Identifying Determinants for Neurobehavioral Morbidity in Tuberous Sclerosis Complex

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Identifying Determinants for Neurobehavioral Morbidity in Tuberous Sclerosis Complex

Identificatie van determinanten voor neuropsychiatrische morbiditeit in Tubereuze Sclerose Complex

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Hold a baby to your ear
As you would a shell:
Sounds of centuries you hear
New centuries foretell.

Who can break a baby's code?
And which is the older-
The listener or his small load?
The held or the holder?

E.B. White

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INTRODUCTION, AIMS AND OUTLINE OF THE THESIS

DIAGNOSIS AND COURSE OF TUBEROUS SCLEROSIS COMPLEX

Tuberous Sclerosis Complex (TSC) is a multisystem disorder characterized by the growth of hamartomas in multiple organ systems.[1, 2] The syndrome was described as early as 1835 by Rayer,[3] and later by von Recklinghousen.[4] In 1880, Bourneville described the syndrome in three patients and was the first to use the term 'tuberous sclerosis' to describe the potato-like consistency and hypertrophic sclerosis of the brain gyri at autopsy.[5] The classical triad of seizures, intellectual disability, and adenoma sebaceum (angiofibromas) was first noted by Vogt in 1908.[6] Since these early descriptions, many other presentations of TSC have been recognized and the diagnostic criteria have been refined to include clinical, radiological and pathological characteristics. The diagnostic criteria for TSC were most recently revised in 1998[3, 7, 8] (Table 1, diagnostic criteria).

TSC has a birth incidence of 1:6000.[1] It can be diagnosed in the pre- or perinatal period if cardiac rhabdomyomas are detected with ultrasound,[9, 10] although the diagnosis is most often made in the first years of life when children present with seizures and/or hypopigmented macules.[7, 11] TSC is associated with benign growths called hamartomas, and other abnormalities, in many different organ systems, including the brain (tubers and astrocytomas), kidneys (angiomyolipoma, cysts, renal cell carcinoma), lungs (nodules and lymphangioleiomyomatosis; LAM), skin (angiofibromas), liver (angiomyolipoma, cysts), pancreas (cysts, neuroendocrine tumors) and bones (sclerotic lesions, cysts).[12-16] With increasing recognition of the age-related tumor growth phenotype,[17-21] routine imaging recommendations have been developed for early recognition and intervention.[2, 22-25]

Although health watches focus on the tumor growth predisposition, often the greatest burden for patients with TSC and their caregivers is the neuropsychiatric comorbidity.[26] Most individuals with TSC have epilepsy, which is severe and difficult to treat,[21, 27] and intellectual and specific learning disabilities are common (50-80% of individuals with

Table 1. Diagnostic Criteria for Tuberous Sclerosis Complex (Roach, 2004),[8]

Major Features	Minor features
Facial angiofibromas or forehead plaque	Multiple randomly distributed pits in dental enamel
Non-traumatic (peri) ungual fibroma	Hamartomatous rectal polyps
Hypomelanotic macules (>3)	Bone cysts
Shagreen patch (connective tissue nevus)	Cerebral white matter migration lines
Cortical tuber	Gingival fibromas
Subependymal nodule	Non-renal hamartoma
Subependymal giant cell astrocytoma	Retinal achromic patch
Multiple retinal nodular hamartomas	Confetti skin lesions
Cardiac rhabdomyoma, single or multiple	Multiple renal cysts
Lymphangiomyomatosis	
Renal angiomyolipoma	

Definite TSC: Either 2 major features or 1 major feature with 2 minor features
Probable TSC: One major feature and one minor feature
Possible TSC: Either 1 major feature or 2 or more minor features

TSC).[28, 29] Behavioral abnormalities such as and autistic features[30-32] and psychiatric disorders such as anxiety, depression attention-deficit-hyperactivity-disorder, self-injurious behavior and aggression, are also frequent.[26, 33-39] These problems are a common reason to seek medical attention[1] and cause significant impairment in daily life.[40] Behavioral problems can be challenging to manage at home and are associated with great parental stress.[26, 41, 42] Increasing recognition of the learning difficulties and psychiatric morbidity has resulted in guidelines for routine cognitive and behavioral assessments, assisting clinicians who have individuals with TSC in their care, and providing guidance to these individuals, their families and their caregivers.[43, 44]

GENETICS AND FUNCTION OF TSC

About 70-85% of individuals with a definite diagnosis of TSC have an identifiable mutation in the *TSC1*[45] or *TSC2*[46] tumor suppressor genes, located on chromosome 9q34 and 16p13, respectively. Over 1700 mutations in TSC1 and TSC2 have been reported, and the TSC1/TSC2 mutation database (http://chromium.liacs.nl/LOVD2/TSC/home.php) is an outstanding resource. Larger 'genomic' mutations are very rare in TSC1 and more common in TSC2, occurring in about 6% of all TSC patients.[47] *TSC1* mutations commonly involve deletion or nonsense mutations (37 and 36%, respectively) while missense mutations are rare (3.1%).[48] Deletion, nonsense, and missense mutations all occur at similar frequencies in *TSC2* (22-27%), while splice and insertion mutations are less common (16 and 9%, respectively).[48]

Although in most cases, TSC is caused by a *de novo* germ-line *TSC1* or *TSC2* mutation, in approximately 30% of cases a patient inherits the condition from one of their parents and it is not uncommon for a parent to be diagnosed after the birth of a more severely affected child.[49] In 10-15% of TSC patients, no mutation can be identified (NMI) using current diagnostic screening methods.[50] A proportion of TSC NMI cases can be explained by mosaicism,[51-53] or occult mutations in parts of *TSC1* or *TSC2* not covered by the normal screening methods. Nonetheless, the existence of a third gene for TSC has not yet been excluded completely. The penetrance of TSC is very high,[54] and the expressivity is extremely variable, even within families or between individuals with identical germline mutations,[55, 56] making diagnosis a considerable challenge.

The mRNA transcripts of *TSC1* and *TSC2* encode two large proteins called hamartin (TSC1; 130 kDa) and tuberin (TSC2; 200 kDa) respectively. TSC1 and TSC2 bind to each other to form a protein complex[57] and both proteins are required for the proper function of this TSC1-TSC2 complex. TSC1 seems to act as a chaperone, to stabilize TSC2 and prevent its degradation.[58, 59] TSC2 contains a GTP-ase activating protein (GAP) domain and the TSC1-TSC2 complex functions as a GAP for the small GTPase Ras Homolog Expressed in Brain (RHEB). The TSC1-TSC2 complex switches RHEB from its active GTP-bound state (Rheb GTP) to its inactive GDP-bound state (Rheb GDP), resulting in downregulation of the mammalian target of rapamycin (mTOR) complex 1 (TORC1) pathway. In patients with a *TSC1* or *TSC2* mutation, lack of a functional TSC1-TSC2 complex results in upregulation of TORC1.

MTOR is a major regulator of cell growth and homeostasis throughout the body, and mTOR dysregulation is also implicated in cancer, diabetes and other neurological disorders.[60, 61] TORC1 is a serine-threonine kinase and in the brain, is involved in patterning, synaptogenesis and the growth of dendrites and axons.[62-64] TORC1 upregulation results in a loss of control of cell growth and proliferation, hence predisposing to tumor formation and other TSC-related morbidity.[65, 66] Findings in TSC may help elucidate the disease mechanisms of related disorders.[65, 66]

Animal models are of particular benefit in the study of brain disease in TSC, since histological investigations before natural death are limited to tissue of tubers and sub-ependymal giant cell tumors. Starting with the Eker rat, rodent models have provided great insights into the biological background of the tumor predisposition phenotype, which has enabled successful clinical trials for treatment of TSC-associated tumor growth, such as sub-ependymal giant cell astrocytomas.[68] Other mouse models have been valuable in understanding seizure disorders in TSC[69-73] but a representative neuroanatomical and neurocognitive phenotype has been difficult to capture. In several mouse models, treatment with mTORC1-inhibitors had a dramatic effect on seizures, cognitive function and survival,[74-77] although the effect on the neuroanatomical phenotype was often only partial. Ongoing trials in animal models as well as in patients with TSC will reveal which manifestations of TSC can be rescued or prevented.

FROM GENOTYPE TO NEUROLOGICAL PHENOTYPE

The exact mechanism by which enhanced TORC1 signaling leads to the neurological phenotype of TSC is unclear. MTOR is expressed during early brain development in progenitor cells in the ventricular zone and in early neuronal cells in the nascent cortical plate. Hypothetically, loss of function of tumor suppressor genes that are expressed in the mTOR pathway, such as *TSC1* and *TSC2*, *PTEN*, and *NF1*,[61, 78] would result in dysregulated mTOR activity, causing disturbed proliferation of neural and astrocytic precursor cells and cytomegaly, compromising neuronal migration and differentiation.

The variability in the phenotypic expression of TSC can complicate diagnosis and makes it difficult to make predictions about disease severity and progression based on genotype. One explanation for this variability can be sought in Knudson's "two hit" hypothesis,[79] suggesting that multiple genetic hits are necessary to lead to the tumor phenotype of TSC, such as tubers. In TSC-associated brain lesions, it was originally presumed that second 'hits' would result in biallelic *TSC1* or *TSC2* inactivation, such as was shown for renal angiomyolipomas.[80, 81] However, so far, loss of heterozygosity (LOH) or other second hits cannot account for all of the TSC-associated brain lesions,[82-85] and there is increasing evidence for additional genetic, epigenetic, environmental, and/or tissue-specific mechanisms,[86, 87] which are likely to have a large role in determining the patient phenotype. Although for visceral organ systems, the mechanism of 'benign metastasis' also plays a role in lesion formation and growth,[88-90] this phenomenon has not been investigated in the brain.

The occurrence of a second event is congruent with the classification of TSC as a focal cortical malformation syndrome.[61, 91] Indeed, mouse models with induced LOH or another

type of 'second hit' approach the neuroanatomical phenotype of patients with TSC most closely.[92, 93] However, other observations of global TORC1 upregulation in some TSC mouse models with haplo-insufficiency[72, 74, 94] hint at primary, TSC-dependent, cell-autonomous neural mechanisms.

TSC BRAIN MANIFESTATIONS

Neuroanatomical abnormalities are often present in patients with TSC, and magnetic resonance imaging (MRI) is helpful for diagnosis (see Table 1). The most prominent imaging features are tubers, radial migration lines (RMLs), sub-ependymal nodules (SENs) and sub-ependymal giant cell astrocytomas (SGCTs).[95] Over the last decade, microstructural and morphometric abnormalities have also been increasingly recognized.[28, 96]

Macroscopic abnormalities

On conventional imaging, tubers, white matter abnormalities and/or SENs are often visible. Tubers, white matter abnormalities and SENs have similar histopathological characteristics, showing generalized cellular disorganization with hypomyelination, giant or balloon cells, dysplastic neurons and astrogliosis.[97-100] Robust staining for the phosphorylated isoforms of ribosomal S6, a marker for TORC1 activity, is a characteristic of the abnormal cells in these TSC-associated lesions.[101]

Tubers are benign, hamartomatous growths that are visible in >80% of patients with TSC and vary in size, location and frequency.[95] Various imaging-based tuber features have been identified, based on their MRI signal characteristics.[102] Although tubers remain stable in size, their appearance on MRI can change over time, becoming increasingly cyst-like and/or calcified.[103, 104]

On MRI, white matter abnormalities are visible in 93-100% of TSC patients as curvilinear bands, wedge-like shapes or focal hyperintensities.[105, 106] Historically, such radial migration lines (RMLs) have been regarded as a distinct feature of TSC neuroanatomy[106-111] and a contributor to neurocognitive morbidity.[112, 113] Over the last decades, studies have focused on tubers[30, 95, 112, 114-117] and microstructural white matter abnormalities (see below); RMLs have not been characterized using sophisticated white-matter specific sequences, and as a consequence, their contribution to the neuroanatomical phenotype remains unclear.

Cerebellar lesions occur in about one-third of patients, and differ from tubers on MRI in both shape and appearance.[118] SENs occur along the wall of the lateral ventricles and in 5-20% of cases will develop into SGCTs.[119] Because of their propensity to grow, SGCTs, particularly those located near the foramen of Monro, can cause increased intracranial pressure, obstructive hydrocephalus, neurological deficits and death.[119]

Microscopic abnormalities

With emerging evidence for white matter dysconnectivity in TSC animal models,[72, 94] white matter microstructure in TSC is increasingly investigated using diffusion weighted and tensor imaging (DWI/DTI). DWI visualizes the diffusion of water molecules, providing information on the microstructural properties of tissue. DTI provides information on the

directionality (anisotropy) of the diffusion in specific white matter tracts. Tubers and RMLs are associated with abnormalities in diffusivity and anisotropy.[120-126] DTI findings in normal-appearing white matter (NAWM) have been conflicting, reporting both normal[121, 124, 125] and abnormal[120, 127-130] microstructural properties. These investigations were often limited by low-resolution imaging or did not exclude regions with RMLs from their measurements. Findings that presumed NAWM abnormalities were often regional[120, 122, 128-132] and correlated with the volume of 'subcortical tubers'[131] suggest that the presence of RMLs interfered with these DTI measurements. Detailed morphological and DTI characterization of both RMLs and NAWM should help clarify this issue.

NEUROPSYCHIATRIC PHENOTYPE OF TSC

Epilepsy and intelligence

Epilepsy is the most common neurological problem for individuals with TSC and occurs in 70-90% of all patients.[21, 133, 134] In most cases, seizures begin in the first year of life, manifesting as infantile spasms (IS) or as early-onset, refractory multifocal seizures.[21] However, seizures may begin at any age into adulthood,[21, 133] and nearly all patients with a single seizure subsequently develop epilepsy.[21] Seizure types often have a focal or multifocal origin with a topographic correspondence between EEG foci and MRI high-signal lesions. This has been interpreted as indicating that the cortical tubers represent the epileptogenic foci[135, 136] even though epileptogenic activity can also be detected in normal-appearing tissue surrounding the tubers.[137]

Epilepsy variables, such as age of seizure onset, IS and refractory epilepsy are closely linked to intellectual functioning.[29, 134, 138, 139] The prevalence of global intellectual disability in TSC is estimated to be between 50-80%, and many individuals with intellectual quotients in the normal range experience specific neuropsychological impairments, such as receptive or expressive language deficits, executive deficits, attentional deficits, and memory deficits.[28, 29, 37, 43, 134, 140-142]

Autistic features

The association of TSC with social withdrawal, impaired social contact, stereotypies and abnormal speech was described by Critchley and Earl in 1932,[143] many years before the first description of infantile autism by Leo Kanner.[144] In patients with TSC, studies have reported prevalences of autism spectrum disorders (ASD) ranging from 16 to 66%,[30, 31, 145] and TSC is estimated to account for 1-4% of all cases of autism.[146] Because clinical studies have been limited by small cohort sizes and dichotomous classifications of autism, intelligence and seizures, the cause and phenotype of autism spectrum disorders (ASDs) in patients with TSC remain unclear. In general, autism in TSC and idiopathic seizure disorders are thought to be strongly related to epilepsy severity and cognitive impairment.[39, 41, 147, 148] However, reports of ASDs in individuals with IQs in the normal range, and observations of imaging phenotypes distinctly associated with ASD in TSC[31, 39, 149] imply that autistic features can occur independently, and could be the result of distinct pathogenic mechanisms.

In addition to TSC, a number of other neurodevelopmental disorders have been associated with ASD, such as Fragile X syndrome, Neurofibromatosis type 1, and epilepsy of unknown origin,[150] and it has been proposed that investigation of autism in these disorders may shed light on the etiology and treatment of idiopathic autism.[151] Little is known about the relative contribution of the genetic and neuroanatomical risk factors versus the influence of cognitive deficits and epilepsy comorbidity on the autistic phenotype. Clinical research comparing the expression of autistic features in neurodevelopmental disorders could address this lack of knowledge by elucidating the relationship with intelligence and epilepsy, identifying autistic phenotypes, and comparisons with idiopathic autism.

Sleep and anxiety

While epilepsy, intelligence, attention deficits, hyperactivity and self-injurious behavior are relatively well-characterized in TSC,[21, 29, 38, 152, 153] much less is known about anxiety and sleep disorders in the pediatric and adult population. It is unclear whether these are primarily due to the genetic mutation, secondary to the chronic conditions associated with TSC, or a combination of these factors. Additionally, there are no clinical guidelines on the diagnosis and treatment of these disorders in TSC.

In other genetic syndromes, such as Williams Syndrome (WS) and Fragile X Syndrome (FXS), it is increasingly recognized that anxiety disorders may be specific for the behavioral phenotype.[154-156] In TSC, the reported prevalence of anxiety disorders ranges from 28 - 48% using DSM-IV criteria,[34, 35, 139, 157] and all types of anxiety disorders have been described. However, little is known about the etiology, phenomenology or treatment of these disorders in TSC.

Sleep disruption is common in children with TSC, and often due to sleep-related epileptic events. Sleep disruption is associated with increased behavioral problems, resulting in higher stress levels for both the patients and their families.[158-160] The impact of sleeping disorders in adults with TSC has not been investigated, and increased clinical characterization will increase awareness of these important issues.[26, 161]

RISK FACTORS FOR NEUROCOGNITIVE MORBIDITY

The vulnerability to neuropsychiatric disorders of patients with TSC, is likely due to direct effects of a *TSC1* or *TSC2* mutation on neuroanatomy and synaptic function.[153, 162,163] Additionally, infantile spasms or other early onset and refractory seizures, could disrupt the development of neuronal circuitry through TORC1-dependent and independent mechanisms.[164] Other factors such as increased rate of life stressors, tumor predisposition, cosmetic impact and other medical comorbidity, are also likely to contribute to psychological dysfunction. Due to the complex phenotype of TSC, it is difficult to disentangle these primary and secondary effects of TORC1 dysregulation.

Genetic risk factors

As a group, TSC patients with a *TSC2* mutation have a worse prognosis than patients with a *TSC1* mutation. *TSC2* mutations are associated with a higher tuber burden,[114] more cyst-like tubers[103] and a more severe neuropsychiatric phenotype, with higher rates of IS

and refractory epilepsy, lower intelligence and a higher risk for autism and self-injurious behavior.[29, 30, 165, 166] Interestingly, the *TSC2* patient population shows a bimodal distribution of intelligence scores,[29, 134] suggesting the existence of distinct subgroups which may differ in underlying pathogenetic mechanisms. In contrast, the *TSC1* patient population shows a normal distribution of intelligence scores, but shifted slightly towards the lower end of the scale. The TSC NMI population appears to be a distinct clinical and pathophysiological subgroup, with a milder neurocognitive profile and differentially affected other organ systems.[29, 134]

There are several possible reasons why individuals affected by *TSC2* mutations are more severely affected than those with *TSC1* mutations. First, the *TSC2* locus may be more susceptible to mutation (explaining why there are more individuals with a *TSC2* mutation compared to a *TSC1* mutation (5:1 ratio) and therefore more likely to suffer a second hit). Secondly, since *TSC2* encodes the catalytic subunit of the TSC1-TSC2 complex which is necessary for the canonical TSC1-TSC2 function, mutations in this subunit will lead to severe loss of function. In contrast, *TSC2* may have some residual RHEB GAP activity even after complete loss of *TSC1*.

Although the phenotypic differences between the *TSC1* and *TSC2* mutation populations are clear and reproducible,[29, 30, 50, 114, 139, 165, 167] it is not yet possible to differentiate TSC1 and TSC2 patients at the individual level. Many severely affected individuals with TSC have a *TSC1* mutation, while other mildly affected individuals have a *TSC2* mutation. Despite the considerable phenotypic variability, there is increasing evidence for genotype-phenotype correlations in TSC and it is possible that the type of mutation may help determine the severity of the disease.

Truncating *TSC1* and *TSC2* mutations are, in general, completely inactivating, resulting in complete loss of TSC1-TSC2 activity and upregulation of TORC1.[168, 169] In contrast, some missense and splice site mutations can result in the production of protein that may have some residual function or activity and may therefore be expected to have less drastic effects on the disease phenotype. The TSC2 GAP-domain is essential for TSC1-TSC2 function.[170] Although some investigations have reported a higher neuroanatomical and neurological phenotype associated with mutations affecting the GAP domain,[50, 114] this was not always observed for the cognitive phenotype.[50, 114] Furthermore, relatively mild phenotypes associated with non-truncating mutations in the GAP domain and elsewhere have been described.[59, 171, 172]

Recent case reports and *in vitro* investigations suggest that *TSC1* missense mutations destabilize TSC1 and may result in a relatively mild clinical phenotype.[171-175] Investigations in larger patient cohorts could elucidate whether these are mutation- or location-specific effects.

Neuroanatomical risk factors

Early diagnosis and intervention may improve neurocognitive prognosis in TSC,[176, 177] inspiring an active search for prognostic neuroradiological biomarkers. Tuber count, burden or location have all been associated with seizure variables, intelligence and ASD[28, 30, 116, 138, 153, 178-181] but, overall, the findings have been variable and sometimes conflicting. Tubers or perituberal areas can be epileptogenic by disturbing neural circuitry[136, 137, 182] and tuber epileptogenicity has been associated with microstructural abnormalities, increased

serotonin synthesis, decreased benzodiazepine receptor expression and increased gamma-aminobutyric acid (GABA) levels.[126, 183-186] Tubers with a cyst-like appearance predict a higher risk of refractory epilepsy and autism.[103]

Although white matter MRI abnormalities were associated with intellectual disability in TSC as early as 1995,[187] a recent study did not find any association between the frequency of radial migration lines (RMLs) and a diagnosis of autism.[30] In the latter study, RMLs were categorized as either fewer than, more than, or equal to three in number, and this may not represent the true distribution of these lesions. Recently, DWI/DTI studies comparing white matter microstructural characteristics reported significant differences between TSC patients with and without autism.[132] Thus far, the white matter phenotype has not yet been delineated sufficiently to be able to distinguish abnormalities in 'normal appearing white matter' from the effects of focal migration abnormalities.

