

Tuberous sclerosis complex: A *Drosophila* connection

Recent findings based on experiments with *Drosophila melanogaster* significantly advance our understanding of a human disease known as tuberous sclerosis complex (TSC). The present note begins with background information and goes on to explain what these findings are.

Tuberous sclerosis complex is a neuro-cutaneous genetic disorder which affects several organs in the human body including the brain, heart, kidneys, eyes, skin, spleen, liver and lungs. TSC is characterized by hamartomas (benign outgrowths, predominantly of a cell or tissue type that occurs normally in the organ) which rarely change to malignancy in the affected organs. Clinical symptoms of TSC include cortical tubers in the brain, seizures, mental retardation, autism, unguis and periungual fibromas, shagreen patches and hypopigmented macules on the skin, angiofibromas on the face, micro or macrocysts and angiomyolipomas in kidneys, cardiac rhabdomyomas, retinal hamartomas, hyper-inflated lungs and dental pits. In a majority of cases clinical symptoms are not evident or overlooked. Seizures are the most frequent clinical symptoms and are present in 86% of TSC patients. Patients who had frequent seizures in infancy develop mental retardation to some extent during the later part of their lives; approximately 55% of TSC patients are mentally retarded. A definite diagnosis of TSC is based on the presence of any one of the following symptoms: cortical tubers or subependymal nodules in the brain as diagnosed by computerized tomography (CT) and/or magnetic resonance imaging (MRI), retinal hamartomas, facial angiofibromas, unguis fibromas, and angiomyolipomas with or without cysts in kidneys as diagnosed by ultrasound, CT scan or MRI (NTSA 1988). A patient may have only one, some or all of the above symptoms. The estimated prevalence of TSC is 1 in 5,000–6,000 live births in western populations, and it occurs in all racial groups. The actual incidence of TSC is not known in India, but it seems to be as common as in western countries. It is estimated that approximately two-thirds of TSC cases are either sporadic or new mutations (i.e. with no previous family history).

TSC is apparently an autosomal dominant disorder which exhibits both incomplete penetrance and variable expression. It shows genetic heterogeneity with two known loci: TSC1 on chromosome 9q34 mapped in 1987 and TSC2 on chromosome 16p13-3 mapped in 1992. Multi-generation families are evenly divided between both loci. Patients with mutations in *TSC1* and *TSC2* genes cannot be separated clinically, suggesting that both genes work probably in the same pathway. The *TSC1* gene, which was isolated in 1997, has 23 known exons (exons 1 and 2 being non-coding) with an approximately 8.6 kb long transcript including an unusually long 4.5 kb 3' untranslated region (3' UTR) with five non-overlapping polyA signals (AATAAA or ATTAAA). The significance of a long 3' UTR and five polyA signals is not yet obvious. *TSC1* encodes a protein, hamartin, of 1164 amino acids (130 kDa protein) with a single potential transmembrane domain at amino acids 127–144 coded by exon 6 and a predicted 266 amino acids long coiled-coil domain (beginning at amino acid residue 730) coded by exons 17–23. The GenBank search shows a possible yeast homologue of *TSC1* encoding a protein of 103 kDa with unknown function.

The *TSC2* gene, which was isolated in 1993, has at least 42 known exons (41 coding and one non-coding leader exon 1a) and codes for a 5.5 kb long transcript with two overlapping polyA signals. The *TSC2* gene encodes a protein, tuberin of, 1784 amino acids (198 kDa protein) which shows stretches of homology of approximately 160 amino acid residues (encoded by exons 34–38) to a GTPase activating protein (GAP) GAP3 (or rap1GAP), a protein involved in signalling pathways. In fact, the eukaryotically expressed tuberin protein has been shown to have a rap1GAP activity *in vitro*. Further, tuberin and hamartin associate physically *in vitro*, suggesting that both proteins play a closely related role. Tuberin has two small coiled-coil domains at amino acids 346–371 and 1008–1021 coded by exons 10

