Tuberous Sclerosis Complex

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Abstract
Tuberous sclerosis complex(TSC) , which affects 1 in 6000 people, is a genetically determined, variably expressed, multisystem disorder that may affect any human organ with well-circumscribed, benign, non-invasive lesions often referred as hamartomas. TSC is caused by genes mutation namely TSC1 and TSC 2 on different chromosome thereby affecting protein synthesis, cell growth and proliferation signaling pathways. The skin, brain, heart, kidney and lungs are the organs more often involved. Most importantly the involvement of the central nervous system is emphasized by the unique and distinctive cerebral pathology found in patient with CNS disability, seizure and learning disability. Pathogenic mutation of these genes causes disorder that varies in extent and severity and therefore the clinical phenotype of the disease presents a high variability. TSC is an important disease not only because it is a relatively common hereditary neurological disease but has great morbidity and mortality in cases if no early diagnosis is made or no proper follow-up and treatment given. In this review, we shall overlook TSC and its clinical features
Keywords: *Tuberous Sclerosis Complex*

Introduction

Tuberous sclerosis complex is an autosomal dominant, multisystem disorder, which affects 1 in 6000 people (Osborne and al 1991), characterized by the development of widespread hamartomatous lesions involving various organs or organ systems, including the brain, skin, kidneys, heart and eyes. The Central Nervous System is almost invariably involved, with most of patient presenting with epilepsy, some patients having intellectual disability or other neuropsychiatric disorders including autism spectrum disorder. TSC 1 and TSC 2 are tumors suppressor genes that are mutated in individuals with tuberous sclerosis complex. TSC is caused by the mutation of TSC 1(130KDa) gene at chromosome 9q34 coding for *tuberin* protein and TSC 2(200KDa) gene at chromosome 16p13.3 coding for *hamartin* protein. The C-terminal domain of TSC 2 contains a small region of homology with GTPase-activating proteins (GAPs), and TSC1 and TSC2 interact to form a complex (Benvenuto et al 2000) (Chong-Kopera etal 2006) that acts as a Gap for the small G-protein RHEB, accelerating the conversion of RHEB from its active GTP-bound state to its inactive GDP-bound form. RHEB-GTP is required for the activation of mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) that stimulate the cell growth by promoting protein translation and lipid synthesis and inhibiting autophagy. In low energy, low glucose conditions, TSC1-TSC2 complex is activated to down-regulated TORC1 complex whereas in response to growth factor, the TSC1-TSC2 complex is inactivated to allow RHEB-GTP-dependent stimulation of TORC1. TSC1 and TSC2 are both required for full TSC1-TSC2 activity. TSC1 stabilises TSC2 and prevent TSC2 ubiquitination and proteosomal degradation, helps maintain the TSC1-TSC2 complex in the correct intracellular localization and regulates TSC1-TSC2 activity through diverse signaling pathways. TSC1 and TSC2 form a stable complex due to interaction between the N-terminal domain (NTD) of TSC2 (amino acids 1-900) and multiple regions in TSC 1. In TSC-associated lesions , loss or inactivation of the TSC1-TSC2 complex results in TORC1 activation and the constitutive phosphorylation of the downstream TORC1 targets including p70 S6 kinase (S6K), ribosomal protein S6 and 4EBP1 (Huang and Manning, 2008). Therefore mTORC1 over activation following the genetic defect determines the cell growth and proliferation responsible for TSC-related lesions, as well as the alteration in neuronal excitability and synaptogenesis leading to epilepsy and neuropsychiatric disorder. About half of these patients are affected by mental retardation, which has been associated with TSC 2 mutation. The cerebral lesion, **cortical tuber**, is the hallmark of a protean autosomal dominant disease with variable expression in one or more organs or tissues. The majority of the patients identified as having the disorder do experience symptoms referable to the CNS. Even in subjects without

neurological symptoms, CNS lesions are present in all brains studied. Thus the brain is anatomically normal, but histological research would likely to reveal lesions.

Based on the mutation involved, its severity and its degree of expression on the phenotype, a causative mutation for the disorder is identified in about 85% of patient with a clinical diagnosis of TSC. Patients with TSC 2 mutations usually present a more severe phenotype, characterized by higher number of tubers, earlier age of seizure onset and higher prevalence of intellectual disability. Genotype-phenotype correlation of the disease may help to determine risk profiles and select patients for targeted treatments but clinical phenotype of the disease presents a high variability, thus making the prediction of the phenotype on an individual basis still challenging. Some of the clinical advances in diagnosing or recognition, imaging, including functional imaging, neuropsychological studies, as well as genetic and histopathological cell markers of maturation and of lineage need to be involved for both clinical and genetic diagnosis.