SENs are generally not thought to directly cause neurocognitive morbidity, although they may cause 'stiffening' of the ventricular walls. In contrast, SGCTs can block the circulation of cerebrospinal fluid, causing increased intracranial pressure, and when untreated, can result in death.[119, 188, 189] TSC patients with cerebellar lesions do not display 'typical' cerebellar symptoms such as ataxia, and although a positron emission tomography (PET) study found increased metabolism in the deep cerebellar nuclei and caudate nuclei to be associated with autism in TSC,[149] the impact of cerebellar lesions on neuropsychiatric morbidity remains unclear.[118, 190]

Epileptogenic risk factors

Various epilepsy features, including early age at seizure onset, infantile spasms (IS), mixed seizure type, and poor seizure and IS control, have been associated with intellectual disability.[29, 114, 115, 138, 153, 180, 187, 191, 192] IS have also been associated with autism in TSC,[30, 192] similar to observations in other genetic syndromes.[193] Furthermore, temporal lobe epileptiform discharges on EEG and early seizure onset have been associated with autism, emphasizing that early recognition and treatment of IS and other seizure types are of great importance to reduce the cognitive consequences.[176, 178, 194] The effect of interictal epileptiform discharges on electroencephalography (EEG) on cognitive function is unclear[195] and no research has been performed on this in TSC.

Gender

Some studies have reported a more severe cognitive phenotype in men with TSC compared with women with the disease.[50, 167] In contrast, attention-deficit-hyperactivity-disorder and autism spectrum disorders seem to affect both genders equally in TSC.[30, 139] For anxiety disorders, both higher rates in women[34] and no gender differences have been reported.[39] More detailed quantification of the TSC phenotype will better address the effect of gender on disease expression.

Age

There has been little investigation on cognitive and adaptive development in children and adults with TSC. In a study of infants with TSC, cognitive development was found to be

slow but relatively stable, although a subset showed large variability.[196] In a parallel study, it was found that children with TSC and autism were more impaired than those without autism.[147] In other neurodevelopmental syndromes, there are indications for intellectual and/or adaptive decline,[197, 198] but cognitive and adaptive development has not been studied in older children and adults with TSC. More information on the natural history of the neuropsychiatric phenotype, including cognitive and adaptive skills, autistic features and anxiety, will be critical for determining the required support for an affected individual to succeed at school, work and in the community.

TREATMENT OF NEUROPSYCHIATRIC MORBIDITY

Advances in pharmaceutical research have made the medical management of epilepsy more successful.[199] Nonetheless, in TSC patients, epilepsy often remains refractory to treatment.[21] Except for vigabatrin for infantile spasms, there is no consensus on the most effective anticonvulsant in TSC.[27] Efficacy of other anti-epileptic drugs (AEDs) including topiramate, lamotrigine and levetiracetam has been reported in small populations of patients with TSC[200, 201] and vagus nerve stimulation, dietary treatments and neurosurgery can also be effective.[27, 202, 203] However, there remains a great need for new treatment options and treatment predictors. For example, clobazam (CLB) has been widely used as an adjunctive therapy in refractory epilepsy in adults and children,[204] but has not yet been evaluated in TSC. Treatment studies including predictors for outcome would help inform and stratify patients for interventions.

Similar to the seizure phenotype, the behavioral problems and other psychiatric morbidities in TSC are often refractory to treatment[26, 35] and the high rates of psychiatric morbidity contrast greatly with the paucity of evidence for guiding treatment in patients with TSC or other genetic syndromes. For TSC, reports are limited to the treatment of sleep disorders with melatonin.[205] Anecdotally, traditional sedatives have been found to be less effective in patients with TSC, and they often worsen the situation by increasing hyperactivity.[206] There have been no studies on selective serotonin reuptake inhibitors (SSRIs), even though retrospective reports suggest that SSRIs are generally the pharmacologic treatment of choice for anxiety in patients with TSC.[34, 35, 207]

Specific TORC1 inhibitors are in clinical use, and have been shown to reduce the growth and/or decrease the volume of subependymal giant cell tumors (SGCT) and renal angiomyolipomas, and improve the appearance of facial angiofibromas,[68, 208] underlining the exciting potential of these drugs as disease-modifying therapy in TSC.

SCOPE AND AIMS OF THIS THESIS

Intellectual disability, epilepsy and autistic features often co-occur in syndromes with a known genetic cause, such as Tuberous Sclerosis Complex (TSC). Since for TSC, the responsible disease mechanisms and even disease-modifying therapies have been rapidly identified, TSC is increasingly used as a model to identify the cause and therapies for idiopathic autism spectrum disorders. However, the clinical phenotype of TSC, such as

autistic features and contributing neuroanatomy, is still poorly understood. Furthermore, other neuropsychiatric morbidity such as anxiety and sleeping disorders may be underdiagnosed and undertreated, in children as well as adults.

This thesis focuses on gene-brain-behavior relationships in the syndrome Tuberous Sclerosis Complex. The objective was to further explore the neuroanatomical and neurobehavioral phenotype of TSC, and elucidate the contribution of specific mutation types. By clarifying some of these mechanisms, it may assist in the treatment and counseling of patients and their caregivers, and serve as a platform for future research.

OUTLINE

In **Chapter 2** and **3**, the relationships between genotype and neurocognitive phenotype are explored, focusing on intellectual outcomes and the occurrence of infantile spasms.

In **Chapter 4**, an MRI-based phenotype is presented, characterizing normal-appearing white matter, radial migration lines, and their association with neurobehavioral morbidity.

The neurocognitive phenotype over time is presented in **Chapter 5**, focusing on cognitive and adaptive development. **Chapter 6** provides insights on the rate and expression of autistic features in TSC and related disorders such as Neurofibromatosis type 1, childhood-onset epilepsy, and idiopathic autism spectrum disorders, and the relationship between these autistic features and other neurocognitive variables.

Chapter 7 and **Chapter 8** provide information on the occurrence and presentation of selected sleep- and stressor-related disorders in adolescent and adult individuals. In **Chapter 9**, an investigation into clobazam treatment for epilepsy in patients with TSC and refractory epilepsy is presented.

Chapter 10 provides a general discussion of the findings of this thesis, and their implications for future research as well as for clinical care.

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GENOTYPE AND COGNITIVE PHENOTYPE OF PATIENTS WITH TUBEROUS SCLEROSIS COMPLEX

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ABSTRACT

Tuberous sclerosis complex (TSC) is an autosomal dominant, multisystem disorder, which affects 1 in 6000 people. About half of these patients are affected by mental retardation, which has been associated with TSC2 mutations, epilepsy severity and tuber burden. The bimodal intelligence distribution in TSC populations suggests the existence of subgroups with distinct pathophysiologies, which remain to be identified. Furthermore, it is unknown if heterozygous germline mutations in TSC2 can produce the neurocognitive phenotype of TSC independent of epilepsy and tubers. Genotype–phenotype correlations may help to determine risk profiles and select patients for targeted treatments. A retrospective chart review was performed, including a large cohort of 137 TSC patients who received intelligence assessment and genetic mutation analysis. The distribution of intellectual outcomes was investigated for selected genotypes. Genotype–neurocognitive phenotype correlations were performed and associations between specific germline mutations and intellectual outcomes were compared. Results showed that TSC1 mutations in the tuberin interaction domain were significantly associated with lower intellectual outcomes ($P<0.03$), which was also the case for TSC2 protein-truncating and hamartin interaction domain mutations (both $P<0.05$). TSC2 missense mutations and small in-frame deletions were significantly associated with higher IQ/DQs ($P<0.05$). Effects related to the mutation location within the TSC2 gene were found. These findings suggest that TSC2 protein-truncating mutations and small in-frame mutations are associated with distinctly different intelligence profiles, providing further evidence that different types and locations of TSC germline mutations may be associated with distinct neurocognitive phenotypes.

INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant, multisystem disorder caused by heterozygous mutations in the tumorsuppressor genes TSC1 and TSC2.[1,2] Their protein products, hamartin and tuberin respectively, interact to form a protein complex that inhibits signal transduction to the downstream effectors of the mammalian target of rapamycin complex 1 (mTORC1), a serinethreonine kinase with major roles in cell growth signaling. Various regions in the TSC2 C-terminal domain, including the GTPase- activating protein (GAP) domain, appear to be important for normal TSC2 protein function in the mTOR pathway regulation of mTOR.[3] Mutations in TSC1 and TSC2 are typically inactivating, resulting in little to no protein activity, leading to upregulation of mTORC1.[4,5] This results in a constitutive growth phenotype with development of hamartomas in various organ systems, including the brain. More than 90% of individuals with TSC show neuroanatomical abnormalities such as tubers, sub-ependymal growths and white matter abnormalities.[6] Most patients are affected by epilepsy, often presenting with infantile spasms (IS) as the initial symptom of the disorder.

The prevalence of mental retardation (MR) in TSC is estimated to be between 44 and 70% and has been associated with tuber burden, tuber/brain proportion, early seizure onset, IS, mixed seizure types, TSC2 mutations and poor seizure control.[7–13] A bimodal intellectual quotient (IQ) distribution in the total TSC population has been suggested[7] and was recently refined as being observed only in the TSC2 population.[10] TSC patients with germline TSC1 and TSC2 mutations have only one fully functional TSC2 allele in all their cells, and this condition could lead to neurocognitive dysfunction through the mechanism of haplo-insufficiency,[14–16] similar to Fragile-X syndrome and Neurofibromatosis type 1.[14–16] However, in TSC there are additional factors which may contribute to cognitive impairment, including loss of heterozygosity, which may contribute to tuber development,[17,18] and effects of early onset and refractory epilepsy. Thus far, no associations have been found between specific TSC mutation types and cognitive outcomes,[10,19] although there are reports on associations with epilepsy and psychiatric features.[10,19–22] As most of these studies have limited power or do not address all mutation types of interest, more extensive investigations are warranted to determine potential correlations between genotype and neurocognitive phenotype in TSC. Furthermore, as mTOR-inhibitors are now under investigation to prevent or reverse neurocognitive morbidity in TSC, more specific information on genotype–phenotype associations will assist clinicians and caregivers in these important treatment decisions. In this study, we use quantitative intelligence outcomes and genetic mutation results of a large TSC patient cohort to explore the intellectual phenotype and associations with the affected gene and specific gene domains, mutation types and locations.

METHODS

Study group

The charts of all 377 patients with a definite diagnosis of TSC who were treated at the Herscot Center for TSC at Massachusetts General Hospital (MGH) were reviewed. TSC patients who had received genetic mutation analysis and neuropsychological assessment at

the MGH Psychology Assessment Center were identified. This study was approved by the Institutional Review Board of MGH.

Cognitive assessment

Comprehensive neuropsychological evaluations, including intellectual functioning, were performed by an experienced neuropsychologist (MP). For all patients, the outcome of the most recent full-scale intelligence quotient (IQ) assessment was selected. These were available by one of the following five neuropsychological measures, according to best practice standards: (1) Bayley Scales of Infant Development – 2nd edition (BSID),[23] (2) Stanford–Binet Intelligence Scale – 5th edition,[24] (3) Wechsler Preschool and Primary Scale of Intelligence – 3rd edition,[25] (4) Wechsler Intelligence Scale for Children – 4th edition[26] and (5) Wechsler Abbreviated Scale of Intelligence – revised.[27] The BSID and Stanford–Binet also provide mental age scores, which are based on a patient's raw score converted to a mental age at which an average child would obtain that score. For the patients who were at the floor of the age-appropriate standardized scores, we calculated developmental quotients (DQs) (mental age/ chronological age x100), where a DQ of 100 would be considered the mean. The presence of MR was recorded for each patient with a score of o70, according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition.

Clinical data

Clinical data were collected from the patient medical records, including information on gender, history of epilepsy and history of IS. We did not have access to clinical data of two patients, and these missing values were excluded when determining percentages in the results.

Genetic analysis

All patients at MGH followed for TSC are offered genetic testing as part of their comprehensive evaluation. Genetic testing of the TSC1 and TSC2 genes, including detecting of large DNA deletions and rearrangements of the TSC2 gene, was performed at Athena Diagnostics (Worcester, MA, USA) or the MGH Neurogenetic Diagnostic Laboratory (Boston, MA, USA). Pathogenic mutations were confirmed by consultation of two TSC mutation databases (website tsc-project.partners.org, chromium.liacs.nl/LOVD2/TSC). Patients with predicted disease-associated mutations of the TSC1 and TSC2 genes were labeled as such. Patients with no definite findings or only polymorphisms were classified as having no mutation identified (NMI). We examined the possible effects of each of the missense mutations identified in these patients using the Alamut Mutation Interpretation Software. A single mutation (TSC2 A460T) was predicted to have a possible effect on splicing, with a score of -33%. However, as this score was <50%, it was considered unlikely to have an effect on splicing. Patients with two pathogenic mutations were excluded. An individual's specific mutation-type and its exon and nucleotide location within the TSC1 or TSC2 gene were recorded.

To examine the neurocognitive impact of mutations in specific gene domains, functional domains of the TSC1 and TSC2 gene products were selected, including the TSC1 tuberin

interaction domain (TID), the TSC2 hamartin interaction domain (HID) and the TSC2 GAP domain.[5,28] Mutations were additionally classified into protein-truncating (PT; nonsense, frame-shift, splice site, large deletions of at least one exon) and non-truncating (missense, small in-frame deletions and insertions) mutations. Protein-truncating mutations were divided into proximal and distal mutations, determined from the middle exon of each gene.

To investigate the effect of gene location of TSC2 missense mutations, these were grouped into three subsets according to the exon (E) location of the affected amino acid: affecting or potentially affecting HID–TID (E1–E22); the GAP domain (E34–E41); and mutations in between these two regions (E23–E33).

Statistical analysis

Statistical analyses were performed using SPSS Version 11.5 (SPSS, Inc., Chicago, IL, USA).

T-tests were used to compare selected genetic mutation domains and types with outcomes on intellectual measures. Owing to relatively small sample size, we restricted analyses in the TSC1 cohort to comparing TID mutations with all remaining mutations and with only proximal PT mutations.

For TSC2 mutations, HID mutations were compared with all other muta-tions and proximal PT mutations (<E22). Additionally, GAP mutations were compared with other distal mutations, PT mutations were compared with missense mutations and small in-frame deletions, and proximal PT mutations were compared with distal PT mutations. To investigate if, within the TSC2 cohort, PT mutations and missense mutations showed distinctly different intellectual profiles, a two-sample Mann–Whitney test was performed.

When not specifically mentioned, all mutation subgroups were compared with the remaining cohort of the respective affected gene. All reported *P*-values used two-tailed tests of significance with a set at 0.05.

RESULTS

Patient characteristics

Of all 377 patients with a definite diagnosis of TSC, 164 (44%) had received IQ/DQ assessment. Genetic testing had been performed on 137 (85%) of these patients, including 66 men and 71 women, with a mean age of 17 years old (range 3–57) and 4 patients under the age of 5. Of this study group of 137 patients, 35 (26%) patients were affected by a pathogenic TSC1 mutation, 81 (59%) patients by a pathogenic TSC2 mutation and in 21 (15%) patients no mutation could be identified (NMI). The distribution of the pathogenic mutations within the TSC1 and TSC2 genes is shown in Figure 1.

Intellectual profiles of TSC1, TSC2 and NMI cohorts

Of the total study group, the mean IQ/DQ was 71.1 (range 7–135). In 36 (22%) patients, conversion to DQ was performed. The prevalence of MR was 23% for the TSC1 population, 57% for TSC2 patients and 29% for the NMI cohort (Figure 1, Table 1). Intelligence scores for the total study group and according to mutation subtype are illustrated in Figure 2a and

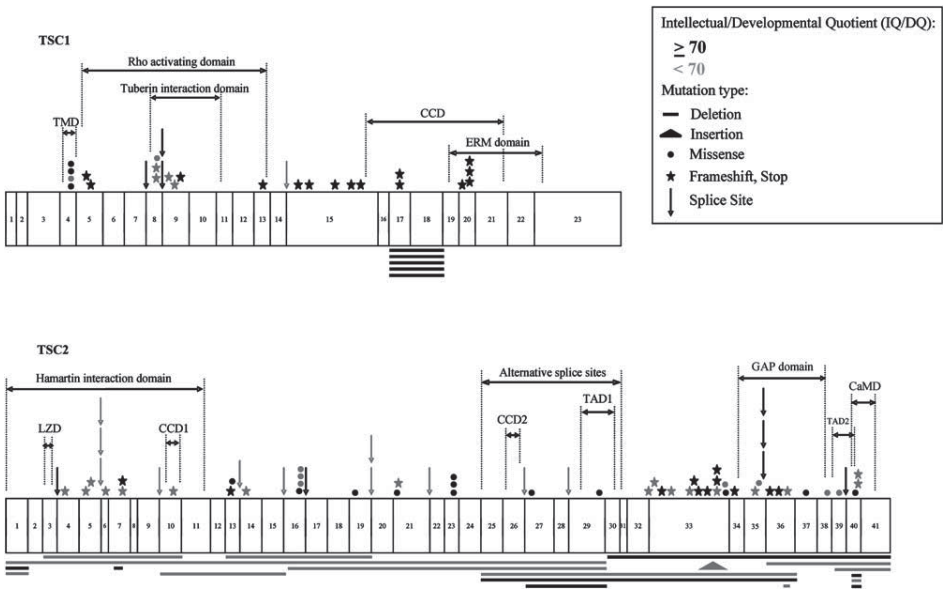

Figure 1. TSC1 and TSC2 gene exon map, depicting mutation types of patients with and without MR. CaMD, calmodulin-binding domain; CCD, coil–coil domain; ERM, ezrin–radixin–moesin; GAP, GTPase-activating protein; LZD, leucine zipper domain; TAD, transcription-activating domain; TMD, transmembrane domain.

confirm the bimodal appearance of the IQ/DQ distribution of the total and TSC2 cohort (Figures 2a and b). The mean IQ/DQ of the TSC1 and TSC2 mutation subgroups was 83 and 64, respectively, where male and female patients showed identical mean IQ/DQs (Figure 3a). The mean IQ/DQ of the total NMI subgroup was 79, with the 13 men showing a mean IQ/DQ of 77, and the 8 women a mean of 84. Of note is that the NMI subgroup consisted of 13 men and 8 women while the TSC1 and TSC2 cohorts each had a slight preponderance of women.

Genotype–phenotype analyses TSC1 mutations

In the TSC1 cohort, the 11 (33%) patients with a mutation in the TID showed a significantly lower mean IQ/DQ of 66 ($P<0.03$) compared with 88 in the remaining TSC1 cohort (for epilepsy characteristics, see Table 1). Compared with patients with proximal PT mutations (<E15) not affecting the TID domain who showed a higher mean IQ/DQ of 84, the IQ/DQs of patients with TID mutations remained lower, although not significantly ($P<0.12$). Of the four TSC1 missense mutations, one patient had an IQ of 37, which lowered the mean of the other three related patients who had IQ/DQs between 72 and 103. Of interest are the relatively high IQ/DQs associated with the three splice site mutations affecting the proximal TID, contrary to the more distal splice site mutation in I14, which was associated with an IQ/DQ of 21. Excluding this latter splice site mutation, no TSC1 patients with a mutation distal of the TID were affected by MR or IS, although most had a positive history of epilepsy (Figure 1 and Table 1).

Table 1. Neurocognitive characteristics per TSC mutation type and domain.

Gene	Domain/type	Mean IQ/DQ (range) P/N	MR P/N	Epilepsy P/N	IS P/N
TSC1	All mutations	83 (7–130)	8/35 (23%)	31/35 (89%)	3/31 (1%)
	TID	66 (7–115)	5/8 (63%)	8/8 (100%)	2/8 (25%)
	Missense	72 (37–103)	2/5 (40%)	5/5 (100%)	2/5 (40%)
TSC2	All mutations	64 (7–134)	46/81 (57%)	72/81 (89%)	44/75 (59%)
	HID	51 (8–134)	14/18 (78%)	17/18 (94%)	10/16 (63%)
	GAP	72 (11–132)	5/11 (46%)	9/11 (82%)	8/10 (80%)
	PT	60 (7–134)	30/47 (65%)	42/47 (91%)	30/47 (73%)
	Prox. PT (E1-E22)	49 (22–91)	30/46 (78%)	9/9 (100%)	5/8 (63%)
	Small in-frame	79 (11–117)	8/23 (35%)	19/23 (83%)	9/22 (41%)
	Missense	78 (11–116)	6/18 (33%)	14/18 (78%)	7/17 (41%)
	Large in-frame deletions	51 (8–101)	9/13 (69%)	13/13 (100%)	6/13 (46%)
NMI	—	79 (12–135)	6/21 (29%)	13/21 (62%)	8/21 (38%)

Abbreviations: E, exon; GAP, GTPase-activating protein; HID, hamartin interaction domain; IQ/DQ, intellectual/developmental quotient; IS, infantile spasms; MR, mental retardation; NMI, no mutation identified; P/N, number of patients in cohort displaying symptoms/number of patients at risk; PT, protein truncating; TID, tuberin interaction domain; TSC, tuberous sclerosis complex.
Characteristics of the NMI cohort are included.

GENOTYPE–PHENOTYPE ANALYSES TSC2 MUTATIONS

Within the TSC2 cohort, the patients with mutations in the HID (n=18, 21%) showed a significantly lower mean IQ/DQ of 51 (P<0.05) (for epilepsy characteristics, see Table 1). The patients with a proximal PT mutation (<E22, excluding HID mutations) showed a similar mean IQ/DQ of 49. Distal PT mutations showed a significantly higher mean IQ/DQ of 69 (P<0.04) compared with proximal PT mutations (Figures 2b and 3b). When PT mutations were compared with small in-frame deletions and missense mutations combined, the latter group had a significantly higher mean IQ/DQ of 76 (P<0.05), which was only slightly higher with an IQ/DQ of 78 when only missense mutation were included in the analysis (P<0.04) (Figures 2b and 3b). The Mann–Whitney test confirmed significantly different intellectual profiles for PT and missense mutations. Mutations in the GAP-domain were associated with a mean IQ/DQ of 72, which was higher than remaining mutations with a mean IQ/DQ of 63, but not significantly so (P<0.38). Although the GAP-related IQ/DQ profile was higher than the mean IQ/DQ of all PTs (P<0.12), it was only slightly higher than distal PTs (Table 1). When missense GAP mutations were compared with the remaining GAP mutations, the mean IQ/DQs were similar (71 vs 68).

