have shown that three to four per cent of autistic subjects may have TSC. Conversely, studies of TSC patients have identified high rates of autism, ranging from 17 per cent to as high as 61 per cent. The pathogenesis of autism in TSC is still unknown. In patients with TSC, autism could be a consequence of any of the following:

- the effect of seizures and/or EEG abnormalities;
- a spectrum of behaviours associated with mental retardation;
- a generalized defect in brain development;
- the location of the cortical and subcortical tubers and/or subependymal nodules;
- the direct effect of an abnormal genetic program.

Among autistic TSC children reported as having autistic spectrum disorders there is a high incidence of infantile spasms and cognitive delay, raising the question of cognitive defects as the primary cause of autism. Early onset generalized epilepsy or epileptiform EEG discharges may have an effect on cognitive function that results in autistic behaviour. However, the presence of infantile spasms in non-autistic TSC patients and the large number of autistic TSC patients without infantile spasms suggest that there may be some other aspects of TSC predisposing to autism or autistic behaviour (1). Because of the presence of easily identifiable cortical lesions in TSC, several studies of autism in TSC have attempted to correlate the behavioural disorder with the location of cortical tubers. Curatolo *et al.* (15) found that patients with TSC and autism with early onset (before the age of 2 years) presented prevalent parieto-temporal cortical lesions, while those with later onset had both frontal and posterior tubers, suggesting that an early dysfunction in the associative areas may be responsible for autistic features. A strong association between temporal tubers and autism in 18 TSC patients was found by Bolton & Griffiths in 1997 (21). Seri and coworkers (22) reported seven autistic patients with temporal lobe lesions and abnormalities of auditory evoked potentials. Weber *et al.* (23) observed an increased number of cerebellar tubers in TSC patients with higher CARS (childhood autism rating scale) scores. Recently, PET studies showed an abnormal metabolism in the lateral-temporal gyri, in the deep cerebellar nuclei, and in the caudate nuclei in TSC children with autism, suggesting that autism may be related to both cortical and subcortical dysfunction (24). An alternative explanation is that autism in TSC reflects more direct effects of an abnormal genetic program. The TSC2 gene product tuberin is highly expressed in frontal and temporal regions, which are the brain areas potentially involved in the behavioural phenotypes of the autistic disorder. Moreover, two TSC patients affected by autism showing 16p13

chromosomal microduplication have been reported (25) (26). Recently the terminal two megabases of the short arm of human 16 chromosome have been sequenced. We have found that a susceptibility locus for autism may exist on chromosome 16p13 (27). The genetic dissection of the short arm of the chromosome 16 in autism will help to localize a susceptibility gene more precisely and clarify its position with respect to the TSC2 locus.

## CONCLUSIONS

Despite the great deal of progress in the last few years, the neurobiological basis of cognitive and behavioural phenotypes in TSC is still poorly understood. Neurobehavioural phenotypes seem to be associated with the nature of the genetic mutation, the extent of brain abnormalities, and the age of onset and type of seizure disorder. The degree to which these factors, independently or jointly, contribute to determining neurological phenotype is still unknown. Improvement in structural and functional imaging techniques and genotype-phenotype correlation studies of a large population may provide new insight into the neurobiological basis of the cognitive and behavioural phenotypes associated with tuberous sclerosis.

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