Mapping and identification of TSC 1

Initial definition of the 1.5Mb candidate region on chromosome 9q34 was achieved by identification of key meiotic recombination events in large TSC 1 families. Many microsaetellite markers from this region were then identified and a cosmid, PAC and BAC contig assembled. Two putative recombinants in unaffected individuals provisionally narrowed the candidate region to 900kb between the markers D9S2127 and DBH. Large deletions or other rearrangements of the region were sought in patients with TSC, but no abnormalities were detected. The TSC 1 region proved to be gene rich with over 30 genes identified in or mapped to the critical region by a variety of techniques. Many of these were assessed as positional candidate for TSC 1 without identification of mutations.

Genomic arrangement at the TSC 2 locus

TSC 2 has a complex genomic structure, comprising 41 coding exons and a non-coding leader exon which are distributed over 44kb of the genome. The sequence predicts the product of 1807 amino acids. Immediately centromeric to TSC 2 is the PKD 1 gene which is mutated in autosomal dominant polycystic kidney disease. TSC 2 and PKD 1 are oriented in 3’ to 3’ and there Polyadenylation signals are separated only by 60bp.

Physiology of TSC 1 and TSC 2

TSC2 codes for a 200 kDa protein of 1807 amino acids, which contains a leucine zipper region (amino acids 75–107), two coiled-coil domains (amino acids 346–371 and 1008–1021), two transcriptional activation domains (amino acids 1163–1259 and 1690–1743), a conserved GTPase activating protein homology region (amino acids 1517–1674) and a nuclear localization signal (NLS; amino acids 1743–1755). It localizes to both cytoplasm as well as the nucleus. It interacts with TSC1 (tuberous sclerosis 1) and forms the TSC1/TSC2 (hamartin/tuberin) protein complex. *TSC1* codes for a 1164 amino acids long cytoplasmic protein of 130 kDa. The TSC1/TSC2 complex is a key negative regulator of the **PI3K-AKT-mTOR pathway.** Mammalian target of rapamycin (mTOR) **mTORC1** phosphorylates EIF 4EBPs (eukaryotic translation initiation factor 4E binding proteins) and RPS6KB1 (ribosomal protein S6 kinase, 70kDa, polypeptide 1; S6K1 (ribosomal protein S6 kinase 1).

Physiological roles of TSC1 and TSC2, which are independent of the PI3K-AKT-mTOR pathway, are termed as ‘non-canonical’. Transcription is one such independent function of TSC2. Small GTPases bind guanine nucleotides and serve as a molecular switch to regulate a number of physiological processes such as cell growth and morphology. Rheb, a small GTPase that belongs to a unique family within the Ras superfamily of GTPases, controls cell growth and proliferation as well as cell size. Unlike most small GTPases that are predominantly in an inactive GDP-bound state, Rheb exists in a high activated state presumably due to a low intrinsic GTPase activity as well as to a limiting amount of Tsc1/Tsc2 GAP protein inside the cell.

mTOR regulates protein synthesis through the phosphorylation and inactivation of the repressor of mRNA translation, eukaryotic initiation factor 4E-binding protein (4E-BP1), and through the phosphorylation and

activation of S6 kinase (S6K1). These two downstream effectors of mTOR whose phosphorylation is inhibited by rapamycin in vivo, can be phosphorylated by recombinant mTOR in vitro. S6K1 or 4E-BP1 phosphorylation is often used as an in vivo readout of mTOR activity. Raptor appears to serve as an adaptor protein that recruits mTOR substrates. It binds S6K1 and 4E-BP1, both downstream effectors of mTOR, and is necessary for the in vitro phosphorylation of 4E-BP1 by mTOR and for the efficient phosphorylation of S6K1. The interaction of Raptor with S6K1 and 4E-BP1 is mediated by a 5 amino acid motif termed TOS (TOR signaling) that is present in the N termini of S6K1 and 4E-BP1. mTOR activity is regulated by growth factors. Insulin and other growth factors dramatically increase the phosphorylation of S6K1 and 4E-BP1 in a rapamycin-sensitive manner. Mutations in the PDGF receptor that prevent the recruitment and activation of phosphoinositide-3-OH kinase (PI3K) also inhibit S6K1 phosphorylation by PDGF. mTOR-Raptor interaction may regulate mTOR activity in response to nutrients. In the absence of nutrients, a tight interaction between mTOR, Raptor, and mLST8 prevents the access of mTOR to its targets. In the presence of nutrients, a conformational change may disrupt Raptor/mLST8 interaction and enables the accessibility of mTOR (or an associated kinase) to its targets, 4E-BP1 or S6K1, which are bound to raptor. The regulation mTOR activity by growth factors is mediated by the PI3K/Akt signaling pathway leading to phosphorylation and inhibition of TSC2 by Akt and to the subsequent activation of Rheb, which activates mTOR by an as yet unknown mechanism. The serine/threonine protein kinase Akt, also known as protein kinase B (PKB), a downstream effector of PI3K, has emerged as a critical mediator of mTOR activity.