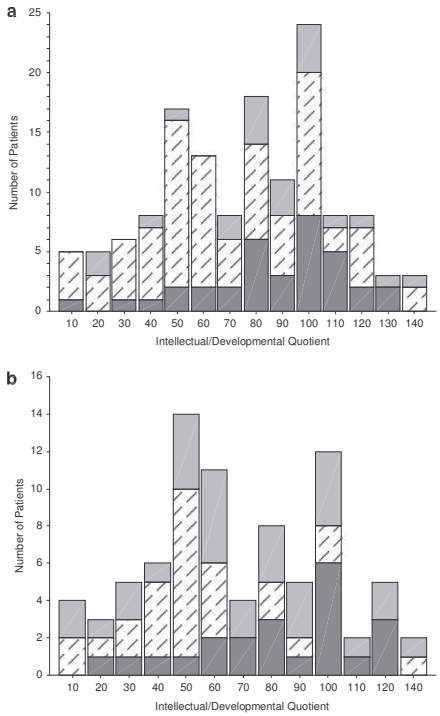

Figure 2. Histograms depicting intelligence distributions of selected TSC mutation groups. (a) Intelligence outcomes of the total TSC cohort, including the TSC1 (dark gray), TSC2 (shaded gray) and NMI (light gray) cohorts. (b) Intelligence outcomes of TSC2 mutation subgroups with missense mutations and small in-frame deletions (dark gray), proximal protein-truncating mutations (shaded gray) and distal protein-truncating mutations (light gray).

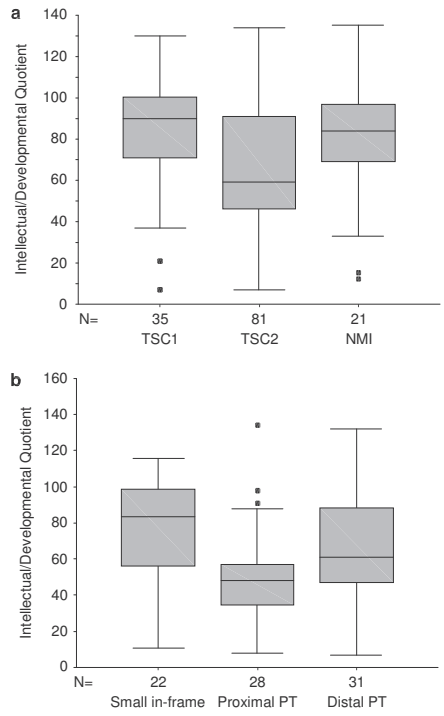

Figure 3. Boxplots depicting intelligence outcomes of selected TSC mutation cohorts. (a) Intelligence outcomes of TSC1, TSC2 and NMI mutation cohort, including the mean intelligence, SD and outliers. (b) Intelligence distributions for TSC2 subgroups with small in-frame deletions, proximal protein-truncating mutations, distal protein-truncating mutations. Small in-frame mutations included missense mutations and deletions o1 exon.

Grouping all TSC2 missense mutations according to their position on the gene (see Materials and Methods) revealed mean IQ/DQs that were relatively lower for proximal and distal missense mutations, whereas missense mutations in the middle of the TSC2 gene were associated with a relatively normal cognitive phenotype, excluding one outlier (Figure 4).

DISCUSSION

The data provided by our large study cohort and quantitative intelligence outcomes are the first to indicate significant relationships between specific mutation types and intellectual outcomes in patients with TSC. We confirm the more severe neurocognitive phenotype of the total TSC2 population, and within this cohort, found subgroups showing significantly

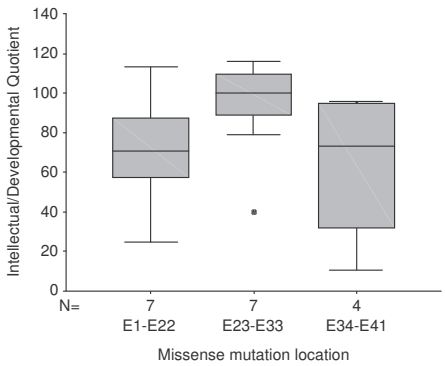

Figure 4. Boxplot depicting intelligence outcomes for all patients with TSC2 missense mutations, specified per location of the mutation on the TSC2 gene. The outlier in the middle group represents a female patient with a neurological history of refractory partial complex seizures and epilepsy surgery; genetic analysis revealed a P1497R mutation.

different intellectual profiles associated with specific genotypes. TSC2 patients with proximal PT mutations and HID mutations showed very similar, significantly lower mean IQ/DQs compared with patients with small, in-frame deletions or missense mutations, confirming case reports finding a milder phenotype in patients with missense mutations.[20,22,29–31] This apparent phenotypic dichotomy corresponds with the reported bimodal IQ/DQ distribution in the TSC2 population and we showed that this bimodal appearance can, at least partly, be explained by the effects of different mutation subtypes. Furthermore, these findings correlate well with functional considerations of the effects of different mutations on the TSC1–TSC2 protein complex. Two pathophysiological mechanisms have been reported in TSC, where truncating TSC1 and TSC2 mutations undergo mRNA nonsense-mediated decay, and what little aberrant truncated protein is produced is likely rapidly cleared from cells in the cytosol with no functional protein production. In contrast, missense and other small in-frame mutations may produce an intact, albeit dysfunctional, protein that remains present in the cell with variable remaining function.[32,33] In addition to this 'all-or-nothing' theory, we found that PT mutations occurring in the latter half of TSC2 were associated with significantly higher intellectual outcomes than PT mutations in the first half of the protein, suggesting that mutations in TSC2 which leave the HID intact may result in production of some functional protein. This suggests a third pathophysiological mechanism, applying to distal TSC2 truncating mutations that leave the HID intact and result in appropriate formation of the hamartin–tuberin complex, but perhaps disrupt functions exerted by domains in the distal part of TSC2, such as GAP-expression, transcription and binding of kinases.[22,34] There was some suggestion that mutations in the GAP domain were associated with a relatively better neurocognitive profile, although this did not reach significance, perhaps because the relatively small sample size limited the power of this observation as we only investigated mutations directly in this domain.

Investigating the location of mutations more in detail, we found compelling clinical support for previous observations that TSC2 missense mutations that do not affect the hamartin–tuberin interaction or the GAP domain produce a milder phenotype, possibly by retaining some GAP activity.[31,35] The single severely affected patient in the 'milder' missense group possibly reflects a remote splice site mutation,[36] a severe 'second hit' in the other allele, secondary effect of seizures or another pathophysiologic phenomenon in this complex syndrome.

Although the TSC1 cohort was relatively smaller, the findings that mutations in the TID were associated with a more severe cognitive phenotype, even when compared with only proximal PT mutations, confirms the importance of unimpaired binding of hamartin and tuberin through their interaction domains. The three patients with splice site mutations in the TID without cognitive impairment were noteworthy and future studies should focus on protein studies of splice site mutations to learn more about their effect. The relatively low mean IQ/DQ of the five TSC1 missense mutations conflicts with previous observations of a relatively mild phenotype,[33,37] possibly due to the fact that all of these missense mutations occurred in the N-terminal region, which is essential for TSC1 function.[32,38] Of note is that, excluding splice site mutations, none of the patients with more distal mutations were affected by MR.

Previous studies have reported a more severe cognitive phenotype in men compared with women with TSC, using dichotomous outcomes such as 'MR'.[19,39,40] However, the nearly identical IQ/DQs in men and women in our large TSC1 and TSC2 cohorts are more consistent with previous data on the prevalence of autism, ADHD and other neuropsychiatric disorders in the TSC population,[41,42] suggesting that genetic effects override gender effects.

The neurocognitive phenotype of patients with TSC is highly variable, because of several effects. Apart from the effect of the genetic mutations, the effect of epilepsy comorbidity, neurosurgery and other anti-epileptic treatments may influence cognitive development in patients with TSC and thus complicate genotype–phenotype associations. Our explorations confirm that cognition and epilepsy are interrelated in TSC, showing greater frequencies of epilepsy and IS in mutation subgroups with a higher prevalence of MR and low mean mutation subgroup. In addition, it is still unclear if second hits are absolutely necessary for the formation of tubers,[17,18] which are also associated with intellectual outcomes.[9,43]

A drawback for this type of study is the use of multiple cognitive measures, which is inherent to the inclusion of different age groups. We accounted for this by using both dichotomous and quantitative outcomes of cognitive functioning, MR and IE, in order to validate and strengthen our findings. As the patients were assessed at different ages, it is unclear if cognitive development is sufficiently stable in patients with TSC to perform such a study. Although thus far there has been little investigation on cognitive development in children and adults with TSC, we recently found that the mean IQ/DQ of a large TSC cohort remained stable over time, albeit showing variability,[44] confirming a previous study in infants with TSC.[45] As this study group IQ/DQs, which may contribute to the large ranges of IQ/DQ per represents only TSC patients who were referred for neuropsychological assessment, this may represent a bias toward more severely affected patients. For this study, we selected intelligence as the primary outcome because these quantitative data provide more precise and powerful information, although this reduced the size of the study cohort.

We limited our statistical analysis to intelligence outcomes per mutation type, as similar investigations on epilepsy parameters are ongoing in a larger sample. Of importance is that some subjects in categories associated with a more severe neurocognitive phenotype had excellent intelligence outcomes. This, together with the described missense mutation finding, limits the use of our findings as prognostic indicators and should remind clinicians to be very cautious in attempting phenotype predictions. Future genotype–phenotype correlations in larger cohorts should expand on our findings and include seizure variables, psychiatric burden and the neuroanatomical endophenotype of TSC. Functional analysis on the biochemical effects of specific missense mutations, small in-frame deletions and splice site mutations may identify more genotype–phenotype correlations.

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3

CENTRAL *TSC2* MISSENSE MUTATIONS ARE ASSOCIATED WITH A REDUCED RISK OF INFANTILE SPASMS

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ABSTRACT

Tuberous Sclerosis Complex (TSC) is an autosomal dominant syndrome with a variable neurocognitive phenotype. Recently, different intelligence profiles were observed for distinct mutation types and locations, suggesting that individuals with missense mutations represent a subgroup with milder neurocognitive outcomes. We applied these recent insights to the analysis of the epilepsy phenotype in a large cohort of patients with TSC. Associations between genotype and a history of epilepsy and/or infantile spasms (IS) were explored retrospectively, using data from 478 TSC patients from the databases of the Tuberous Sclerosis Alliance and the Herscot Center at Massachusetts General Hospital. Absolute and relative risks for IS and other types of epilepsy were calculated for various mutation classes, selected according to type and location. As expected, *TSC2* mutations were associated with a significantly higher occurrence of IS and other epilepsy types. However, missense mutations located in the central region of *TSC2* (exons 23 - 33) were associated with a significantly reduced incidence of IS. Our study further delineates the epilepsy phenotype in TSC patients. Identifying distinct epilepsy phenotypes for specific mutation subgroups may help identify relevant biomarkers and assist clinicians in making treatment decisions.

INTRODUCTION

Tuberous Sclerosis Complex (TSC) is an autosomal dominant, multisystem disorder caused by mutations in the *TSC1* and *TSC2* genes. Disruption of the TSC1-TSC2 protein complex results in upregulation of signal transduction through the mammalian target of rapamycin (mTOR) complex 1 (TORC1), resulting in a constitutive growth phenotype with development of hamartomas in various organ systems, including the brain.

Epilepsy is the most common neurological symptom of TSC and occurs in 90% of patients, often manifesting as infantile spasms (IS) or as early onset, intractable multifocal seizures.[1] IS are often the first presenting seizure type and occur in one-third of patients with TSC, often evolving into refractory epilepsy. Since IS are associated with a higher risk for cognitive impairment and autism,[2, 3] early recognition and treatment are of great importance.[4]

IS and epilepsy are generally more frequent and more severe in patients with *TSC2* mutations, compared to individuals with *TSC1* mutations.[4] However, this distinction may be an over-simplification, as recent reports indicate that specific *TSC2* mutations are associated with a less severe epilepsy phenotype.[5-8] Here, we explore the associations between specific *TSC1* and *TSC2* genotypes and epilepsy phenotypes using data provided by a large TSC patient cohort.

METHODS

Clinical and genetic information on 1034 individuals with TSC from 15 institutions in the USA was obtained from the TS Alliance Natural History Database. Information on a history of epilepsy and a history of IS was classified into present, not present, or unknown. Since electroencephalographic (EEG) characteristics are not a diagnostic criterium for IS (Commission on Pediatric Epilepsy of the International League Against Epilepsy, 1992), and EEG data were not consistently available, these were not retrieved. Specific data on seizure types other than IS, or intelligence outcomes were not consistently available. Complete data on IS, other seizure types, and results of genetic testing were available for 440 (43%) individuals and were supplemented with information from 77 patients from the Carol and James Herscot Center for TSC at Massachusetts General Hospital (MGH). Of the total group, patients with an unclear seizure history (14), only one seizure (2), incomplete results of genetic mutation analysis (20), or genetic mosaicism (3) were excluded. Pathogenic mutations were classified using ALAMUT mutation interpretation software, SIFT amino acid substitution analysis software,[9] functional testing,[10] and by comparison with the *TSC1* and *TSC2* Leiden Open Variation Database (chromium.liacs.nl/LOVD2/TSC).

Patients with definite pathogenic *TSC1* or *TSC2* mutations were labeled as such. Patients with only polymorphisms, unclassified variants or no identified variants were classified as no mutation identified (NMI). Nonsense, frame-shift and splice-site mutations, as well as deletions of at least one exon were classified as protein terminating (PT) mutations. Missense mutations and small in-frame deletions or insertions were classified as non-terminating (NT) mutations. NT *TSC2* mutations were divided into 3 groups according to the location of the pathogenic change:[8] 1) a proximal group containing changes to exons 1 - 22, that were likely to disrupt the TSC1-TSC2 interaction, 2) a distal group containing changes

to exons 34 - 41 that were likely to affect the GAP domain, and 3) the remaining central group with mutations mapping to exons 23 - 33. NT mutations to this central region have been shown to affect the activity of the TSC1-TSC2 complex, but do not appear to affect TSC1-TSC2 binding, and map outside the GTPase activating protein (GAP) domain.[10, 11] Statistical analyses were performed using SPSS Version 11.5 (SPSS, Inc., Chicago, IL). Chi-square analyses were performed with alpha set at 0.05. This study was approved by the review boards of the MGH and the TSA Database.

RESULTS

Patient characteristics

A complete epilepsy history and complete genetic analysis was available for a total of 478 individuals, consisting of 233 (49%) males and 245 (51%) females with a mean age of 7.6 years (range 1.5 to 63.1 years). Of this group, 99 (21%) had no history of seizures, 141 (30%) had a history of IS and other seizure types, 222 (46%) had a history of epilepsy without IS, and 16 (3%) had a history of IS followed by seizure freedom.

Genotype-phenotype analyses

A pathogenic *TSC1* mutation was identified in 110 (23%) individuals, a *TSC2* mutation was identified in 269 (56%) individuals, and in 99 cases (21%), no mutation was identified (NMI). Patient characteristics per mutation cohort are listed in Table 1. Absolute risks of IS

Table 1. Patient characteristics per TSC mutation cohort.

	TSC1 (N = 110)	TSC2 (N = 269)	NMI (N = 99)
Mean age (range) Mean age at diagnosis (range)	8.1 (2.0—16.2) 9.2 (−0.4 to 59.5)	4.8 (1.9—57.3) 2.2 (−0.5 to 43.8)	10.1 (1.5—63.1) 7.6 (0.0—55.7)
Female	59 (53.6%)	132 (49.1%)	54 (54.5%)
IS	16 (14.5%)	122 (45.4%)	19 (19.2%)
Other epilepsy	88 (80.0%)	219 (81.4%)	57 (57.6%)
IS + epilepsy	16 (14.5%)	108 (40.1%)	17 (17.2%)
Epilepsy no IS	72 (65.5%)	110 (40.9%)	40 (40.4%)
IS only	0 (0.0%)	14 (5.2%)	2 (2.0%)
No history of epilepsy	22 (20%)	37 (13.8%)	40 (40.4%)
PT mutations	104 (94.5%)	185 (68.8%)	0 (0%)
NT mutations	6 (5.5%)	84 (31.2%)	0 (0%)
Missense mutations	6 (5.5%)	67 (24.9)	0 (0%)
In-frame insertions	0 (0%)	0 (0%)	0 (0%)
In-frame deletions	0 (0%)	17 (6.3%)	0 (0%)

TSC, tuberous sclerosis complex; NMI, no mutation identified; IS, infantile spasms; PT, protein terminating; and NT, non-terminating.

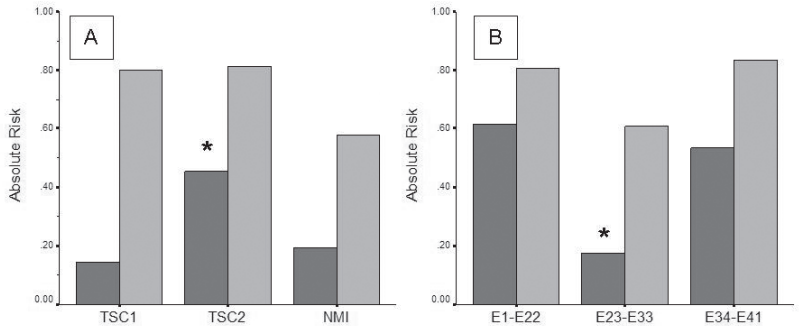

Figure 1. Bar-graph depicting the incidence of Infantile Spasms (green) and other types of epilepsy (blue) for A) individuals with a TSC1 or TSC2 mutation or with no mutation identified (NMI), and B) individuals with a non-terminating mutation in exon 1 - 22 (E1-E22), exon 23 - 33 (E23-E33) or exon 34 - 41 (E34-41) of the TSC2 gene. * $P<0.01$

or other forms of epilepsy per mutation group were estimated (see Table 1, Figure 1A), and relative risks (RR) were calculated with chi-square tests. *TSC2* mutations were significantly associated with a higher risk for IS compared to *TSC1* mutations (RR 1.56, $P<0.0001$) and NMI (RR 2.36, $P<0.0001$). *TSC2* mutations were also associated with a significantly higher relative risk for other epilepsy types compared with the NMI group (RR 1.41, $P< 0.0001$), but not compared with the *TSC1* mutation group (RR 1.08, $P<0.43$) (Figure 1). Compared with the NMI patients, the *TSC1* mutation cohort did not have a significantly higher risk for IS (RR 1.06, $P<0.24$), but did show a higher risk for other types of epilepsy (RR 1.34, $P<0.0001$).

Next, relative risks were computed for more specific mutation types. Only six patients had a *TSC1* missense mutation, which are known to be rare,[12, 13] and three of these patients were related. Therefore, no detailed analyses were performed. For patients with a *TSC2* mutation, there were no significant differences in IS or epilepsy outcomes between individuals with a PT or NT mutation ($P<0.46$ and $P<0.10$, respectively). However, when the NT mutations were sub-divided according to their location in the *TSC2* gene (Figure 2), proximal and distal *TSC2* mutations showed a significantly higher risk of IS compared with mutations in the central region (exons 23 - 33) of the gene (proximal: RR 3.52, $P<0.001$; distal: RR 1.77, $P<0.008$) (Figure 2). Although higher risks for other seizure types were also observed, they did not reach significance for either proximal (RR 1.3, $P<0.10$) or distal *TSC2* NT mutations (RR 2.35, $P<0.06$), compared to the central NT mutation group.

From the 16 patients with a history of IS that resolved without the occurrence of other types of epilepsy, 14 individuals had a mutation in *TSC2* and 2 individuals were NMI. Of the *TSC2* mutations, 7 were PT and 7 were NT, the latter in proximal and distal locations. Strikingly, although not statistically significant ($P<0.11$, OR 0.14 – 1.30) due to the small sample size, 22% of the patients with a *TSC2* NT mutation and IS became seizure free, compared to only 8% of the patients with a *TSC2* PT mutation and IS.

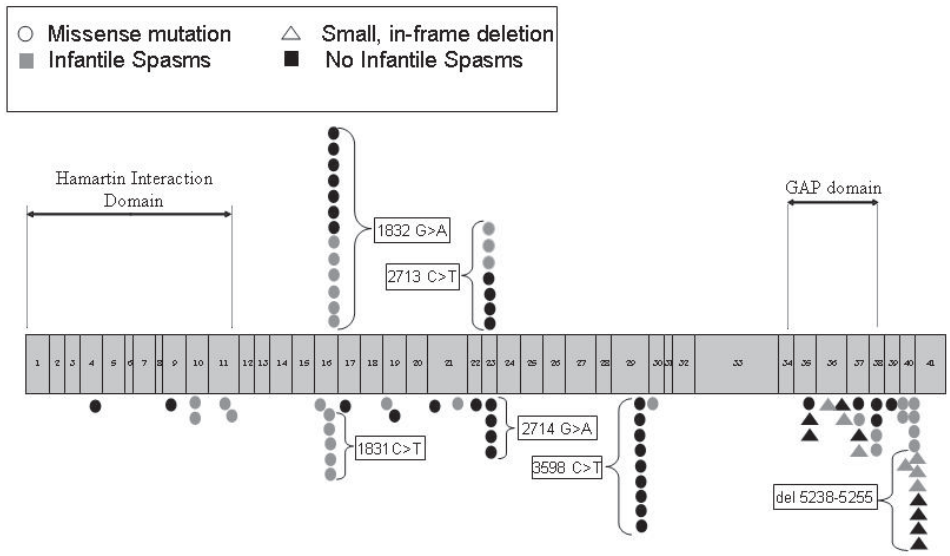

Figure 2. Exon map of the TSC2 gene, showing missense mutations and small in-frame deletions identified in patients with (grey) and without (black) a history of infantile spasms.