and 26, respectively. Rap1GAP is known to stimulate the hydrolysis of active GTP-bound rap1a and rap1b to their inactive GDP-bound forms. Both rap1a and rap1b are members of the ras superfamily of small GTP-binding proteins whose functions include the transduction of mitogenic signals from plasma membrane receptors to the nucleus (Cheadle *et al* 2000). By converting GTP-binding proteins to their inactive forms, GAPs can function as negative regulators of cellular processes including cell proliferation (Cheadle *et al* 2000). Other important domains in tuberin include four potential tyrosine kinase phosphorylation sites that could be involved in signalling and a leucine zipper consensus domain from amino acids 81–102 coded by exon 3 (Cheadle *et al* 2000). Tuberin does not seem to have any transmembrane domain. Alternate splicing of exon 25, the first 3 bp of exon 26 and exon 31 have been shown in human, mouse, rat and *Fugu* (Cheadle *et al* 2000). Both *TSC* genes are expressed widely in different tissues. The loss of heterozygosity (LOH) at *TSC1* and *TSC2* loci in *TSC*-associated hamartomas (tumours), and patients with germline mutations showing somatic mutations in hamartomas, suggest that both genes function as tumour suppressors. In other words, although the disease seems to display an autosomal dominant mode of inheritance in pedigrees, the hamartomas develop due to the loss of gene function of both alleles in a somatic cell. Lines for *Tsc1* and *Tsc2* knockout mice have been established by gene targeting (Onda *et al* 1999; Kobayashi *et al* 2001). Heterozygous *Tsc1* mutant (*Tsc1*^{+/}/*Tsc1*⁻) mice developed renal and extra-renal tumours such as hepatic hemangiomas with the loss of wild-type *Tsc1* allele in tumours, and homozygous mutant (*Tsc1*⁻/*Tsc1*⁻) mice died *in utero* (Kobayashi *et al* 2001). *Tsc2* heterozygotes (*Tsc2*^{+/}/*Tsc2*⁻) demonstrate incidence of multiple bilateral renal cystadenomas, liver hemangiomas, and lung adenomas by 15 months of age, and similar to *Tsc1* null embryos *Tsc2* homozygotes died *in utero* (Onda *et al* 1999). Thus, both *Tsc1* and *Tsc2* have a role akin to that of classical tumour suppressor genes.

Mutation analysis of patients has revealed a total of 337 mutations in both genes: 105 in *TSC1* and 232 in the *TSC2* gene (Gilbert *et al* 1998; Cheadle *et al* 2000). Of these 337, 20 mutations in *TSC1* and 21 mutations in *TSC2* are recurrent and the rest are private mutations. Seventy-three (47%) mutations in the *TSC1* gene are single-base substitutions, and 82% of those are nonsense mutations. In the *TSC2* gene, 50% of the mutations are point mutations, and nonsense mutations account for 38% of the total. Protein truncating mutations (nonsense, splicing and frameshift) account for 98% and 77% of *TSC1* and *TSC2* mutations respectively. *TSC1* mutations have been identified in 10–15% of sporadic cases, whereas *TSC2* mutations make up for 70% of the cases (Cheadle *et al* 2000). A total of 33 and 81 different polymorphisms have been identified in *TSC1* and *TSC2* genes respectively (Cheadle *et al* 2000).

Orthologues of *TSC1* gene have been cloned and characterized in mouse, rat and *Drosophila*. The rat hamartin with 1163 amino acids shows approximately 86% identity with human hamartin. The *Drosophila Tsc1* protein encodes a protein of 1100 amino acid residues and is 22% identical (46% similar) to human hamartin (Cheadle *et al* 2000). The predicted coiled-coil and transmembrane domain, and a stretch of 133 amino acids close to the potential transmembrane domain are conserved in human, mouse, rat and *Drosophila*. Orthologues of *TSC2* gene have been cloned from rat, mouse, *Drosophila* and the Japanese pufferfish, *Fugu rubripes*. The *gigas* mutant in *Drosophila* results from mutation of the orthologue of the *TSC2* gene (Ito and Rubin 2001). Human and *Drosophila* tuberins are 26% identical (46% similar) with the highest level of conservation (53% identity) being in the GAP-related domain (Cheadle *et al* 2000).

Recently, two groups have reported independently that the *Drosophila* homologues of human *Tsc1* and *Tsc2* regulate cell growth, cell proliferation and organ size (Potter *et al* 2001; Tapon *et al* 2001). Tapon *et al* (2001) have demonstrated that inactivating mutations in *Drosophila Tsc1* or *Tsc2* genes cause an identical phenotype which is characterized by enhanced growth and increased cell size with no change in ploidy level. Co-expression of both wild-type *Tsc1* and *Tsc2* genes restricted tissue growth and reduced cell size and cell proliferation in mutant cells from wings and eyes, and over-expression of either protein alone in the wings and eyes had no effect (Potter *et al* 2001). This could account for why a mutation in only one of the *TSC* genes is enough to cause the disorder. This also explains why the clinical manifestations of *TSC1* and *TSC2* mutations are indistinguishable: both proteins work in the same pathway. Potter *et al* (2001) have shown that *Drosophila* eye cells mutant for *Tsc1* are dramatically increased in size yet differentiate normally. The individual facets of the eyes as well as the interommatidial bristles were significantly longer in mutant cells of the eyes

(Tapon *et al* 2001). Mutant clones of *Tsc1* have additional cell divisions and a shortened G₁ phase, and consistent with mammalian findings, *Tsc1* and *Tsc2* proteins bind to each other (Potter *et al* 2001). Further, Potter *et al* (2001) proposed a model in which *Tsc1* and *Tsc2* interact with each other to antagonize the insulin signalling pathway in regulating cell proliferation, cell growth and organ size.

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