Pathophysiology of TSC 1 and/or TSC 2

Under pathological condition, **Mutations or deletions** in either the *TSC1* or *TSC2* genes cause TSC. To develop TSC, it is sufficient to have a mutation in either *TSC1* or *TSC2*. Inactivating mutations of either TSC 2 or TSC 1 leads to loss of GAP function of TSC 2. As a consequence, intracellular Rheb bound to GTP (active form) cannot be reverted to inactive GDP-bound form resulting in heightened signal transduction through mTORC1. The mTOR signaling pathway plays a role in regulating cell growth and metabolism, where mTOR helps initiate translation of messenger RNA into proteins through its downstream substrates 4E-BP1 and S6K which are important for cell growth, survival, cell-cycle progression and cellular metabolism. Regulators help to control the mTORC where positive regulator helps to transmit signals along the pathway and negative regulators, like hamartin and tuberin inhibit signaling to this pathway. Therefore mutation in either of TSC 1 or TSC 2 destabilizes the TSC1-TSC2 complex ceasing its inhibitory effect upon mTORC1 leading to a cascade of other downstream kinases and translational factors that stimulate protein translation, uncontrolled cell growth, proliferation and influence neuronal excitability and precipitate epileptogenesis. These alterations are associated with widespread hamartomas in several organs, including the brain, heart, skin, eyes, kidney, lung, and liver. S6K1 is highly phosphorylated in mammalian cells lacking a functional TSC1 or TSC2 provide potential links between Akt and TSC1/TSC2 and between mTOR and TSC1/TSC2. Further analyses of TSC2-deficient cells, as well as TSC1 and TSC2 overexpression experiments, clearly demonstrate that the TSC1/TSC2 heterodimer is an upstream negative regulator of mTOR

Genetic Mutation

Missense mutation

We classify missense mutation as pathogenic if they meet the following conditions-(1) they are present in a sporadic TSC patient or a founding family member, (2) they are not present in the unaffected parents of that patients, and (3) the TSC patient DNA sample has been completely screened for other changes in all exons of TSC 1 and TSC 2.

Single base substitution

In TSC 1, 48% of mutations are single based substitution, 82% of which are nonsense mutation. Most of the nonsense mutations are recurrent C to T transitions at 6 of the 7 CGA codons (encoding Arginine).

Insertion and deletions

In TSC1 mutation spectrum includes many insertions of less than 28bp and small deletions of less that 23bp. Nearly all small insertion/deletions (indel) cause frame shift in TSC 1. Most small insertion arose from the duplication of an adjacent base or region. Both a deletion and an insertion were seen to affect the 23b regions between bases 1671 and 1693, which is flanked by 9bp repeats.

Polymorphisms

In TSC 1 33 different polymorphisms have been identified. 30% occur in intronic sequence, 39% are silent and 30% cause missense changes, four of which are nonconservative according to the BLOSUM62 matrix (Henikoff and Henikoff 1992). In TSC 2, 81 different polymorphisms have been identified, consisting of one 3bp exonic deletion, two deletions in intronic sequences, 32% single base changes in intronic regions, 23%missense changes of which six were non-conservative and 40% silent changes in coding sequence.

Germline mutations of the *TSC1* and *TSC2* genes cause the familial syndrome of tuberous sclerosis complex. Those patients suffer from hamartomas and tumors in various tissues such as kidney angiomyolipoma, cardiac rhabdomyoma, subependymal giant cell astrocytoma (SEGA) and increased risk for renal cancer.

CLINICAL FEATURES OF TSC

There are no known pathognomonic signs for TSC since no single clinical feature is unique to the disease. In addition, many features known to be present in some individuals with TSC, such as epilepsy and intellectual disability, are too common in the general population to help establish the diagnosis. A constellation of features is therefore necessary for a diagnosis of TSC, with more specific features contributing more heavily to the diagnosis and an increasing number of features making the clinical suspicion of TSC more likely. Approximately 96% of patients who suffer from TSC have one or more kinds of skin lesions, an estimated 90% have symptoms or signs of cerebral pathology, 84%have seizures and 60% renal pathology. The phenotype of the patients affected varies according to the number and size of the lesions, the organ or organs involved, and sometimes the exact location of the lesions.