DISCUSSION

The large cohort provided by the combination of two large databases allowed us to investigate the risk of epilepsy and IS associated with specific TSC germline mutation types. In addition to confirming a significantly higher occurrence of IS in patients with a *TSC2* mutation,[1] we observed that non-terminating mutations located in the central region of the *TSC2* gene (exons 23 - 33) are associated with a significantly lower risk for IS, and a trend towards a lower prevalence of any type of epilepsy. This profile is similar to that of patients with a *TSC1* mutation or with NMI. Combined with the recent evidence for higher mean intellectual outcomes for patients with non-terminating mutations in this region of the *TSC2* gene,[8] these observations suggest that patients with a NT mutation between exons 23 and 33 of the *TSC2* gene are likely to have a less severe neurocognitive profile. Although for specific *TSC2* amino acid changes, variable epilepsy profiles were observed, the consistent absence of IS in patients carrying the *TSC2* c.2714G>A (p.R905Q) and c.3598C>T (p.R1200W) variants confirms the previously reported milder phenotype in these patients,[5, 14] while the presence of IS in all patients with a *TSC2* c.1831C>T (p.R611W) mutation suggests a generally more severe phenotype, consistent with *in vitro* observations that this mutation causes severe impairment of TSC1-TSC2 binding and function.[15] Unfortunately, we did not have sufficient numbers of patients with a *TSC1* missense mutation to allow any statistical comparisons to be made.

A previous study on intellectual outcomes in TSC showed more distinct associations with genotype, such as differences between proximal and distal PT mutations as well as

differences between PT and NT mutations.[8] The occurrence of epilepsy and IS seem less strongly associated with germline mutation types, which may be due to the dichotomous outcomes in this study. It is also likely that stochastic effects, such as loss of heterozygosity (LOH) and other somatic, second-hit mutational events are important determinants for tuber development[16, 17] and the epilepsy phenotype in TSC, while the cognitive phenotype may be more directly influenced by the nature of the germline mutation. This is also consistent with a recent report that found a stronger relationship between tuber count and IS than between tuber count and developmental outcomes.[18]

A major strength of this study is that it was performed on an extremely large TSC cohort which provided sufficient power for detailed genotype-phenotype comparisons. Although hypsarrhythmia is the EEG pattern classically associated with IS, the diagnosis of IS in TSC is a clinical diagnosis, and hypsarrhythmia need not be present for this diagnosis to be made. The data on IS were provided by TSC experts who are unlikely to misdiagnose IS. Due to possible overestimation in pediatric epilepsy samples, the prevalence of IS in TSC has been difficult to determine and the rate of IS in 33% of patients in this cohort may be the most unbiased so far.

Unfortunately, the available data were insufficient to analyze associations between genotype and the complete epilepsy phenotype, or to distinguish other seizure types associated with poor neurodevelopmental outcomes, such as Lennox-Gastaut syndrome. Furthermore, we were limited by the lack of systematic data on intelligence outcomes, and it will be interesting to explore associations between genotype and more specific neurocognitive profiles, including response to anti-epileptic treatments.

Finally, it is important to note that some subjects in categories associated with a more severe epilepsy phenotype had excellent outcomes and vice versa. This limits the clinical use of our findings as prognostic indicators and close monitoring of each patient diagnosed with TSC remains justified. Nevertheless, the increased recognition of TSC patients with less severe neurological phenotypes may assist in the search for biomarkers and the selection of patients for preventative treatment. Functional analysis on the biochemical effects of specific missense mutations, small in-frame deletions and splice site mutations will help characterize the effects of mutations and identify candidates for more specific genotype–phenotype correlation studies.

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4

THE NEUROANATOMICAL PHENOTYPE OF TUBEROUS SCLEROSIS COMPLEX: FOCUS ON RADIAL MIGRATION LINES

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ABSTRACT

The contribution of radial migration lines (RMLs) to the neuroanatomical and neurocognitive phenotype of tuberous sclerosis complex (TSC) is unclear. The aim of this study was to perform a comprehensive evaluation of the neuroradiological phenotype of TSC, distinguishing RMLs from normal-appearing white matter (NAWM) using diffusion tensor imaging (DTI) and volumetric fluid-attenuated inversion recovery imaging.

Magnetic resonance images of 30 patients with TSC were evaluated. The frequencies of RMLs, tubers, and subependymal nodules (SENs) were determined for every hemispheric lobe. Cerebellar lesions and subependymal giant cell tumors were counted. DTI metrics were obtained from the NAWM of every hemispheric lobe and from the largest RML and tuber. Analyses of variance and correlations were performed to investigate the associations between neuroanatomical characteristics and relationships between RML frequency and neurocognitive outcomes. NAWM DTI metrics were compared with measurements of 16 control patients.

A mean of 47 RMLs, 27 tubers, and 10 SENs were found per patient, and the frequencies of these lesions were strongly correlated ($P<0.001$). RML fractional anisotropy and mean diffusivity were strongly inversely correlated ($P= 0.003$). NAWM DTI metrics were similar to the controls ($P=0.26$). RML frequency was strongly associated with age of seizure onset ($P=0.003$), intelligence outcomes (P=0.01), and level of autistic features ($P=0.007$).

A detailed neuroradiological phenotype is presented, showing that RMLs are the most frequent neuroanatomical lesion, are responsible for white matter DTI abnormalities, and are strongly associated with age of seizure onset, intelligence outcomes, and level of autistic features.

INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder caused by mutations in the tumor suppressor genes TSC1 or TSC2. Loss of function of the TSC1–TSC2 protein complex causes upregulation of the mammalian target of rapamycin (mTOR) pathway, resulting in a tumor growth phenotype. Brain manifestations of TSC are variable in severity are characterized by cortical tubers, white matter (WM) abnormalities, and periventricular subependymal nodules (SENs) which may evolve into subependymal giant cell tumors (SGCTs).[1-3] Neurocognitive morbidity is often severe, including early onset seizures (90 %), cognitive impairment (50 %), and psychiatric disorders (70 %).[4,5] Early diagnosis and interventions may improve neurocognitive prognosis, inspiring an active search for neuroradiological prognostic biomarkers.

Historically, neuroimaging studies in TSC have focused on morphological tuber characteristics and their relationships with neurocognitive morbidity, reporting variable and conflicting results.[6,7] With emerging evidence for WM dysconnectivity in TSC,[8] imaging investigations shifted to WM microstructure using diffusion tensor imaging (DTI), which is sensitive to the molecular movement of water and provides information on WM integrity or pathology.

It was established that radial migration lines (RMLs) were associated with abnormal DTI values[9-14] (Figure 1), consistent with the histopathological findings of disrupted myelination in these areas.[15,16] Although these observations suggest that RMLs may contribute to neurocognitive morbidity in TSC, these lesions have only rarely been acknowledged as prognostic biomarkers[17,18] and no quantitative evaluations have been performed.

Instead, recent studies have focused on DTI investigations in normal-appearing white matter (NAWM) with conflicting observations, reporting both normal[10,13,14] and abnormal

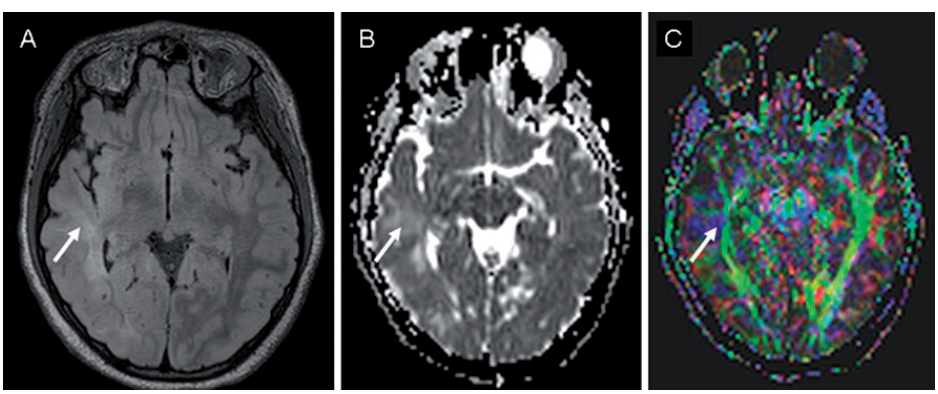

Figure 1. Appearance of radial migration lines in a patient with TSC. A) Axial volumetric FLAIR, showing RML extending from the temporal horn of the right lateral ventricle to the right superior temporal gyrus (arrow). B) ADC map, showing increased mean diffusivity in the area of the RML, suggesting disruption of the white matter microstructure (arrow). C) FA color map, showing aberrant orientation of white matter fibers in the area corresponding with the RML (arrow).

NAWM microstructure.[9,19-22] However, most investigations were limited by small sample sizes, low-resolution fluid-attenuated inversion recovery (FLAIR) imaging, or did not exclude regions with RMLs from their measurements. Findings that presumed that NAWM abnormalities were often regional[9,11,20-24] and correlated with the volume of "subcortical tubers"[23] suggest that the presence of RMLs interfered with these DTI measurements.

We hypothesized that, in TSC, the contribution of RMLs to the neuroradiological phenotype is underrecognized, that RMLs are responsible for microstructural WM abnormalities, and that RMLs contribute to the neurocognitive phenotype of TSC. To explore the first hypothesis, a detailed morphological evaluation was performed, quantifying the distribution and frequencies of RMLs, tubers, SENs, and SGCTs. Secondly, RMLs and NAWM were interrogated with DTI under guidance of volumetric FLAIR imaging in TSC patients and control patients. Lastly, RML characteristics were compared with quantitative data on seizures, intelligence and autistic features.

METHODS

Subjects

All subjects were followed at the multidisciplinary TSC clinic of the study institution and had a definite diagnosis of TSC.[1] The inclusion criteria were (1) the availability of a recent magnetic resonance imaging (MRI) study that met the study protocol criteria (see succeeding sections), free of motion or technical artifacts, obtained since 2009 and (2) at least 4 years of age, as after this myelin remodeling is limited. Exclusion criteria were neurosurgery within the last year. This study was approved by the institutional review board of the hospital.

Clinical information

Medical records were reviewed and the following information was recorded: date of birth, gender, history of seizures, history of infantile spasms (IS), age of onset of seizures, and history of neurosurgery. When available, full-scale intelligence quotients or developmental quotients (IQ/DQs) were retrieved.

Behavioral measures

In the context of a larger study on TSC and neurobehavioral morbidity, psychometric questionnaires were prospectively distributed between 1 October 2010 and 1 April 2012. For this study, we retrieved scores of the Social Communication Questionnaire (SCQ), when available. The SCQ[25] is a reliable, validated screening instrument for ages 4 years and older. It yields a level of autistic features, based on current diagnostic criteria for the Autism Diagnostic Interview—Revised.[26] The scale is completed by a caregiver, and since it can also be used for nonverbal probands, it is suitable for all TSC patients.[27] For this study, the T scores of the SCQ-Lifetime were used.

Image acquisition

All MRIs were performed without gadolinium on a 3.0-T system (TimTrio; Siemens, Erlangen, Germany) as part of the diagnosis or annual routine follow-up. The imaging protocol included

3D volumetric T1-weighted sequence (flip angle, 7°; TR, 2,530 ms; TE, 1.64 ms; inversion time, 1,100 ms; voxel size, 1.0×1.0×1.0 mm; section thickness (ST), 1.0 mm; acquisition matrix, 256 ×256; field of view (FOV), 26 cm; sagittal); T2-weighted turbo spin echo (TR, 6,380 ms; TE, 97 ms; voxel size, 0.4×0.4×4.0 mm; ST, 4.0 mm; matrix, 442×512; FOV, 17–22 cm; 0 gap; axial), 3D turbo spin echo FLAIR (TR, 5,000 ms; TE, 355 ms; time to inversion, 1,800 ms; voxel size, 0.5×0.5×1.0 mm; ST, 1 mm; matrix, 388 ×384; FOV, 19×19 cm; sagittal), and susceptibility-weighted imaging (SWI) (TR, 26 ms; TE, 19 ms; voxel size, 0.9×1.0×1.5 mm; ST, 1.5 mm; matrix, 182 ×256; FOV, 17×23 cm). Diffusion tensor images were acquired in an axial plane and contained the following parameters: TR, 8,440 ms; TE, 90 ms; voxel size, 2.0× 2.0×2.0 mm; ST, 2 mm; imaging matrix, 128 ×128; FOV, 26 × 26 cm; 0 gap; diffusion directions, 25; b value, 1,000 s/mm2. Images were eddy current corrected and processed to create fractional anisotropy (FA) and mean diffusivity (MD) maps.

Morphologic MRI evaluation

MRIs were evaluated by one of two radiologists: a pediatric neuroradiologist with 11 years post-fellowship experience (PC) and a radiologist specifically trained for the evaluation of TSC patients (LOT). Evaluations were performed after a review of five test cases where methods and lesion classification were established by consensus (AvE, LOT, and PC).

For the identification and counting of RMLs and tubers, the volumetric FLAIR images served as the primary data set, were reformatted on an AGFA PACs workstation, and viewed simultaneously in the axial, coronal, and sagittal planes. The axial T2, magnetization-prepared rapid gradient echo, and SWI sequences were consulted for clarification and to distinguish calcifications, cysts, and SENs.

Tubers were defined as cortically located lesions hyperintense to the normal cortex on the T2/FLAIR images that produced thickening or blurring of the normal cortex. RMLs were defined as curvilinear or band-shaped lesions traversing the deep WM and hyperintense to the NAWM on FLAIR or T2-weighted images (Figure 1a). Tubers and RMLs were further evaluated for associated disruption of the gyral folding pattern and cystic and calcified components, and a nomenclature for morphological configurations was adopted (Figure 2).

SENs were defined as lesions originating from the ventricle wall and protruding into the ventricular lumen. SGCTs were defined as SENs that measured >1 cm; although this definition is clinically insufficient, it served our quantitative purpose. For patients with a history of SGCT resection, this SGCT was also counted. Cerebellar TSC lesions were defined as high-signal findings on the T2-weighted images. The hemispheric and lobar distributions of RMLs, tubers, and SENs were calculated.

Quantitative MRI analysis

Elliptical regions of interest (ROIs) were manually placed by an experienced radiologist (JJ) in consensus with a TSC research fellow with 2 years of experience with TSC neuroanatomy (AvE) after establishing a substantial interrater correlation with a pediatric neuroradiologist (PC) (see the "Statistical analysis" section). Using volumetric FLAIR images as anatomical reference, maximally sized ROIs (range, 20–100 mm2) were placed on apparent diffusion coefficient (ADC) maps on (1) the largest RML and its contralateral WM region, (2) the

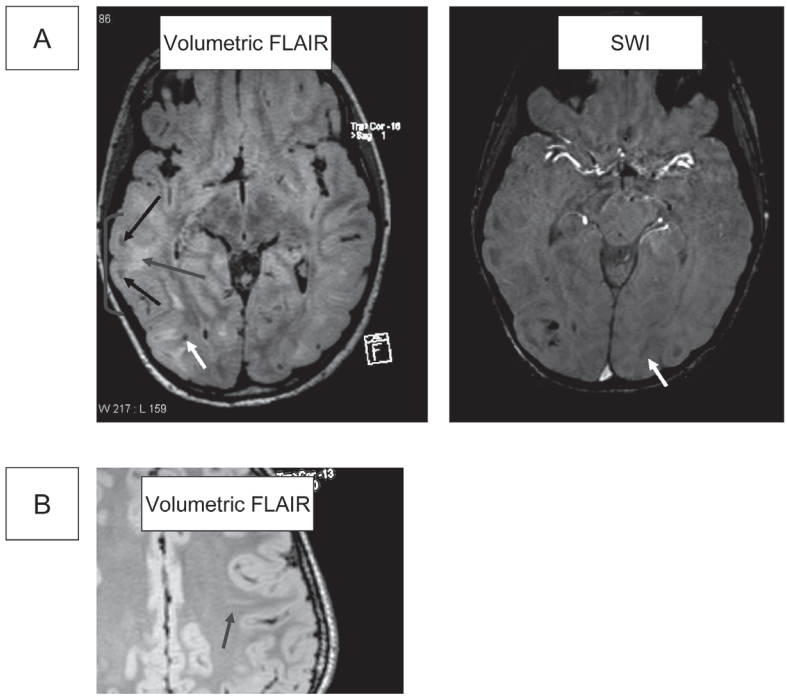

Figure 2. Images and adopted nomenclature of various morphological abnormalities in patients with TSC. A) RML terminating in tuber (grey arrow) and gyral folding disruption (grey bracket). Disruption of the gyral folding pattern was defined as local simplification of the number of gyral folds compared to normal adjacent and contralateral cortex. Note the evidence for hypocellularity in both tubers (black arrows), previously referred to as 'cystic' tubers, defined as low signal iso-intense with CSF on T1 and FLAIR sequences and high signal on T2 weighted sequences. This can be distinguished from mineralization (white arrow), defined as low signal on the susceptibility (SWI) and/or T2-weighted images. Note the confluence of the RMLs in the deep white matter (grey arrow); RMLs that shared part, but not all, of their neuroanatomy were counted as two lesions. B) Image of a subtle isolated RML in a female patient with an IQ of 112 and no neuropsychiatric comorbidity. MRI showed 16 small RMLs, no tubers and no SENs.

largest tuber, and (3) the NAWM of every cerebral lobe in both hemispheres (OsiriX Dicom Viewer, Geneva). Because the frontal lobe is disproportionally large, two ROIs were placed per frontal hemispheric lobe: in the prefrontal WM and the more posterior WM (Figure 3). All ROIs were transposed on identical sections on the FA maps.

After excluding lobes where no NAWM could be visually detected, the mean MD and FA values of each lobe were averaged for each patient to approach a whole-brain index of NAWM MD and FA indices of the cerebral lobes. Cases where the largest RML or tuber were too small to allow a margin of at least 2 mm around the ROIs were excluded from the analysis of DTI metrics due to possible partial volume effects. Cystic or mineralized lesions were avoided as these have aberrant values. Lobes affected by neurosurgery were excluded from all analyses.

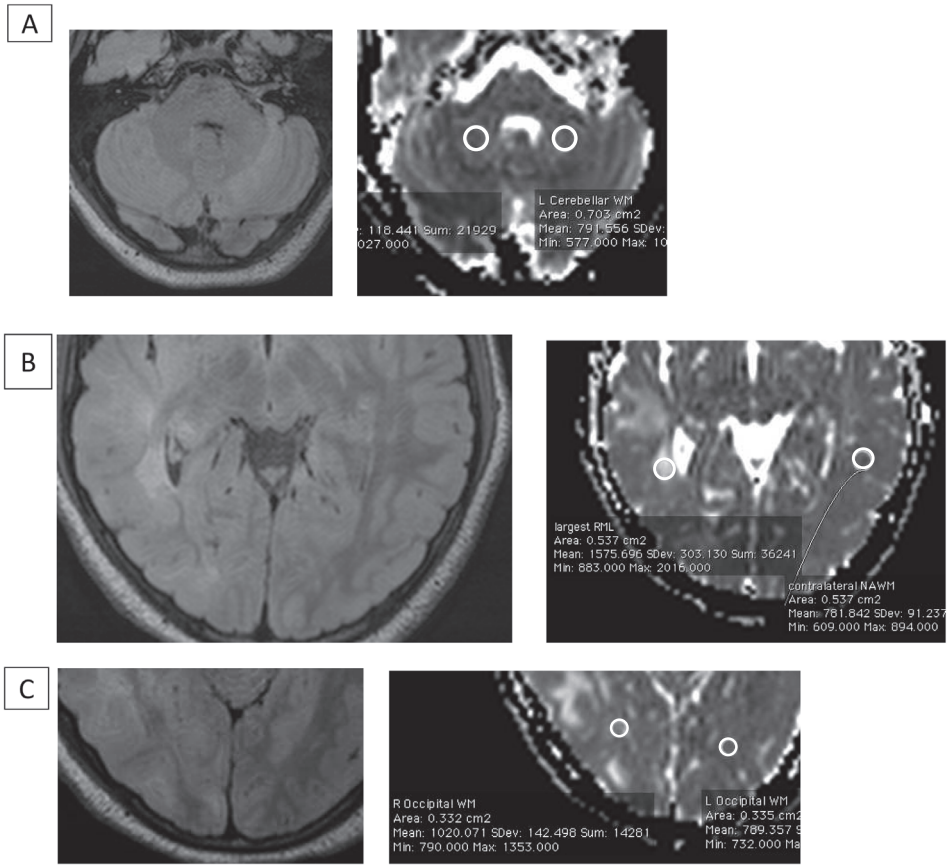

Figure 3. Images showing examples of selected ROIs in one patient. A) ROIs in bilateral cerebellar NAWM, B) ROIs in largest RML and contralateral NAWM, and C) ROI placement in bilateral occipital WM. Due to the severe RML burden, no NAWM could be visually detected in the right occipital lobe and these DTI measurements were excluded from the analyses.

The MD and FA measurements of the same ROIs were obtained for 16 healthy control patients, including 7 children (mean age, 9 years; range, 4–15 years) and 9 adults (mean age, 36 years; range, 29–41 years). Control images were retrospectively retrieved, and inclusion criteria included a normal MRI study using the same imaging protocol and the absence of neurological deficits or developmental delay.

Statistical analysis

To determine interrater agreement for the assessment of RMLs, tubers, and MD and FA values of tubers, RMLs, and NAWM, intraclass correlation coefficients (ICCs) were determined for a subset of 10 MRIs. An ICC of 0–0.20 indicated poor agreement; an ICC of 0.21–0.40, fair agreement; an ICC of 0.41–0.60, moderate agreement; an ICC of 0.61–0.80, substantial agreement; and an ICC of 0.81–1.00, nearly perfect agreement.[28]

Analyses of variance (ANOVAs) and correlations were performed to (1) explore relationships between frequencies of RMLs, tubers, SENs, SGCTs, and cerebellar lesions, (2) test the association between the MD and FA of the largest RML and these macrostructural lesion frequencies, (3) compare RML frequency with intelligence outcomes, seizure variables, and age at MRI, and (4) compare NAWM DTI metrics of the cerebral lobes of TSC and control patients. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Confidence intervals were 95 %, and α was set at 0.05.