Similarly the age of the patient is another important factor to be considered, as there are lesions that don't appear until a certain age (angiomyolipomas), while other hamartomas that appear during fetal life disappear during infant (rhabdomyomas). Clinical features of TSC are most commonly seen in the skin, eyes, brain, kidneys, heart and lungs. Additional features have been reported in the gums, teeth and bones, as well as in most other major organs. The physical findings can vary greatly since TSC can affect different organ systems in different ways at different times in the individual's life. Neurological and dermatological abnormalities are the most common physical findings, each occurring in as many as 90 to 95 percent of individuals with TSC.

Most common involvements in patients are; Heart, brain, psychiatric, lung, kidney, skin, eye and other organ involvement.

HEART INVOLVEMENT

Tuberous sclerosis complex is a disorder characterized by the harmatomas of many cutaneous and visceral organs. Cardiac involvement in TSC patients is usually related to a type of cardiac harmatoma and rhabdomyoma, with the consequences; dysrhythmias, cardiac failure and death. Cardiovascular problems are most frequent cause of death among children with TCS below 10 years of age.. Cardiac rhabdomyomas represent the earliest detectable hamartoma in TSC and, interestingly are the only lesion in TSC which may regress with age. TSC is detected by non-invasive echocardiography. The incidence of these tumors in TSC has been reported to vary from 47 %- 67 % (Webb et all 1993, 60%; Gibbs1985:64%: Muhler et al 1994;67%) . Childhood tumor regression is the rule. In other words, in most cases, cardiac tumors are their largest at birth and may shrink or disappear as the individuals grow older. The tumors represent the earliest detectable hamartomas in TSC, frequently evident in the neonatal period or even prenatally. A second peak in the incidence of these tumors may occur during puberty. Electrocardiograms (EKGs) are used to detect abnormal heart rhythms or arrhythmias. Any symptoms should be monitored by a cardiologist who is aware of the risks

involved for individuals with TSC. In a large proportion of patients with cardiac rhabdomyomas, the tumors are multiple. Most TSC patients usually do not exhibit any clinical manifestation. If symptoms occur, they are largely a consequence of tumor size and location within the heart. These symptoms explained by one or more of the mechanisms: obstruction of inflow or outflow tract, secondary to an obstructing intracavitary tumor, myocardial involvement with secondary deterioration of ventricular functions: and cardiac rhythm abnormalities.

BRAIN INVOLVEMENT

Brain and Neurologic Function: Several types of brain lesions are seen in individuals with tuberous sclerosis complex (TSC); some people will have all the lesions, whereas others will have no brain involvement at all. The basic pathologic findings in TSC include: **Cortical tubers** (from which TSC is named) can be thought of as a "birth defect" on the brain. They are small areas in the cortex (the outer layer of the brain) that do not develop normally. It is thought that the presence of cortical tubers, which disrupts the normal "wiring" of the brain, is what causes seizures in individuals with TSC. **Subependymal nodules** develop near the walls of the cerebral ventricles (the cavities in the brain that contain cerebrospinal fluid). Typically, these nodules accumulate calcium within the first few months or years of life. Because of this calcification, they can be easily detected with a computed tomography (CT) scan. The subependymal nodules are not directly responsible for neurological problems. **Subependymal giant cell astrocytomas (SEGAs)**. This type of tumor develops in approximately 15 percent of individuals with tuberous sclerosis. Typically, SEGAs do not occur in very young children, and the chance for their growth decreases after age 20. The most common brain manifestation is epilepsy or seizures**.** Seizures occur in approximately 85 percent of individuals diagnosed with TSC. Epilepsy has been identified as a risk factor for autism in TSC. Studies reviewed suggested an increased incidence of autism in TSC patients with epilepsy, with infantile spasms presenting a greater risk.

LUNG INVOLVEMENT

The three main pulmonary lesions found in tuberous sclerosis complex (TSC) are lymphangioleiomyomatosis (LAM), multifocal micronodular pneumocyte hyperplasia, and clear cell tumor of the lung. LAM is the most common. The average age of onset is 32–34 years of age, and lung involvement is essentially, although not exclusively, a manifestation of TSC in women. Its involvement is estimated between 1-2.3% of all patients. Clinically it is characterized by progressive dyspnea and recurrent pneumothorax. Other manifestations include cough, chest pain, hemoptysis or chylothorax. The prognosis of LAM is considered poor, respiratory failure is often severe and progressive. Sporadic LAM is rare but its clinical, radiological and histopathological features are same as TSC-associated pulmonary LAM, thus supporting hypothesis that they share common pathogenic mechanism. Pulmonary LAM has distinctive gross appearance, on gross examination the lungs appear voluminous and heavier than normal lung, multiple cysts varying in size. Lung transplantation is considered the only effective therapy for end stage LAM.