RESULTS

Interrater agreement

For 10 MRIs, the ICCs between PC and LOT were r=0.71 for RMLs and r = 0.80 for tubers, indicating substantial agreement. The remaining MRIs were reviewed by both PC (10) and LOT (10).

For DTI indices, the interrater correlation between PC and JJ/AvE was 0.93 for RML MDs, 0.80 for contralateral NAWM MD, and 0.74 for mean lobar NAWM MD values, indicating substantial to nearly perfect agreement. These agreements were the same for FA values. The MD/FA measurements by JJ/AvE were used for further analyses.

Patient characteristics

The MRIs of 30 patients (13 males and 17 females) were included, with a mean age at time of MRI of 15.5 years (range, 5–51 years). Of these patients, 23 (77 %) had a history of epilepsy, 8 (29 %) had a history of IS, and 11 (37 %) had refractory epilepsy. Eight patients had a history of neurosurgery: six received epilepsy surgery and two underwent SGCT resection. Twelve (40 %) of the patients had an intellectual disability (IQ<70). Of the 28 patients who received genetic mutation analysis, 11 (39 %) showed a TSC1 mutation, 15 (54 %) had a TSC2 mutation, and in two (7 %) patients, no mutation was identified.

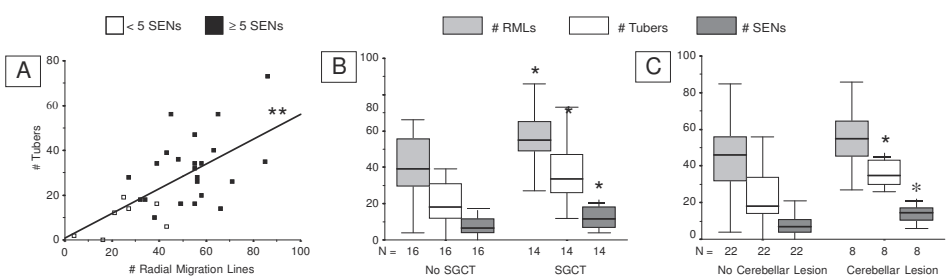

Figure 4. Graphs depicting associations between morphological neuroanatomical characteristics of the study group. A) Scatterplot showing the correlation between the frequency of RMLs and tubers, showing categories of SEN frequencies. B) Boxplot depicting the association between the (history of) SGCT and frequencies of RMLs, tubers and SENs. C) Boxplot depicting the association between cerebellar lesions and frequencies of RMLs, tubers, and SENs. Box = 25th–75th percentile, horizontal line = 50th percentile. *, $P<0.01$; **, $P<0.001$

MORPHOLOGICAL AND MICROSTRUCTURAL RELATIONSHIPS

The neuroradiological characteristics of the study group are listed in Table 1. All patients showed RMLs and 29 (97 %) patients showed tubers. Fifty-seven percent of RMLs ex tended into a tuber. Isolated tubers were rare (3 %), small, and not cystic or mineralized. RML frequency, tuber frequency, and SEN frequency were significantly correlated ($P \leq 0.001$, r>0.56 for all comparisons; Figure 4A).RMLs, tubers, and SENs were equally distributed over the hemispheres. Compared with normal lobar proportional volumes,[29] RMLs and tubers seemed to be slightly overrepresented in the frontal and occipital lobes (Figure 5). A trend was observed between the age at MRI and the frequency of RMLs (P=0.06).

After excluding five patients where partial volume effects may have influenced RML ROI measurements, a significant negative correlation was found between the MD and FA of the largest RML (P=0.003, r=−0.56). Interestingly, the MD of the largest RMLs were associated with higher frequencies of RMLs (P=0.10, r=0.33), tubers (P=0.05, r=0.39), and SENs (P=0.006, r=0.55), although only the latter comparisons were significant. The largest RML FA showed no significant associations with lesion frequencies (P>0.20 for all comparisons).

Table 1. Neuroanatomical characteristics of the study group.

	N±SD (range)
Morphological characteristics	
RMLs	47±19 (4–86)
Tubers	27±16 (0–76)
Tuber–RML units	28±16(0–76)
Without gyral distortion	6±4 (0–23)
With gyral distortion	18±12 (0–40)
Cystic tubers	3±5 (0–19)
Mineralized	1±0.8 (0–6)
Isolated tubers	0.43±0.7 (0–5)
Isolated RMLs	20±15(2–48)
SENs	10±6 (0–22)
No. of patients with SEN >1 cm	13 (43 %)
No. of patients with cerebellar lesion	7 (23 %)
DTI characteristics (mean and standard deviation)	
MD tuber (×10−6 mm2/s)	1,196± 225 (909–1,863)
MD RML (×10−6 mm2/s)	1,120± 190 (826–1,576)
FA RML	0.23±0.06 (0.31–0.37)
MD NAWM (×10−6 mm2/s)	758± 60.0 (633–844)
FA NAWM	0.44±0.12 (0.31–0.53)

N number, SD standard deviation, NAWM normal-appearing white matter

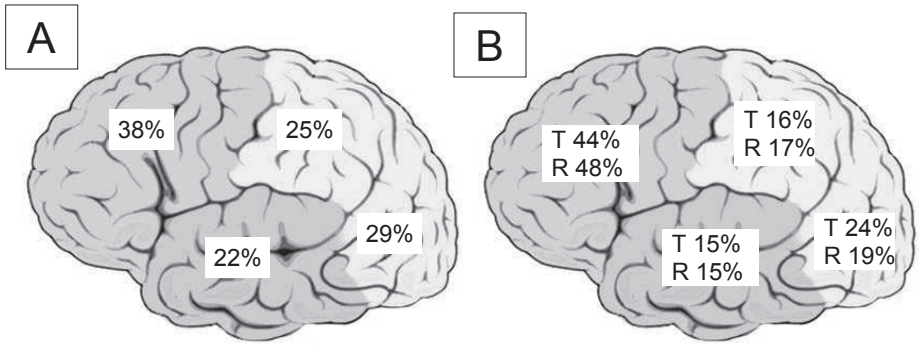

Figure 5. Proportional distribution of RMLs and tubers in the study sample, compared with normal proportional volumes of cerebral lobes. Sagittal view of schematic drawings of two brains, depicting A) proportional volumes of lobes in humans,[29] and B) proportional distribution of RMLs (R) and tubers (T) of the study sample (N=30). The proportional volume of the insular lobe is 1.7 %, and RMLs and tubers were similarly distributed (both 1%).

There was a trend towards co-occurrence of SGCTs and cerebellar lesions (P=0.06). Patients with SGCTs showed significantly more RMLs (P=0.01), tubers (P=0.005), and SENs (P=0.01), but no significant differences in DTI metrics of RMLs or tubers (P>0.10 for all comparisons) (Figure 4B). Patients with cerebellar lesions showed significantly more tubers (P=0.007) and SENs (P=0.01), but no significant differences in RML frequency (P=0.17) or differences in DTI values of RMLs and tubers (P>0.4 for all comparisons) (Figure 4C).

Relationship between RMLs and the neurocognitive phenotype

Highly significant relationships were observed between frequency of RMLs and quantitative neurocognitive outcomes such as age of seizure onset, IQ/DQs, and rate of autistic features (Table 2). Associations with dichotomous variables such as a history of IS were weaker.

Table 2. Results of statistical analysis showing bivariate associations between RML frequency and neurodevelopmental outcomes, depicting p values for ANOVAs and correlations.

	RML frequency
Age at MRI	0.06 (r=−0.34)
History of epilepsy	0.05
History of infantile spasms	0.12
Age of seizure onset	0.003 (r=−0.60)
IQ/DQ	0.01 (r=0.48)
Rate of autistic features[a]	0.007 (r=0.56)

Clinical data were available for all patients, IQ/DQ scores were available for 26 patients, and SCQ scores were available for 22 patients ANOVA analysis of variance, r correlation coefficient, IQ/DQ intelligence/developmental quotient

[a] As measured by the SCQ (see the "Methods" section)

NAWM characteristics

Of all supratentorial hemispheric lobes, no NAWM could be detected in 105 (35 %) lobes of 25 patients. After exclusion of these lobes, TSC and control patients did not show significant differences for mean supratentorial lobar NAWM MD and FA values (P=0.26 for both comparisons) (Figure 6). The DTI metrics of cerebellar NAWM were normal and similar for cerebellar lobes with our without a lesion (P>0.48 for all comparisons).

DISCUSSION

Our findings suggest that RMLs are the most frequent neuroanatomical lesion in TSC patients and are strongly associated with neurocognitive morbidity. The combination of DTI and high-resolution FLAIR imaging allowed these focal migration abnormalities to be distinguished from normal brain parenchyma, demonstrating that RMLs are responsible for DTI abnormalities in the cerebral lobes.

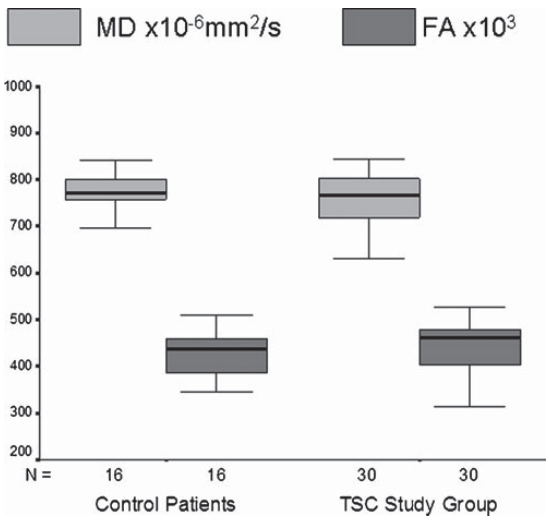

Figure 6. Boxplot comparing DTI metrics of normal-appearing white matter (NAWM) of TSC patients and control patients. No statistical difference was found between the two groups. Box = 25th–75th percentile, horizontal line = 50th percentile.

Characteristics of RMLs

The observed average frequency of 47 RMLs compellingly resembles the mean of 46 morphological abnormalities found in a recent neuropathological study.[30] Only about half of these RMLs terminated in a tuber, and RMLs without cortical involvement were generally smaller. In the RMLs, the MD, reflecting the magnitude of water diffusion regardless of the direction, and the FA, reflecting the degree of directionality of the diffusion, were abnormal and inversely correlated. This is similar to previous observations in TSC[13,14,31] and

in WM associated with other malformations of cortical development.[32] The observation that the NAWM DTI metrics of the cerebral lobes of TSC patients were similar to that of control patients underlines that WM abnormalities in TSC are not diffuse and reflect focal migration abnormalities rather than other causes of disconnectivity. In this regard, we prefer "radial migration line" to previous nomenclature such as "subcortical tubers," hamartomas, or radial glial bands. Although the observed trend towards more RMLs at a younger age is probably due to referral bias, future research should investigate features of RMLs in utero and in early childhood.

Contribution of RMLs to the neuroradiological phenotype

The strong correlations between the frequencies of RMLs, tubers, and SENs confirm that these lesions originate from a common biological dysfunction in the periventricular zone. Since these lesions show also histological similarities[15,16,33] and evidence of mTOR dysregulation,[34] RMLs, SENs, and tubers should be regarded as an inextricable triad representing aberrant proliferation and migration in TSC. Solely identifying patients by tubers or SENs may lead to a misleading conclusion of a "negative" MRI at a time where intervention may greatly improve neurodevelopmental prognosis. Hence, we advocate combining tubers and RMLs into one major diagnostic criterion.

The significant associations between the MD of the largest RML and lesion frequencies support histological observations that both lesion frequency and severity may covary with mTOR dysregulation.[30] Furthermore, patients with SGCTs and cerebellar lesions showed more RMLs, tubers, and SENs, suggesting that lesion frequency may help stratify patients at risk for SGCT growth. However, due to partial volume effects, only the largest RMLs could be interrogated with DTI metrics, limiting our analyses between lesion frequency, severity, and growth.

The exact mechanisms by which enhanced mTOR signaling leads to migration deficits are unclear. mTOR is expressed during early brain development in progenitor cells in the ventricular zone and in early neuronal cells in the nascent cortical plate. Loss of function of regulatory genes that are expressed simultaneously with mTOR, such as TSC1 and TSC2, PTEN, and NF1,[35,36] would result in dysregulated mTOR activity, causing disturbed proliferation of neural and astrocytic precursor cells and cytomegaly, compromising neuronal migration and differentiation.

Relationship between RMLs and the neurocognitive phenotype

The frequency of RMLs was strongly associated with the age of seizure onset and a history of seizures, underlining that WM migration deficits are a robust biomarker for these important prognostic outcomes. Although this association could be explained by the strong relationship between RML and tuber frequency, and a subcortical origin of seizures is controversial, our observations add to evidence of epileptic discharges in regions without tubers[37] or outside of tubers.[38] Together with observations of seizures in a TSC mouse model with only subcortical lesions[39] and neurosurgical experience that relatively large resections of cortex and WM are often necessary to achieve seizure freedom,[40] our findings suggest that WM should be included in investigations in epilepsy studies.

Compellingly, RML frequency was also strongly associated with the level of intelligence and rate of autistic features, concordant with observations that these variables are inextricably linked with age at seizure onset.[41] Together with our findings of normal NAWM microstructure, our results suggest that, in TSC, neurocognitive deficits are suggest that, in TSC, neurocognitive deficits are caused by focal migration and proliferation abnormalities, rather than primary, global synaptic dysfunction primary, global synaptic dysfunction. By interfering with the development of neural circuitry, RMLs could impair neurotransmission, resulting in seizures and deficits in intelligence, communication, and social skills. The regional distribution of RMLs may particularly affect longer-ranging WM tracts, concordant with a previous observation that impaired WM integrity of the corpus callosum was associated with autistic features.[24]

Since quantifying RMLs was extremely laborious, the observed strong associations with neurocognitive outcomes and DTI and FLAIR abnormalities should encourage research on automated methods for evaluating whole-brain WM diffusivity or hyperintensity indices as potential biomarkers. DTI may well detect brain abnormalities before conventional MRIs in early childhood or even prenatally, which is particularly critical in the context of administration of neuroprotective therapies.

Limitations

Although our study population was relatively large, the number of our analyses exceeded that allowed for this sample size and should be regarded as exploratory. Distinguishing NAWM from multifocal dysplasia is complex in TSC; the variable distribution of RMLs does not allow standardized ROI placement or using a template, which may introduce data variability. However, we established acceptable interrater correlations and further increased the confidence level of our validity for the NAWM DTI metrics by including one ROI in every lobe, and even two ROIs in the frontal lobe, and taking the average of these measurements. This is the first study to exclude lobes without visible NAWM, making this the most detailed NAWM analysis in TSC. Since NAWM was detected in every patient and all indices contributed equally to the analyses, selection bias was limited.

Since most of the study patients had a positive history of epilepsy, observed DTI changes could be related to sequelae of chronic seizures, such as neuronal loss and gliosis. However, the normal DTI measurements in the NAWM of our patients suggest that aberrant FA and MD values in patients with TSC are relatively specific for the presence of RMLs. We did not address the FA of tubers to limit our comparisons as FA abnormalities in tubers have been well established and RMLs were the focus of this paper.

CONCLUSION

This study suggests that, in TSC, disruptions in cerebral neural circuitry are not diffuse, but primarily determined by focal migration abnormalities. RMLs are the most pervasive neuroanatomical feature, are the major contributor to WM microstructural abnormalities, and are strongly associated with neurocognitive morbidity. These observations pave the way for studies on whole-brain DTI parameters as a biomarker for neurocognitive morbidity in TSC.

ACKNOWLEDGMENTS

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5

COGNITIVE AND ADAPTIVE DEVELOPMENT OF PATIENTS WITH TUBEROUS SCLEROSIS COMPLEX: A RETROSPECTIVE, LONGITUDINAL INVESTIGATION

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ABSTRACT

The aim of the work described here was to systematically analyze the developmental trajectory of patients with tuberous sclerosis complex (TSC). A retrospective longitudinal chart review was performed, selecting patients who received multiple neuropsychological assessments. Intellectual/Developmental Quotients, Adaptive Behavior Composite scores, and clinical data were collected. On available EEGs, interictal epileptiform discharges were counted. Sixty-six (18%) patients with TSC received multiple cognitive and adaptive development assessments. The mean intelligence of this study group remained relatively stable, albeit variable. Significant decline in adaptive functioning was observed, associated with lower age at seizure onset. Patients who underwent neurosurgery prior to baseline testing showed cognitive improvement. Developmental declines were significantly associated with increased numbers of antiepileptic drugs, with a trend toward association with mutation type and interictal epileptiform discharges. This study suggests that the developmental course of patients with TSC may be altered by epilepsy comorbidity and neurosurgery, underlining the need for early and effective interventions in this population.

INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder that arises from germline mutations in either the TSC1 or TSC2 gene, which encode hamartin and tuberin, respectively.[1] These proteins function as tumor suppressors by forming a heterodimer complex that inhibits the mammalian target of rapamycin complex 1 (mTOR1) pathway.[2,3] Loss or dysfunction of these proteins results in growth of benign tumors called hamartomas in various organ systems, including the brain. Clinical manifestations associated with brain involvement are common and include epilepsy, cognitive impairment, and psychiatric disorders. The prevalence of cognitive impairment in TSC is estimated to be between 44 and 70% and has been associated with various epilepsy features, including early age at seizure onset, infantile spasms (IS), mixed seizure type, TSC2 mutations, and poor seizure and IS control.[4-12] In a study of infants with TSC, development was found to be slow but relatively stable, although a subset showed large variability.[13]

Increased knowledge of the developmental course and the influence of epilepsy comorbidity will provide more information for patients and assist clinicians in important treatment decisions, especially now that early (preventative) treatments with mTOR inhibitors are being considered. Therefore, our primary aim was to determine if the generally stable development reported in infants with TSC is also the case for older age cohorts. Furthermore, we wanted to determine if, and which, factors influence cognitive and adaptive development in patients with TSC, exploring the effect of genetic background, age at seizure onset, neurosurgery, and autism, as well as dynamic epilepsy characteristics such as interictal epileptiform discharges (IEDs) and antiepileptic drugs (AEDs) used for treatment.

METHODS

Study group

Charts of all patients with a definite diagnosis of TSC who were treated at the Herscot Center for TSC at Massachusetts General Hospital were reviewed. Patients who had received neuropsychological assessment (NPA) at least twice were identified and the results of the earliest and most recent NPAs (NPA1 and NPA2) were obtained.

This study was approved by the institutional review board of Massachusetts General Hospital.

Neuropsychological assessment

Neuropsychological assessment is routinely offered as a part of clinical care for patients with TSC at the time of diagnosis and at the time of changes in their neuropsychiatric morbidity or developmental trajectory. Comprehensive NPAs are performed by an experienced neuropsychologist at the Psychology Assessment Center of Massachusetts General Hospital (M.P.) and include assessment of cognitive and adaptive functioning. For the study cohort, we gathered test scores from measures of intelligence and adaptive skills at two time points (NPA1 and NPA2). Intellectual Quotient (IQ) or Developmental Quotient (DQ) was derived from one of the following six neuropsycholog ical measures, selected in conformity with the

mandate of best clinical practice: (1) Bayley Scales of Infant Development, Second Edition (BSID-II);[14] (2) Stanford–Binet Intelligence Scale, Fifth Edition;[15] (3) Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI);[16] (4) Wechsler Intelligence Scale for Children, Fourth Edition (WISC);[17] (5) Wechsler Abbreviated Intelligence Scale—Revised (WAIS-R);[18] and (6) Differential Abilities Scales.[19] To assess adaptive functioning, the Survey Form of the Vineland Adaptive Behavior Scale[20] was used, yielding standardized scores for communication, daily living skills, socialization, and, for children less than 6 years old, motor skills, resulting in a total Adaptive Behavior Composite (ABC) score. Both IQ and ABC scores have a mean of 100 and a SD of 15. For patients with outcomes on the floor of the standardized scores, we calculated DQs (mental age/chronological age × 100), where a DQ of 100 would be considered the mean.

Clinical data

Clinical data were obtained from the clinic records of the consultation with the referring child neurologist (E.T.), which was always close in time before each NPA. Information was collected on gender, age, and results of genetic mutation analysis. The diagnosis of autism spectrum disorders (ASDs) was made by our affiliated psychiatrist and M.P. based on DSM-IV criteria. Epilepsy was characterized based on age at seizure onset, IS, and number of AEDs used for seizure control. Data on epilepsy surgery and subependymal giant cell tumor (SGCT) resection surgery were obtained.

Electrophysiological data

For patients who received an EEG within a year of each NPA, available records were reviewed by an experienced and blinded neurophysiologist (C.C-S.). EEGs were obtained using a 19-channel montage with the standard 10–20 electrode placement system. For routine clinical EEGs, the entire recording was reviewed and manually scored. For longer recordings, the first 20 minutes of the wake and sleep states were reviewed and scored. Studies were screened for IEDs. Counted discharges were averaged to a value per minute. Regional scores were summed to generate a whole brain score, portraying a quantitative index of the overall interictal activity of the EEG.

Statistical analysis

Statistical analysis was performed using SPSS for Windows (Version 11.5).

To determine change in developmental outcomes, a paired t-test was performed to compare the IQ/DQ and ABC outcomes at NPA1 and NPA2. We preferred total change in developmental outcomes over time-dependent outcomes as the latter would inappropriately imply linearity in gains or declines. Because the NPAs were performed at different time intervals, changes in IQ/DQ and ABC were calculated and correlated with the IQ/DQ change/year and ABC change/year to justify use of the former as primary outcomes.