KIDNEY INVOLVEMENT

Tuberous sclerosis complex (TSC) can present itself as five different lesions in the kidneys: angiomyolipomas, cysts, malignant angiomyolipomas, oncocytomas, and renal cell carcinoma. Renal angiomyolipomata, or angiomyolipomas, are of a greater concern because these lesions are associated with abnormal blood vessels that can lead to bleeding. Renal angiomyolipomata occur in approximately 50-80 percent of TSC patients, and approximately 80 percent of individuals will have involvement of both kidneys. Lastly, renal cell carcinoma, an extremely rare association with TSC and angiomyolipoma, is a cancerous growth of the kidney. Although it is very rare, such a lesion must be kept in mind.

EYE INVOLVEMENT

It is thought that about half of patient with TSC demonstrate ocular pathology. Retinal hamartomas, retinal pigmentation and vascular changes, optic nerve atrophy, glaucoma and coloboma of the iris, lens choroid and retina are among the frequent ophthalmological manifestations. Detection of the lesions may be difficult by fundoscopic inspection and if patient is unable to cooperate, sedation and indirect ophthalmoscopy may be required. The incidence of retinal hamartomas, the most frequent retinal lesions in TSC, ranges from 4-76%. They are more frequent in patients with TSC 2 gene mutations. Usually mulberry-like hamartomas are located at the disc margin, but they may also occur in the central part of the retina, the size of lesions may vary 0.5-4 disc diameter. The periphery of tumor is flat and semitransparent whereas its central part is calcified and nodular. Histopathologically, retinal lesions are composed of astrocytic proliferation, which in small lesions is limited to the superficial layers of the retina, the nerve fibers and ganglion layers. Larger hematoma may involve the whole section of the retina or optic disc. Origin of retinal hamartomas in TSC is thought to be identical to that of the CNS tumors, a proliferation of undifferentiated "glioneurocytes" with the development of variable neuronal and glial features. According to Robertson (1999) in the majority of cases there is no need for biopsy and clinical and roentgenologic information is sufficient for the diagnosis of retinal hamartoma in TSC.

PSYCHIATRIC AND BEHAVIORAL INVOLVMENT

The behavior of a child with tuberous sclerosis complex (TSC) can often be the most difficult problem for parents and family. Aggression, sudden rage, hyperactivity, attention deficit, acting out, obsessive-compulsive behavior, repetitive behaviors, staying in their "own world," being nonverbal even at an age when most children are speaking, and other autistic behaviors have all occurred in children with TSC. Learning disability is believed to occur in 40% of individuals. The likelihood of learning disability developing appears to be associated with the nature of the genetic mutation, extent of the brain abnormality, and the age of onset and type of seizure. The degree to which these factors jointly or independently contribute to shaping developmental outcome is still the subject of research. The mechanism involved are likely to be different according to the nature of the cognitive impairment, perhaps structural and functional disruptions to the development of different neural networks resulting in distinct, partly overlapping cognitive impairments.

DERMATOLOGICAL INVOLVEMENT

Tuberous sclerosis complex (TSC) is prominent neurocutaneous syndrome having neurological symptoms and signs accompanied by dermatological manifestations. The diagnostic triad of TSC, including facial angiofibromas was described by Campbell (1905), but is better known as Vogt's triad (Vogt 1908). According to Gomez (1987), 96% of patients with TSC have one or more of the main skin lesions of the disease (facial angiofibromas, ungal fibroma, shagreen patch, hypomelanotic macules). Therefore a thorough skin examination of individuals at risk for TSC is the best and the easiest method to make the diagnosis in most of cases. A Wood's lamp may facilitate this examination. The cutaneous finding diagnostic of TSC are included in the 1998 revision of TSC diagnostic criteria. Occasionally an individual with TSC coincidentally will have café au lait spots (areas of skin darker than the surrounding skin, but lighter and usually larger than a mole), but these skin lesions are not diagnostic of TSC. A child with three or more or an adult with five or more café au lait spots may be diagnosed with neurofibromatosis, another genetic condition.

Conclusion

In conclusive words TSC is variably expressed and affects multisystem. TSC is caused by genes mutations namely TSC1 and TSC 2 on different chromosomes thereby affecting protein synthesis, cell growth and proliferation signaling pathways. The skin, brain, heart, kidney and lungs are the organs more often involved expressing a myriad of phenotypic symptoms