Because of the different sample size, predictors of changes in intelligence and adaptive functioning were examined using a general linear model for each outcome. Levene's test was included to investigate homogeneity. Gender, mutation type, and a positive history of IS were included as predictors, and age at seizure onset and age at NPA1 were defined as

covariants. To evaluate the influence of changes in IEDs and number of AEDs on changes in cognitive and adaptive outcomes, Pearson correlations were performed. Missing values were excluded when determining percentages in the results. To compare changes in IQ/DQ and ABC in groups with and without a diagnosis of ASDs or a history of neurosurgery, t-tests were performed.

In the general population with cognitive impairment, one would expect 6% to show an IQ change of more than 10 points when reevaluated with the Wechsler scale.[21] To optimize the sensitivity and relevance of our findings, we interpreted a change of at least 15 IQ/DQ points as significant. For all tests, a confidence interval (CI) of 95% was used. We interpreted associations as significant for α values ≤ 0.05; α values ≥ 0.05 and ≤ 0.1 were considered trends.

RESULTS

Study group

Sixty-six (18%) patients with TSC in our clinic had undergone at least two NPAs, with a mean interval of 3.9 years (range: 0.5–9.3). Of these 66 patients, henceforth called the study group, all (100%) had completed multiple intelligence tests and 41 (62%) had received multiple adaptive functioning assessments. Demographic and clinical data of the study group are summarized in Table 1.

Course of cognitive and adaptive development

The mean IQ/DQ change for the total study group was – 2 points, which was not significant (P<0.29, CI: –1.9 to 6.3) and showed large variability, ranging from a relative decrease of 48 to an increase of 52 points, with a SD of 16.7 (see Figure 1, left). Twenty (30%) patients had a significant IQ/DQ change of > 15 points, including 9 patients with an increase and 11

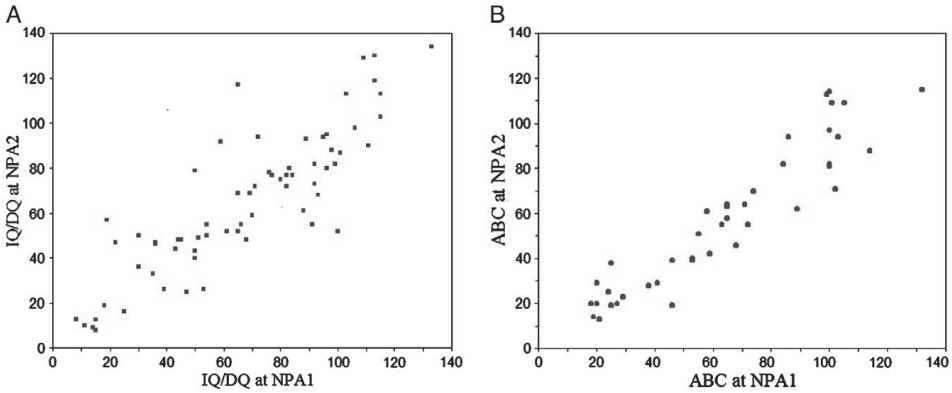

Figure 1. Scatterplot depicting (A) intellectual outcomes and (B) adaptive functioning scores at the first and second evaluations. IQ/DQ, Intellectual/Developmental Quotient; ABC, Adaptive Behavior Composite; NPA, neuropsychological assessment.

Table 1. Clinical characteristics of (A) the total study cohort and the subgroups with a (B) significant relative increase or (C) significant relative decrease in cognitive outcomes.

	A Total study group (N = 66)	B Cognitive improvement (N =9)	C Cognitive deterioration (N = 11)
Gender (male)	32 (48.5%)	3 (33%)	9 (82%)
Characteristics at NPA1			
Mean age (years)	5.8 (0.5–20)	2.8 (0.9–8.4)	3.4 (0.5–12.5)
Mean Intellectual/Development Quotient	67 (8–133)	60 (19–113)	85 (47–111)
Mean Adaptive Behavior Composite score	66 (18–132)	74 (18–132)	84 (46–114)
Mean interval between NPAs (years)	3.9 (0.5–9.3)	5.3 (2.2–7.7)	3.9 (0.9–7.2)
Result of mutation analysis			
TSC1 mutation	14 (21%)	1 (11%)	3 (27%)
TSC2 mutation	39 (59%)	7 (78%)	8 (73%)
No mutation identified	10 (15%)	1 (11%)	—
Not performed	3 (5%)	—	—
Epilepsy variables			
History of epilepsy	58 (88%)	8/9 (89%)	11/11 (100%)
History of infantile spasms	32 (48%)	4/8 (50%)	7/11 (64%)
History of intractable epilepsy	36 (55%)	4/4 (50%)	6/11 (55%)
Mean age at seizure onset (years)	1.3	1.3	0.84
Mean change in number of IEDs/min (N = 23)	1.55 (– 56.3 to 23.5)	– 0.42 (– 4.17 to 1.51)	2.72 (– 20.93 to 23.51)
Mean change in number of AEDs	0.15 (– 3 to 2)	– 0.25 (– 3 to 2)	0.5 (– 1 to 2)
Neurosurgery before NPA1	13 (20%)	3 (33%)	1(9%)
Neurosurgery between NPAs	8 (12%)	1 (11.1%)	2 (18%)

Ranges and percentages provided in parentheses. NPA, neuropsychological assessment; IEDs, interictal epileptiform discharges.

patients with a relative decline (see Table 1). Of note is that 9 of the 11 (82%) patients with a cognitive decline were male. The mean ABC change between NPAs was significant (P<0.0001, CI: 3.2–10.4), with a mean relative decrease of 7 ABC points (see Figure 1, right).

When the total change in IQ/DQ and ABC was divided by the time interval, the change per year was very strongly correlated with the total change (both showed correlations of 0.74, P<0.0001), justifying the use of the outcome change in IQ/DQ and change in ABC as primary endpoints in our regression analysis. Age at seizure onset was shown to have a significant effect (P<0.002) on the change in adaptive scores (Figure 2) and showed a trend toward association with mutation type (P<0.09) (Figure 3).

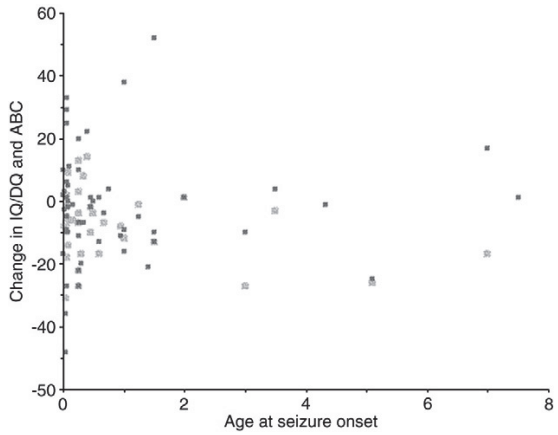

Figure 2. Scatterplot depicting the relationship between age at first NPA and changes in intellectual (dark gray) and adaptive functioning (light gray) outcomes. IQ/DQ, intellectual/developmental quotient; ABC, Adaptive Behavior Composite.

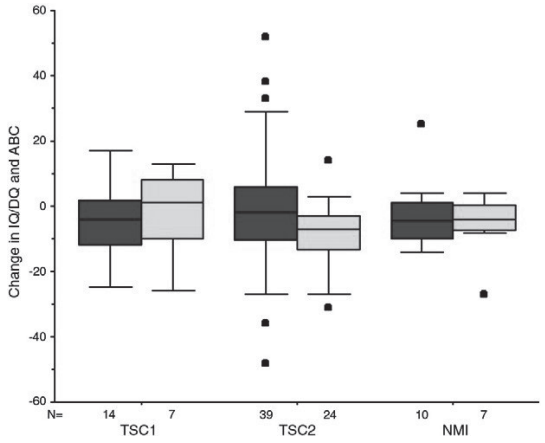

Figure 3. Boxplot showing changes in intellectual and adaptive changes per TSC mutation type, including standard deviations and outliers. Dark blue: change in Intellectual/ Developmental Quotient (IQ/DQ); light blue: change in Adaptive Behavior Composite score (ABC); NMI, cohort with no mutation identified.

Levene's test and the regression model revealed no significant variance in factors included in the regression analysis and no significant predictors of changes in IQ/DQ. Gender, age at NPA1, and a positive history of IS were not significantly associated with change in ABC.

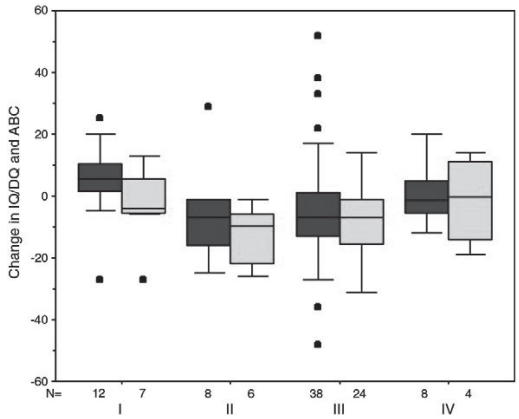

Figure 4. Boxplot depicting changes in intellectual and adaptive functioning outcomes in subgroups I (neurosurgery before NPA1), II (neurosurgery between NPAs), III (epilepsy and no history of neurosurgery), and IV (a negative seizure history). The numbers of available intellectual and adaptive functioning outcomes for each cohort are noted underneath the x axis.

TSC mutation subgroups

Because the regression analysis hinted at an association between mutation type and adaptive development, genetic subgroups were compared (see Figure 3). These groups had a similar mean age at NPA1. The group of patients with a TSC1 mutation showed a mean decline of 5 IQ/DQ and 2 ABC points (SD = 12 and 14, respectively). The TSC2 mutation subgroup showed a mean loss of 1 IQ/DQ and 8 ABC points (SD = 20 and 11, respectively), and the no mutation identified (NMI) group showed a mean loss of 2 IQ/DQ and 6 ABC points (SD = 11 and 10, respectively). There were four pairs of siblings in the study group, and identical genetic mutations were identified in three pairs. Within siblings, IQ/DQ changes were extremely variable, ranging from 13 to 69, and in all but one pair IQ/DQ increased in one sibling and declined in the other.

Subgroup with neurosurgery

Thirteen patients underwent neurosurgery before the first NPA, including 10 (77%) for epilepsy surgery and 3 (23%) for subependymal giant cell tumor (SGCT) resection. Over a mean interval between NPAs of 4.7 years, this group displayed a significantly different developmental trajectory, showing a gain of 7 IQ/DQ points versus a loss of 4 points for the group without a history of neurosurgery ($P<0.02$). There were mean gains of more than 7 IQ/DQ points for the patients who had epilepsy surgery and 5 points for the patients who had SGCT surgery (Figure 4). There was no significant difference in change in adaptive scores between these two groups ($P<0.31$), although the group with a positive surgery history showed a decline of 3 ABC points, which was less than the 8 ABC points of the remaining cohort. For the patients for whom two ABC measurements were available, the 7 patients who had had epilepsy surgery showed a mean loss of 5 ABC points, whereas one patient who underwent SGCT surgery had a gain of 9 ABC points.

Eight (12%) patients from the study group underwent neurosurgery between NPAs (Figure 4): five received epilepsy surgery and three underwent SGCT resection; none of these patients had prior neurosurgery. All three SGCT surgery patients showed a relative decline in IQ/DQ (range: –10 to – 25) and ABC (range: –12 to – 26), possibly related to postsurgical temporary obstructive hydrocephalus just before NPA2 in one patient and extreme anxiety during NPA2, which might have underestimated performance, in a second patient; for the third patient, no clear cause was evident from the medical records. The five patients who underwent epilepsy surgery between NPAs showed very variable, but no mean change in, IQ/DQ (range: –22 to + 29), but a mean loss of 9 ABC points (range: –22 to – 1).

Age at NPA1 was very similar (4.8) for both the group with neurosurgery at baseline and the group with recent neurosurgery, indicating that the first group was operated on at a younger age than the latter.

Epilepsy characteristics

Among the study group, 58 (88%) patients had a positive history of epilepsy at baseline assessment and 3 (5%) developed epilepsy between NPAs. The various epilepsy variables are summarized in Table 1. The regression model showed a significant relationship between younger age at seizure onset and change in adaptive outcomes, which could not be explained by a significantly younger age at first examination ($P<0.24$). Plotting of the data showed that greater variability of IQ/DQ and ABC changes was related to younger age at seizure onset (Figure 2). A significant negative correlation was found between number of AEDs and cognitive change ($P<0.02$) as well as change in adaptive functioning ($P<0.01$). Complete information on medication use was available for 62 (94%) patients. Thirteen (21%) of these patients did not use AEDs at the time of both NPAs, including the 8 patients with no history of epilepsy. At NPA1, 14 (23%) patients used valproate, 12 (19%) lamotrigine, 9 (15%) levetiracetam, 8 (13%) carbamazepine, 5 (8%) vigabatrin, 4 (6%) gabapentin, and 3 (5%) topiramate. At NPA2, the AED regime had been changed in 54 of the 62 patients; 7 (11%) patients started using AEDs, 5 (8%) patients ceased AED treatment, 6 (10%) used a decreased number of AEDs, and 12 (19%) used an increased number of AEDs. At NPA2, 23 (37%) patients used lamotrigine, 19 (31%) levetiracetam, 8 (13%) valproate, 6 (10%) carbamazepine, 4 (6%) gabapentin, 3 (5%) topiramate, and 3 (5%) vigabatrin.

All eight patients without a history of epilepsy had IQ/DQ values > 65 at NPA1. This group showed average declines of 0.5 IQ/DQ and 1.5 ABC points, compared with 2.6 and 7.5 points for the group with a positive history of epilepsy, respectively (Figure 4).

EEG analysis

For 23 patients, there were available EEGs obtained within a year of both NPAs. This EEG subgroup showed a mean increase in number of IEDs of 1.55 discharges/minute (see Table 1). The group that lost more than 15 IQ/DQ points (N = 5) showed an average increase of 2.7 discharges/minute at EEG2, whereas the group with a significant IQ/DQ increase (N = 4) showed an average IED decrease of 0.4/minute (Table 1); similar phenomena were observed for available ABC scores. When changes in IQ/DQ and ABC were correlated with

changes in the number of IEDs per minute per whole brain, no significant associations were found ($P<0.86$ and $P<0.38$, respectively).

Subgroup diagnosed with autism spectrum disorders

Twenty-seven (41%) patients from the study group were diagnosed with an autism spectrum disorder. At time of the first evaluation, the group with ASDs had a lower mean IQ/DQ of 50 versus 79 for the group without ASDs ($P<0.00$) and a mean ABC of 49 versus 80 ($P<0.00$). Compared with the group without ASDs, the group with ASDs showed no significant difference in IQ/DQ or ABC score changes ($P<0.33$ and $P<0.28$, respectively).

DISCUSSION

The current study is the largest longitudinal investigation of intellectual and adaptive development in patients with TSC to date, and identifies significant associations with neurosurgery and epilepsy characteristics. For the whole study group, mean cognition remained relatively stable, although one-third of patients showed a significant change in cognitive performance, confirming previous observations in infants with TSC.[13] Supported by large SDs of IQ/DQ changes, this evidence suggests that the cognitive trajectory of patients with TSC can be highly variable. Cognitive decline was associated with increased numbers of AEDs, which likely reflects poor epilepsy control. Refractory epilepsy is common in patients with TSC, and epilepsy has been associated with poor cognitive outcomes.[22] Developmental decline can also occur in patients with severe and early-onset epilepsy, warranting early and aggressive treatment of seizures. However, the neurocognitive side effects of anticonvulsants could also impair function,[23] striking a delicate balance for clinicians. The medication use of the study cohort shows a slight shift toward second- and third- generation AEDs in accordance with best practice, but this study type did not allow for investigation of the effects of specific AEDs on the developmental trajectory.

The significant relative decline in adaptive scores in the total study population was the most consistent finding of our study. This indicates that patients fall increasingly behind in their neurodevelopment, which is not to be confused with the term regression, as the latter implies loss of skills instead of slower gain. Adaptive decline was significantly related to younger age at seizure onset and increased number of AEDs, confirming the association between early seizure onset and detrimental developmental outcomes.[6,24] The observations that the patients with significant changes in developmental outcomes were relatively younger and that this variability stabilizes as age advances emphasize that infancy is a critical time when influences such as seizures can alter brain development and future outcomes in patients with TSC. The observation that patients with low baseline cognitive outcomes increased their scores and patients with relatively higher outcomes showed deterioration (Table 1) could indicate regression to the mean, which is related to large SDs, although plotting of the data showed no evidence for this statistical phenomenon (Figure 1).

Interestingly, the subgroup who had undergone neurosurgery before the first evaluation showed a significant improvement in intellectual performance, which occurred regardless of surgery indication and could not solely be explained by a larger interval between NPAs.

Whereas findings in general pediatric epilepsy surgery patients have been inconsistent,[25,26] studies in epilepsy surgery patients with TSC have reported excellent long-term seizure outcomes and quality of life,[27,28] confirming our observations, although referral or reporting bias should be considered. In contrast, the TSC subgroup who underwent neurosurgery between NPAs showed variable and little mean change in developmental outcomes. This may reflect referral bias or a pattern of initial postoperative variation followed by improvement in neurosurgical patients with TSC. The compelling observation that the three patients with a history of SGCT surgery showed intellectual improvement after neurosurgery warrants further study focusing on the neurocognitive consequences of SGCT growth.

Although theoretically IEDs may influence cognitive performance, there are few investigations of this clinically relevant issue.[29] Interestingly, we found that the frequency of IEDs was increased on available EEGs of patients who manifested significant cognitive decline, and that it was decreased in the group with relative improvement, although this was not significant. The epilepsy phenotype of TSC is relatively severe and the effect of seizures may overshadow potential cognitive effects of IEDs. As the EEG study sample was relatively small, future research should provide more information on the effect of interictal discharges on cognition to guide clinicians in the decision on treating this phenomenon.

Although cognitive deficits in TSC are related to altered signaling caused by the genetic mutation, change in IEDs was extremely variable within siblings affected by the same genetic mutation, suggesting that there is no genetic pre-determination for developmental gain or decline. Effects of the relatively more severe seizures and psychiatric manifestations in patients affected by TSC, TSC2 mutations in particular,[30] may override inherent cognitive abilities. This is reflected by the variability in outcomes in the TSC2 cohort which could not solely be explained by young age at NPA. Interestingly, the NMI cohort showed the most stable developmental trajectory, reflecting the previously reported milder neurological phenotype of patients with NMI.[31]

Notably, a remarkably large proportion of men showed cognitive and adaptive decline versus women (9:2), a phenomenon previously shown in males with other autosomal dominant syndromes,[32] although the small subgroup limited our statistical inferences.

In patients with autism, declines and variability in adaptive behavior scores have been shown across all ages,[33] more so than in individuals with learning disabilities without autism.[34] In this population, the subgroup with ASDs did not manifest a significantly different course in intellectual or adaptive outcomes. This could be due to the low baseline cognitive and adaptive functioning of this cohort, or a potential nuanced difference may be obscured by the effect of the relatively severe epilepsy type found in patients with TSC.

There are several limitations inherent to this type of study. Although previously seizure frequency has been suggested to be of importance in cognitive development in children with TSC-related epilepsy,[35] this variable was difficult to determine retrospectively. Part of the variability in the cognitive outcomes could be caused by interscale validity variability, especially at young age. However, the use of multiple cognitive measures was inherent to the inclusion of different age groups and similar to other longitudinal studies on cognitive development.[36] Applying a rigorous cutoff of 15 IEDs and finding a similar proportion of

patients (29%) showing significant change on the Vineland Scale, which is used across all age groups and the predictive quality of which has been validated,[20] support our findings. Furthermore, our regression analyses showed no significant effect of age at evaluation on change in developmental outcomes. Because of the exploratory nature of this study, our effort to address multiple variables of influence on the developmental trajectory may have diminished statistical power and prospective studies should confirm our findings.

Although patients with TSC are routinely offered neuropsychological assessment at the time of diagnosis, most patients do not receive neuropsychological assessment multiple times unless this is clinically indicated. Hence, this cohort represents a relatively small proportion of our large clinic population and may be biased toward the more severe end of the neuropsychiatric spectrum of TSC as a result of referral bias. This is reflected by the relatively large proportion of pediatric and neurosurgical patients. However, the relatively higher IQ/DQs and stable development that was observed in patients not affected by epilepsy is likely to be an authentic reflection of development in the TSC population with milder neurological involvement. Although the investigated patients did not have equal time intervals between NPAs, the observation that the change in IQ/DQ correlated significantly with the IQ/DQ change per year suggests this method is applicable. The average test–retest interval of 4 years allows sufficient time for factors such as physical, psychological, and environmental changes to affect an individual's intellectual ability. The retrospective nature of our study and the large number of variables did not allow for comparisons between subdomains of cognitive and adaptive development.

Low intellectual quotient and adaptive skills are defining features of intellectual disability. Our findings of variable cognitive outcomes and relative decline in adaptive scores confirm the need for repeated routine neuropsychological assessments in patients with TSC[37] and multiple assessments in case of neurosurgery or changes in neuropsychiatric morbidity. The association between epilepsy variables and developmental outcomes suggests that early diagnosis and treatment of epilepsy may prevent these encephalopathic effects and improve developmental outcomes in patients with TSC.

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6

UNDERSTANDING RELATIONSHIPS BETWEEN AUTISM, INTELLIGENCE, AND EPILEPSY: A CROSS-DISORDER APPROACH

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ABSTRACT

As relationships between autistic traits, epilepsy and cognitive functioning remain poorly understood, these associations were explored in the biologically related disorders Tuberous Sclerosis Complex (TSC), neurofibromatosis type 1 (NF1), and epilepsy. The Social Reponsiveness Scale (SRS), a quantitative measure of autistic traits, was distributed to 180 patients with TSC, NF1 and childhood-onset epilepsy of unknown cause (EUC), and compared with SRS-data of 210 individuals with idiopathic autism spectrum disorders (ASDs) and 130 of their unaffected siblings. Scores and trait profiles of autistic features were compared with cognitive outcomes, epilepsy variables and genotype. Regression models showed a significant association between rate of autistic features, intelligence outcomes and epilepsy variables but not with a specific underlying disorder or genotype. The rate of autistic features was strongly associated with intelligence outcomes in TSC and epilepsy patients; in NF1 patients these relationships were weaker. For all study groups, autistic trait subdomains covaried with neurocognitive comorbidity, with endophenotypes similar to that of idiopathic autism. Our data show that in TSC and childhood-onset epilepsy, the rate and phenotype of autistic features are inextricably linked with epilepsy and intelligence outcomes. Such relationships were weaker for individuals with NF1.

Tuberous Sclerosis Complex (TSC) and neurofibromatosis type 1 (NF1) are biologically related syndromes caused by mutations in the tumor suppressor genes *TSC1, TSC2* and *NF1*, respectively. Mutations in these and other genes, such as the Fragile-X mental retardation (*FMR1*) gene, can result in disruption of the mammalian target of rapamycin (mTOR) pathway and are associated with learning disabilities, epilepsy and autistic features. Dysregulation of the mTOR pathway accounts for 10-15% of individuals with autism and may also represent a final common pathway for epilepsy and autism of unknown cause.[1, 2]

Autism is a complex neurobehavioral disorder that includes impairments in social interaction, language and communication deficits, and rigid, repetitive behaviors. Currently, the group of autism spectrum disorders (ASDs) is subclassified into autism, Asperger syndrome and pervasive developmental disorder not otherwise specified (PDD-NOS).[3] Autism associated with neurogenetic syndromes can be classified as 'complex autism' which is characterized by lower intelligence quotients (IQ) and higher rates of epilepsy comorbidity.[4] Because of the heterogeneous etiology but high heritability of ASDs, disorders of a known genetic etiology associated with autism are attractive research models. However, although TSC and NF1 animal models display various neurocognitive features of these syndromes,[5, 6] autistic features have been difficult to reproduce. Clinical investigations of autistic features in TSC, NF1 and epilepsy involved small cohorts, dichotomous ASD outcomes, and lack of phenotypic characterization.[7-9] Little is known about the relative contribution of the genetic risk factor to the autistic features and phenotype, versus the influence of cognitive impairment and epilepsy comorbidity. Increased understanding of the neurodevelopmental manifestations of these disorders will allow for more precise associations to be tested between biomarkers and specific traits, especially now that promising treatments are under study.[10] In the current study, quantitative data on autistic features were compared with epilepsy and intelligence outcomes of large cohorts of patients with TSC, NF1, and childhood-onset epilepsy of unknown origin (EUC), and compared with data of individuals with and without idiopathic ASDs.

METHODS

Patient Recruitment

Individuals with TSC and NF1 were recruited at the outpatient clinics of Massachusetts General Hospital (MGH). Patients with non-familial, childhood-onset EUC with normal brain imaging results were recruited at the outpatient adult and pediatric epilepsy clinics of MGH. Subjects with sporadic, idiopathic ASD and their non-affected siblings were recruited through the Autism Consortium (AC), a research and clinical collaboration that includes 6 Boston-area medical centers. Only AC individuals that had been assigned a diagnosis of ASD based on both the Autism Diagnostic Interview-Revised (ADI)[11] and Autism Diagnostic Observational Schedule-Generic[12] (ADOS) and for whom the Social Responsiveness Scale[13] was available, were included (see Figure 1).

Assessment of autistic features

The Social Responsiveness Scale (SRS)[13] is a widely used, reliable and validated[14] psychometric measure that assesses autistic traits in children of age 4 and older; an adult version is available for research purposes.[15] The scale is completed by caregivers or companions and contains 65 items spanning five domains; 1) social awareness, 2) social cognition including information processing and interpretation, 3) social and reciprocal communication, 4) social motivation, including questions on social anxiety and avoidance, and 5) autistic preoccupations and mannerisms such as stereotyped behavior. Raw scores are converted to gender-based T scores; total T-scores > 60 are highly suggestive of any ASD, and scores > 75 are highly suggestive for autism.

Clinical Information

Medical records were reviewed and the following information was recorded: age at evaluation, gender, history and types of seizures, age of seizure onset, refractory epilepsy (as defined by the International League Against Epilepsy,[16] number of anti-epileptic drugs (AEDs), and presence of a formal ASD diagnosis according to the DSM-IV criteria[3] or ADI/ADOS evaluation.[11, 12] Full-scale intellectual/developmental quotients (IQ/DQs) were available by various measures, according to best practice standards. As full-scale IQ often does not completely reflect academic difficulties[17] and was not available for most NF1 patients, the presence of a learning disability or special education services was recorded, and cognitive function was categorized as 1) no learning problems, 2) specific learning disability (LD), or 3) global intellectual disability (ID, IQ<70). For TSC patients, results of genetic mutation analysis were obtained and categorized as TSC1 and TSC2 mutations, and NMI (no mutation identified). The mutation type was sub-grouped into protein terminating (including nonsense, splice-site, and frameshift mutations, and insertions and deletions ≥ 1 exon) and non-terminating (including missense mutations and small, in-frame insertions and deletions < 1 exon) and the location of the mutation on the TSC2 gene was recorded. This study was approved by the internal review board of MGH and by the Autism Consortium.

Statistical analysis

Total SRS scores for all cohorts were compared with t-tests. Factors associated with total SRS score for the neurodevelopmental disorders were examined using a step-wise linear regression, including gender, type of disorder (TSC, NF1 or epilepsy), history of epilepsy, and refractory epilepsy as fixed factors and cognitive category (ID, LD, no learning disability) and age of epilepsy onset as covariates. Since it has been argued that in studies of neurodevelopmental disorders, IQ should not be included as a covariate,[18] we repeated this analysis without the cognitive variable.

To distinguish the effect of genetic background from epilepsy in the TSC cohort, another model was constructed with total SRS score as a dependent, and mutation group (TSC1, TSC2, NMI) and epilepsy as fixed factors. T-tests were performed to compare SRS outcomes for cognitive categories in NF1, gender, and for specific TSC2 mutation groups and infantile spasms (IS) in the TSC population. Correlations were performed to explore

the association between SRS scores and IQ/DQ and age at evaluation. SRS T-scores were used for all analyses. An alpha of 0.05 was applied with confidence intervals of 95%.

RESULTS

Patient Characteristics

In total, 180 SRS scales were collected at the MGH outpatient clinics, including 64 TSC patients, 50 NF1 patients, and 66 individuals with EUC, henceforth called the study groups. The AC database yielded SRS data of 210 AC probands, henceforth called the 'AC-ASD' group, and 130 AC unaffected siblings, henceforth called the 'AC-siblings' (Figure 1). IQ/DQ scores were available for 45 (69%) TSC patients, 42 (81%) EUC patients, 3 (6%) NF1 patients, 118 (56%) AC-ASD patients and 68 (52%) AC-siblings (see Table 1 for clinical characteristics for all cohorts). Information on epilepsy and developmental/educational trajectory were available for all study groups, however AC database information on the epilepsy type of probands and developmental history of siblings was incomplete. Non-responder characteristics are listed in Figure 1.

Distribution of autistic features

For all study groups, mean SRS scores were significantly lower than the AC-ASD group ($P<0.0001$) and significantly higher than the AC-siblings ($P<0.0001$). The frequencies of patients with an SRS score higher than 60, which is suspect for ASD, was 36 (56%) for the TSC cohort, 20 (40%) for the NF1 cohort, and 35 (53%) for the EUC cohort. The percentages higher than 75, which are suspect for autism, were 24 (38%), 9 (18%) and 13 (20%), respectively. SRS scores for AC probands and siblings were similar to those found previously.[19] Although the NF1 and EUC groups showed normal distributions of autistic features, the TSC population displayed a bimodal distribution (Figure 2). SRS total scores were not significantly correlated with age ($P<0.2$ for all groups).

Association with neurocognitive phenotype

The first regression model showed a significant association between total SRS score and IQ category ($P<0.0001$), refractory epilepsy ($P<0.003$), and epilepsy ($P<0.02$), and the combination of TSC diagnosis and refractory epilepsy ($P<0.001$), but no significant association with TSC, NF1 or EUC ($P<0.42$). Excluding cognitive category from the regression analysis, the relationship between SRS score and epilepsy variables remained the same, and a significant association with a combination of male gender and refractory epilepsy emerged ($P<0.05$).

Correlations showed highly significant inverse relationships between IQ/DQ and SRS total scores for TSC and EUC (Figure 3A). Although patients with NF1 and learning disabilities showed higher SRS scores than normal-learning patients, this difference was not significant ($P<0.11$, CI -8 to 5) (Figure 3B). The TSC cohort showed significantly higher SRS scores in patients with refractory epilepsy ($P<0.0001$, CI 13 – 29), IS ($P<0.02$, CI 2 - 21), or epilepsy without IS ($P<0.01$, CI 4 – 25). The age of epilepsy onset was significantly inversely correlated with the SRS scores (r=-0.33, $P<0.02$). In the EUC study group, SRS

scores were not significantly associated with age of onset of epilepsy (r=-0.15, $P<0.25$) or refractory epilepsy ($P<0.16$, CI -6.1 – 4.3). Due to low number of epilepsy patients, no statistical analyses were performed for the NF1 group and epilepsy variables.

Table 1. Clinical characteristics of study and control groups.

	TSC N= 64	NF1 N= 50	EUC N= 66	AC-ASD N= 210	AC-controls N= 130
Gender					
Male (%)	27 (42%)	31 (62%)	32 (48%)	167 (79%)	60 (46%)
Female (%)	37 (58%)	19 (38%)	34 (52%)	43 (21)	71 (54%)
Age at evaluation (range)	22 (4-62)	25 (4-63)	16 (4-49)	9 (4-22)	10 (4-21)
Epilepsy	53 (83%)	3 (6%)	100%	15 (7%)	0 (0%)
Mean age onset (years)	2.1 (0-21)	2.0 (0-5)	6 (0-17)	3 (0-12)	-
Refractory	26 (49%)	0 (0%)	25 (38%)	5 (42%)	-
Mean # AEDs	1.5 (0-4)	1 (0-1)	1.6 (0-5)	2 (1-5)	-
Seizure type (%)					
IS	23 (36%)	-	-	3 (20%)	-
Prim gen	-	1 (33%)	34 (52%)	3 (20%)	-
Absence	-	-	8 (23%)	1 (33%)	-
Tonic/Clonic	-	-	32 (60%)	-	-
TC + absence	-	1 (100%)	4 (11%)	2 (67%)	-
Other	-	-	2 (6%)	-	-
Focal Simple	4 (8%)	-	4 (6%)	-	-
Focal Complex	49 (92%)	2 (67%)	28 (42%)	-	-
Unknown	-	-	-	12 (80%)	-
Mean IQ/DQ	76 (15-134)	101 (88 - 113*)	80 (40-128)	95 (34-148)	111 (83-145)
Intellectual Disability (%)	30 (47%)	3 (6%)	21 (32%)	40 (19%)	0 %
Learning Disability (%)	12 (19%)	23 (46%)	13 (20%)	103 (49%)	n.a.
ASD diagnosis	15 (23%)	1 (2%)	10 (15%)	100%	0 (0%)
Autism	15 (100%)	-	4 (44%)	188 (89%)	-
Other ASD	-	1(100%)	6 (56%)	22 (11%)	-
SRS total score (mean + range)	67 (36 – 95)	60 (34-95)	62 (35-95)	81 (43-95)	49 (34-95)
Social awareness	59 (30-95)	53 (30-77)	54 (30-81)	72 (36-95)	45 (30-88)
Social cognition	65 (36-95)	60 (36-95)	62 (36-95)	79 (45-95)	49 (36-95)
Social communication	65 (37-95)	58 (36-95)	60 (37-95)	79 (39-95)	51 (37-95)
Social motivation	64 (37-95)	59 (37-95)	61 (37-95)	73 (37-95)	51 (37-95)
Mannerisms	69 (41-95)	65 (40-95)	64 (40-95)	82 (42-95)	50 (40-95)

TSC, Tuberous Sclerosis Complex; NF1, Neurofibromatosis type 1; EUC; childhood-onset epilepsy of unknown cause; AC, autism consortium; ASD, autism spectrum disorder; AED, anti-epileptic drugs; IS, infantile spasms; TC, tonic-clonic; IQ/DQ, intellectual quotient / developmental quotient; MR, mental retardation (IQ<70); SRS, social responsiveness questionnaire; n.a., no information available. Numbers and percentages in subgroups are reflected as a fraction of the total of the subgroup. *only 3 IQ scores were available for the NF1 cohort.

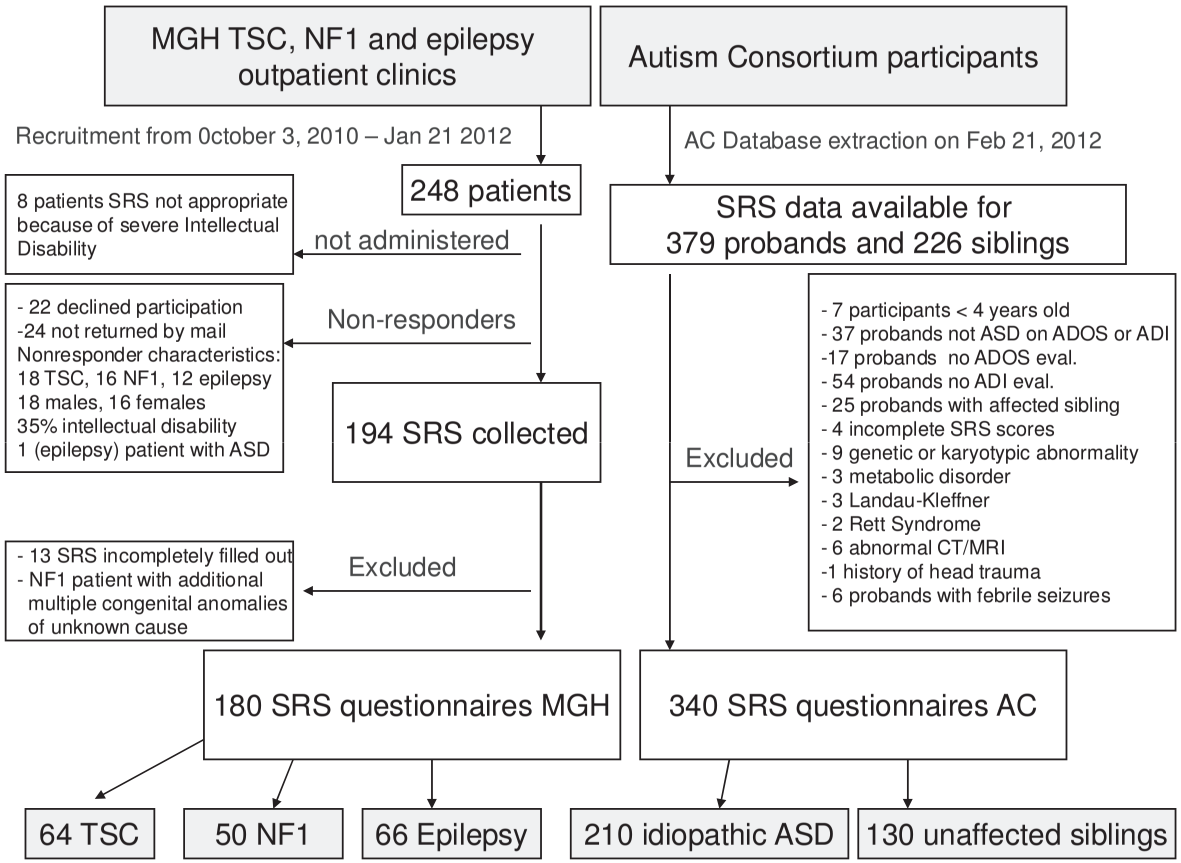

Figure 1. Flowchart depicting derivation of analyzed study cohorts. MGH, Massachusetts General Hospital; TSC, tuberous sclerosis complex, NF1, neurofibromatosis type 1; AC, autism consortium; SRS, social responsiveness scale.

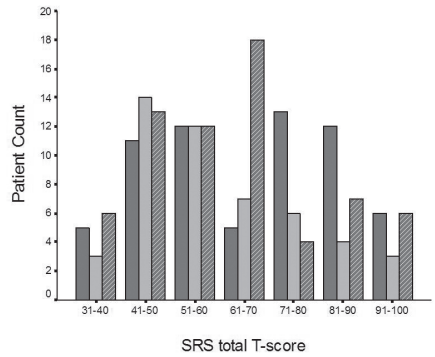

Figure 2. Histogram showing distribution of SRS scores for patients with TSC (red), NF1 (orange) and epilepsy (green). Note the bimodal distribution pattern in the TSC cohort. An SRS total score > 75 indicates a high suspicion of autism, while a score between 60 and 75 is indicative of another ASD such as PDD-nos.

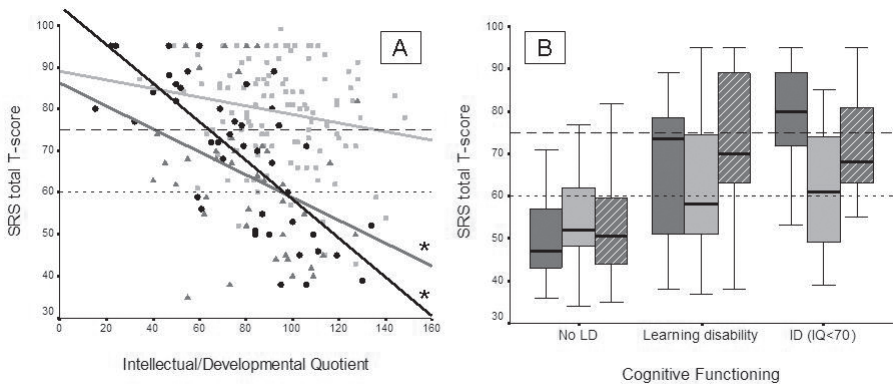

Figure 3. Graphs showing relationship between cognitive outcomes and rate of autistic features. A) Scatterplot of IQ/DQ outcomes and SRS scores for the TSC (black), epilepsy (dark gray) and AC-ASD (light gray) cohorts. * $P<0.05$. B) Boxplots showing SRS total scores per cognitive category for individuals with TSC (dark gray), NF1 (light gray) and epilepsy (shaded gray)

Association with genotype and gender

The TSC-specific regression model showed a significantly stronger relationship between the SRS score and epilepsy ($P<0.02$) than between SRS score and the TSC mutation group ($P<0.67$). The TSC1 group scores were significantly lower than the TSC2 cohort ($P<0.04$, CI 0.6 - 21), and similar to the seven patients with no mutation identified (NMI). The four patients with TSC2 missense mutations showed mean SRS scores that were 10 points lower than the 17 individuals with TSC2 protein terminating mutations.

No significant differences in mean SRS scores were observed between males and females with TSC, NF1, or EUC. When the association between gender and refractory epilepsy was explored, significantly more autistic features were observed for male EUC patients with refractory epilepsy compared with females (P<0.05, CI 0 -28) but no such differences were observed in TSC patients (P<0.98, CI -11 to 11).

Phenotype

Within each study group, the SRS subdomains (Social Awareness, Social Cognition, Social Communication, Social Motivation, and Mannerisms) correlated strongly with each other (r>0.40, P<0.005 for all groups). The study groups and AC-ASD group showed similar SRS trait profiles with relatively higher scores for the social cognition and mannerisms domains, and less affected social awareness domains (Figure 4A). This profile was attenuated for individuals *without* LD or ID (Figure 4B) or without epilepsy, who all showed mean domain

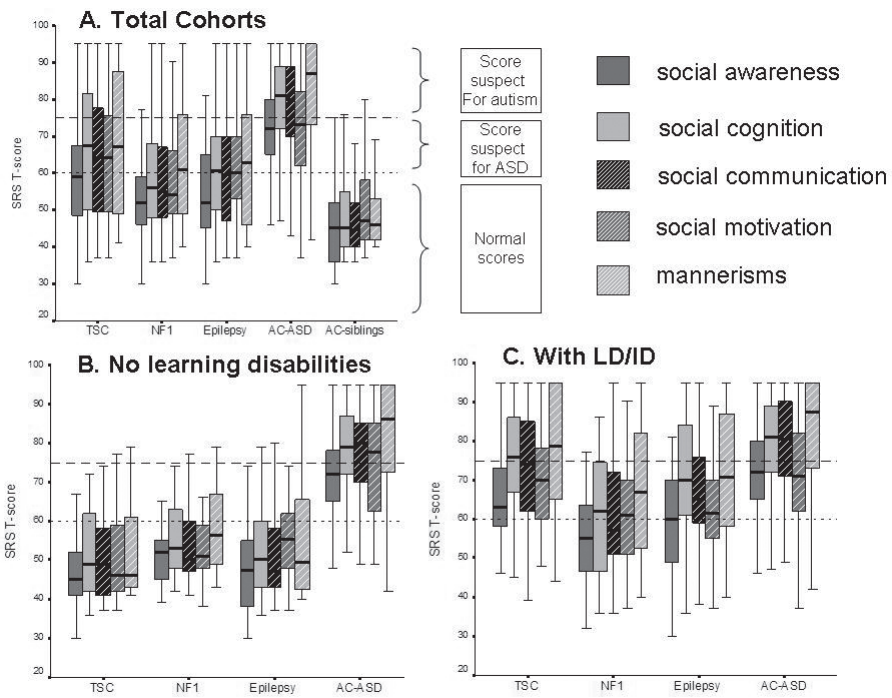

Figure 4. Boxplots depicting SRS trait profiles for selected study cohorts. Each box shows the median and quartiles within a category. Reference lines indicate thresholds above which T-scores are clinically suspect for autism (>75, striped line) or other ASD (between 60 and 75, dotted line). A) SRS scores for all cohorts. B) SRS trait scores for individuals without ID or LD, with TSC (N=22), NF1 (N=22), epilepsy (N=32) and idiopathic ASD (N=67). C) SRS trait scores for individuals with a learning disability (LD) or intellectual disability (ID) with TSC (N=42), NF1 (N=27), epilepsy (N=34) and idiopathic ASD (N=142).

Table 2. Results of statistical analysis.

2A) Associations with SRS score for different variables per study group, depicting *P*-values for ANOVA's and correlations.

	TSC SRS score	NF1 SRS score	EUC SRS score
ANOVAs			
IQ category[a]	<0.001	0.25	<0.001
epilepsy	0.001	n/a	-
refractory epilepsy	<0.001	n/a	0.40
infantile spasms	0.018	n/a	n/a
Correlations			
IQ/DQ score	<0.001	n/a	0.01
age onset epilepsy	0.035	n/a	0.24

a) no learning problems, specific learning disability, or global intellectual disability (see methods). ANOVA, analysis of variance; IQ, intellectual quotient; DQ, developmental quotient; n/a, not analyzed due to small patient samples or insufficient data

2B) Variables in the linear regression models.

	B[a]	*P*-Value	95% CI of B[b] Lower	Upper
Multi-disorder model				
Study group:				
TSC	17.3	0.31	-8.6	2.8
EUC	23.7	0.14	-7.5	54.9
NF1	0[c]	-	-	-
IQ category:				
Normal learning	-16.8 ± 5.0	0.001	-26.7	-6.9
Learning disability	0[c]	-	-	-
Intellectual disability	30.1 ± 17.4	0.079	-3.6	65.6
epilepsy	10.8 ± 21.4	0.613	-31.6	53.2
refractory epilepsy	2.0 ± 1.5	0.19	-1.03	5.0
age onset epilepsy	-0.28 ± 0.3	0.40	-0.9	0.38
TSC-specific model				
Mutation type:				
TSC1	-0.12 ± 9.1	0.99	-18.7	18.4
TSC2	-6.4 ± 8.3	0.45	-23.2	10.5
NMI	-4.2 ± 10.6	0.70	-25.7	17.3
Unknown	0[c]	-	-	-
IQ/DQ	-0.27 ± 0.1	0.015	-0.49	-0.06
epilepsy	20.7 ± 8.7	0.023	3.0	38.4
refractory epilepsy	15.7 ± 5.6	0.008	4.3	27.0
infantile spasms	6.3 ± 4.8	0.196	-3.4	15.9

[a]) The coefficient of that variable in the overall model
[b]) 95% confidence intervals surrounding the B
[c]) This group was designated as the reference category

scores in the normal value ranges although NF1 patients without LD showed relatively high scores on the mannerism domain. For individuals *with* LD or ID, this trait profile became more pronounced (Figure 4C). Lastly, to approximate a true autistic phenotype, individuals with SRS T-scores > 60 were selected and more striking trait differences were observed; study groups showed relatively less affected social awareness and relatively more mannerisms, although the profiles remained similar to that of the AC-ASD cohort. In general, the social communication domain seemed consistently in the average of the other traits, regardless of the neurocognitive profile.

The TSC cohort showed strong correlations between autistic trait domains and IQ/DQs on all domains, while this relationship was weaker for the EUC and AC-ASD cohorts, and absent in the AC-sibling group (Table 2). For the EUC and AC-ASD cohorts, lower intelligence outcomes were generally associated with more impaired social cognition, social communication and mannerisms but less associated with social awareness and social motivation domains. In the TSC2 cohort, the social cognition domain was relatively impaired, while for the TSC1 and NMI groups the social motivation domain was more severely affected, similar to the EUC groups without learning disabilities.

DISCUSSION

To our knowledge, this study is the first to compare autistic rates and traits in large cohorts of biologically related disorders. Across disorders, similar mechanistic and phenotypic relationships were apparent, suggesting that autistic features covary more directly with cognitive and epilepsy co-morbidity than with the genetic background.

Relationships with intelligence and epilepsy

Contrary to weak associations observed in idiopathic autism,[20] TSC and EUC patients showed highly significant inverse associations between IQ and total SRS score, implying that cognitive impairment and autistic features are part of the same neurocognitive construct. In other words, the more severe and global the cognitive impairment, the more likely the underlying brain dysfunction also affects the widely distributed networks responsible for social behavior, language, and cognitive flexibility. This relationship seemed less robust in NF1 patients, perhaps due to lack of quantitative IQ data, less variability in neurocognitive outcomes, or to weaker statistical relationships between epilepsy, IQ, and autism for individuals with borderline to normal intelligence[21] such as observed in NF1 patients.

Although the relationship between epilepsy and autism has been recognized since the description of the first autistic patients,[22] the underlying mechanism is still unclear.[23] TSC patients showed highly significant associations between SRS scores and various epilepsy variables such as age of seizure onset, IS and refractory epilepsy, providing a useful model for understanding relationships between epilepsy and autism. Such relationships were weak in the EUC cohort, perhaps due to the heterogeneous etiology in this group. Although we confirmed that TSC and NF1 showed no gender differences in autistic features, as their autosomal inheritance would imply, male EUC patients with refractory epilepsy represented a vulnerable subgroup, perhaps reflecting gender-specific mechanisms of idiopathic autism.

Table 3. Correlations between intelligence outcomes and SRS subdomain scores for study groups. The NF1 group was not included due to lacking IQ scores.

	Social awareness	Social cognition	Social communication	Social motivation	Mannerisms
TSC IQ/DQ	-0.85**	-0.92**	-0.96**	-0.85**	-0.91**
Epilepsy IQ/DQ	-0.24	-0.42*	-0.35*	-0.22	-0.43*
AC-ASD IQ/DQ	-0.10	-0.30**	-0.15	-0.11	-0.11
AC-sibling IQ/DQ	-0.06	-0.16	-0.20	-0.20	-0.18

* significant to $P<0.05$ level; ** significant to $P<0.001$ level

Our results suggest that, regardless of the underlying disorder, children who have a history of severe or refractory epilepsy at an early age, represent a subgroup associated with insults to the developing brain manifesting in a more severe autistic phenotype.

Relationship with genetic background

Since genetic mutation analysis is not a routine part of NF1 patient care, our genotype-autistic phenotype analyses were limited to the TSC cohort. TSC patients without learning disabilities or epilepsy showed normal SRS scores, suggesting that TSC germline mutations do not exert a uniform effect on cognitive and autistic features. We confirmed that TSC2 patients as a group are more affected by autistic features.[8, 24] and identified a relatively milder autistic phenotype in patients with TSC2 missense mutations, as has recently been observed for other neurocognitive morbidity. Interestingly, the distribution of SRS scores in the TSC population showed the same bimodal distribution as has been observed for intelligence outcomes, confirming that these variables are inextricably linked in TSC. Although there are some indications for genotype-neurocognitive phenotype relationships in these syndromes,[26] direct links between autism and NF1 and TSC gene loci have not been identified[27] and it is still unclear if heterozygous mutations can cause brain disruption or whether a "second hit," with loss of heterozygosity (LOH) is necessary.

Autistic trait profiles

A key question in investigating autistic phenotypes is whether the symptom clusters are independent or rather covary together. Our observations suggest that in TSC, NF1 and EUC, the autistic traits covary together significantly, and are strongly dependent on neurocognitive comorbidity. Individuals with NF1, TSC, and childhood-onset epilepsy, showed similar profiles of autistic traits. There was a trend for more severely impaired social cognition and mannerisms with relative preservation of social awareness, reminiscent of autistic traits described in Fragile-X patients. The mannerisms subdomain was most consistently affected for all study groups with cognitive impairments, possibly reflecting the sub-group of mannerisms whereby the sensory and motor repetitive behaviours are generally associated with lower IQs.[28] Mannerisms were also strikingly present in NF1 patients without learning disabilities, which is a different autistic feature than the previously

reported social skill deficits in patients with NF1[29] and may reflect symptoms of a different behavioral characteristic such as anxiety. The social motivation domain was relatively more affected within the normal-learning EUC population and the TSC1 and NMI subgroups, resembling the social alienation and interpersonal sensitivity that has previously been reported in normal to mildly affected individuals with TSC, epilepsy, Fragile-X syndrome, and idiopathic ASD patients.[30-33] Altogether, the inter-disorder similarities and intra-syndrome phenotypic heterogeneity suggest there is no mTOR-specific autistic phenotype, and that the debate in Fragile-X syndrome whether these autistic- like behaviors truly represent autism or are a 'phenocopy' of autism[34] may be irrelevant, since the autistic features seem to overlap at a neural level for all of these disorders. Future research should further explore the neural determinants of the variable neurocognitive manifestations in these disorders, such as synaptic disruption[2, 35] and neuroanatomical manifestations such as hamartomas and white matter abnormalities.[36, 37]

Limitations

Our samples were representative, and nonresponders were similar to participants. However there are also limitations. Of note is that in this study, the SRS was used to explore quantitative relationships, not as a diagnostic tool. Since research on the biological determinants of the SRS subdomains is limited, we performed few statistical comparisons on these domains and focused on clinically relevant observations. Secondly, as disruption of the mTOR pathway has been implicated in idiopathic cases of epilepsy and autism, our observations on phenotypic profiles may not reflect distinctly biologically different neurodevelopmental disorders. Thirdly, as a group, siblings of autistic individuals show a higher rate of (subclinical) autistic features[19] and do not represent the normal population. However, for our purposes the AC-siblings were a suitable comparison group, as they were all clinically evaluated for autistic features, familial ASDs were excluded, and showed SRS rates very similar to a normal population. Furthermore, we did not investigate relationships between autistic features and seizure frequency or specific anticonvulsants, which may impair neurocognition,[38] and future studies should explore this. Lastly, for both AC cohorts, the age at evaluation was lower than for the study groups; although in idiopathic ASD, autistic symptoms may decrease over time, there is no information on how this would affect the phenotype.

CONCLUSION

Our observations suggest that the autistic features in TSC, NF1, and childhood-onset epilepsy are an inextricable part of the neurocognitive phenotype and seem determined at a neural rather than the genetic level. Mouse models showing cognitive and epilepsy morbidity in these syndromes may inherently explain the pathophysiology of autistic features, suggesting that therapeutic interventions for neurocognition may also improve autistic features.[10] These mechanistic and phenotypic observations may extend to other mTOR-related syndromes, and perhaps 'complex autism' in general.

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CHARACTERIZING SLEEP DISORDERS OF ADULTS WITH TUBEROUS SCLEROSIS COMPLEX; A QUESTIONNAIRE BASED STUDY AND REVIEW

7

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ABSTRACT

An adult cohort with Tuberous Sclerosis Complex was investigated for the prevalence of sleep disturbances and the relationship with seizure variables, medication and psychological functioning. Information on 35 adults was gathered using four questionnaires, including the Epworth Sleepiness Scale (ESS), Sleep and Epilepsy Questionnaire (SEQ), Sleep Diagnosis List (SDL), and the Adult Self-Report Scale (ASR). In addition, clinical, genetic and electrophysiological data were investigated.

Of 35 respondents, 25 had a history of epilepsy. A subjective sleep disorder was found in 31% of the cohort. Insomnia scores showed a significant positive correlation with obstructive sleep apnea syndrome and restless legs syndrome scores. Significant correlations were found between daytime sleepiness and scores on depression, antisocial behavior, and use of mental health medication. Subgroup using anti-epileptic medication showed high correlation between daytime sleepiness, attention deficits and anxiety scores.

INTRODUCTION

Tuberous sclerosis complex (TSC) is a genetic disorder affecting 1 in 6,000 live births, and is caused by mutations in the tumor-suppressor genes TSC1 or TSC2. These genes encode a protein complex that inhibits mTOR kinase signaling by inactivating the Rheb GTPase,[1, 2] a regulator of cell growth. In TSC, mutations in these genes lead to benign tumors affecting multiple organs, including the skin, kidneys and heart. In more than 90% of TSC patients, the brain is involved,[3] often resulting in significant neuropsychiatric morbidity including seizures, mental retardation, autism, Attention-Deficit-Hyperactivity-Disorder (ADHD), depression and anxiety.[4, 5]

Seizure disorders are present in 70-90% of TSC patients, most often presenting in the first year of life.[6, 7] Most seizures are of the 'partial onset' subtype but generalized tonic-clonic, myoclonic and infantile spasms are also frequent. In TSC, reported EEG findings during sleep are increased epileptiform activity during non-REM sleep and, contrastingly, less epileptiform activity during REM sleep,[8] just as has been described in epilepsies of other causes.[9] Similar to frontal lobe seizures resulting from other etiologies, in TSC, nocturnal seizures are often associated with complex motor phenomena (tonic posturing, clonic movement of one limb).

Research on sleep in TSC has been very limited, and has involved children only. Hence, little information is available on the sleep architecture of TSC patients, how sleep states are altered by the underlying neurological condition and epilepsy, and how sleep disorders affect the individual's wake life.[10-12] The sleep disturbances in TSC are likely more complex than simply being secondary to epilepsy. In addition to the obvious neuroanatomical abnormalities, there are more possible contributing factors including mental retardation and psychiatric comorbidity like autism and hyperactivity, which, in turn, could affect the development of circadian rhythms and bedtime routines.[13] In addition, anticonvulsant medications, often prescribed in patients with TSC, can also affect sleep.[9]

In children with TSC, severe sleep problems are frequent after the onset of epileptic spasms and are often due to sleep-related epileptic events. Sleep disorders, including night waking, early waking, seizure-related sleep problems, and excessive daytime sleepiness, have previously been recognized as a frequent cause and result of stress in the more severely affected TSC patients and their families.[11] In a postal survey of 300 children with TSC, a high prevalence of sleep disorders was reported, with problems with settling (60%) and night waking (62%) being the most common.[12, 14] A follow-up questionnaire-based study looking at sleep disorders and the relationship with epilepsy in TSC found the degree of sleep disruption to be significantly higher in the group with seizures than in the siblings and matched controls and also associated with a high level of behavioral disturbance.[11] There is only one controlled polysomnographic study, comparing 10 children with TSC and partial epilepsy with healthy controls.[10] In 9 TSC cases sleep architecture abnormalities were found, including shorter total sleep time, fragmentation by frequent awakenings, and a decrease in REM sleep.[10] Although the sample was small, there was a tendency for children with TSC and seizures to show a more disturbed sleep architecture. Although sleep disturbances were seen with and without the occurrence of nocturnal seizures and/or the presence of EEG

abnormalities, sleep disorders seemed to be mainly due to sleep-related epileptic events and improved after a seizure-free period. Shorter awakenings not related to epileptic seizures were identified that were not detected by the caregivers, suggesting that the prevalence of sleep disorders in TSC patients is underestimated.[10] To our knowledge, there have been no studies evaluating sleep disorders in adults with TSC.

The present study explores the relationship between TSC and sleep disorders in an attempt to describe the prevalence and severity of sleeping problems in this patient group and the relationship with seizure features, medication and psychological functioning.

METHODS

The study is a questionnaire-based exploration of sleep in adult patients with TSC. Inclusion criteria were adults (age ≥ 18 years old) with a clinical diagnosis of definite TSC, followed at the TSC clinic at Massachusetts General Hospital in Boston; the presence of epilepsy was not a criterion for enrollment. No control patients were recruited. There was no selection on the basis of cognitive abilities or psychiatric comorbidity. Information on epilepsy intractability was obtained from our clinical database, using the definition: 1) having used three or more medications for the treatment of epilepsy or 2) having undergone epilepsy surgery, or 3) one or more seizures per day despite therapy.

Questionnaires

Participants were sent the following questionnaires, to be completed by the patient and with assistance of a caregiver as needed: the Sleep Diagnosis List (SDL)[15] derived from the Sleep Diagnosis Questionnaire,[16] Epworth Sleepiness Scale (ESS),[17] the Adult Self Report Scale[18] which is derived from the Child Behavior Checklist (CBCL),[19] and the Sleep and Epilepsy Questionnaire (SEQ).

The SEQ is an eight-page questionnaire with 24 questions based on the patient intake for of the Department of Sleep Medicine at our hospital, to assess aspects of epilepsy severity and sleep. It includes questions on seizure presence, type, frequency and severity, including treatment of these seizures in the past and present. It also contains questions on the subjective presence of sleeping problems and relationship with AEDs, psychiatric drugs, as well as experiences with treatment of sleep disorders. The ESS poses eight questions regarding the chance of a person dozing off during day-to-day activities, answered on a 4-point scale from 0 = never doze to 3 = high chance of dozing. A total score ≥ 10 is generally interpreted as daytime sleepiness. The SDL has been validated in epilepsy populations and consists of 30 randomly distributed questions covering five common sleep disturbances (e.g. insomnia, sleep apnea, narcolepsy, periodic leg movements and parasomnia) in the past six months, rating answers on a 5-point scale from 1=never to 5 = very often or always. A total mean score ≥ 3 in a category indicates the presence of a sleep disturbance. The ASR has been validated in adults with chronic conditions, has been identified as highly consistent with DSM-IV criteria, and is used to assess people's perceptions of their own psychological functioning over the last six months. It consists of about 140 questions, including demographic information and rating behaviors and functioning, thus gathering

information on psychological syndromes (Anxious/Depressed; Withdrawn; Somatic Complaints; Thought Problems; Attention Problems; Aggressive Behavior; Rule-Breaking Behavior, Intrusive) and DSM-oriented scales (Depressive Problems; Anxiety Problems; Somatic Problems; Avoidant Personality Problems; Attention Deficit/Hyperactivity Problems; Antisocial Personality Problems).

TSC mutational analysis

Genetic testing was performed at Athena Diagnostics (Worcester, MA, USA) or the MGH Neurogenetic Diagnostic Laboratory (Boston, MA, USA). Patients in whom genetic testing was negative are classified herein as NMI (no mutation identified). Patients with disease-associated mutations of the TSC1 and TSC2 genes were recorded. Mutations were not further classified.

Electroencephalography

Electroencephalography records were investigated for patients who had undergone surface EEG monitoring with a standard international 10-20 system of electrode placement, consisting of at least 25 minutes of recording. Only EEGs performed in the last 5 years were included in our analysis. When more than one EEG record was available, the EEG closest in date to the filling in of the questionnaires was used. Only information on the presence of regional epileptiform features consisting of spikes, sharp waves, and spikes and waves was gathered. Distinct localizations were not distinguished.

Statistical analysis

Statistical analyses were performed using SPSS Version 11.5 (SPSS, Inc., Chicago, IL). Complete (sections of) questionnaires were used for analysis. For the analysis of the SEQ and ARS, 90% completion of a section was considered sufficient for analysis. ESS and SDL scores were excluded if one item was missing.

Initially, a Pearson correlation between the outcomes of the ESS and the SDL-subscales insomnia and RLS was performed to investigate their relationship. A multivariate linear regression established the influence of gender, age, seizure history, and TSC mutation on the outcome of the ESS and SDL subscales.

To explore the influence of epilepsy on sleep in TSC patients, we selected the group with a positive history for seizures and investigated the effect of a history of intractable epilepsy, seizure frequency, number of AEDs and the presence of interictal spikes on the ESS and SDL outcomes.

For secondary analysis, continuous variables were analyzed by two-sample T-test and limited Pearson correlations were performed.

All reported p-values used two-tailed tests of significance with α set at 0.05. Data points unknown due to unavailable answers or histories were excluded from all statistical tests. Due to our small sample and abundance of variables, we had to restrict our statistical analysis.

Ethics

This study has been approved by the Institutional Review Board (IRB) of our hospital.

RESULTS

Participant characteristics

Of the 146 questionnaires that were sent out to adult TSC patients, 36 patients returned the questionnaires, resulting in a relatively low response rate of 25%. The scores of two SEQs and one ASR were not included because of insufficient information. Of note is that, in the remainder, questions on education and mental health were most often not filled out. The ESS and SDL were fully completed by all participants. The demographic characteristics of patients included in the analyses are listed in Table 1.

Table 1. Patient characteristics. The total responder group consisted of 35 patients, of which 20 were currently using AEDs. Fifteen and eleven responders scored high on the Epsworth Sleepiness Scale (ESS) and Sleep Diagnosis List (SDL).

	No AED (N=15)	**AED use (N=20)**	**ESS ≥10 (N=15)**	**SDL≥3 (N=11)**
Men (N=10)	2	8	3	3
Women (N=25)	13	12	12	8
Single (N=19)	9	10	8	5
Employed (N=24)	13	11	10	8
Mean age	33	33	34	34
Lower secondary education or less (N=11)	5	6	3	3
Pre-university education or higher (N=29)	9	10	10	6
Positive seizure history (N=25)	5	20	9	10
AED polytherapy (N=14)	-	14	6	5
TSC2 mutation (N=12)	3	9	3	2

Of the 25 patients with a seizure history, 13 (52%) had a history of intractable epilepsy, and 7 (28%) had a history of status epilepticus. Seizure frequency ranged from daily to less than one a month. Of these 25 patients, about half (49%) of the patients had their last seizures more than 6 months ago, and 4 (11%) patients had the last seizure within the last month. None of the respondents experienced multiple seizures per day. Six patients (17%) were on AED monotherapy and 14 (40%) were on AED polytherapy. The most common AEDs currently used were as follows: carbamazepine (CBZ; 49%), lamotrigine (LTG; 40%), valproate (VPA; 34%), phenytoin (PT; 34%), lorazepam (LZP; 31%), phenobarbital (PB; 31%), levetiracetam (LVT; 26%), gabapentin (GBP; 26%), topimarate (TPM; 23%), oxcarbamazepine (OXC; 20%), benzodiazepine (BZD; 20%), zonisamide (ZNS; 14%) and clonazepam (CLN; 14%).

Regarding mental health, 17/31 (55%) patients indicated on the SEQ that they had visited a mental health professional over the last year, and 15 (50%) of the cohort were taking medication for mental health concerns, with 9 on more than one psychiatric drug.

Sleep parameters

Within the SDL, insomnia scores showed a significant positive correlation with the obstructive sleep apnea syndrome (OSAS) score (r= 0.512, *P* <002) and restless legs syndrome (RLS) score (r=0.372, *P*<0.028). The raw narcolepsy score was positively correlated with RLS (r=0.480, *P*<0.004). There was only one responder with a narcolepsy score ≥ 3. This patient also had RLS and insomnia scores above the threshold. As this person was on AED polytherapy, mental health medication and on the SEQ reported to 'be a zombie' because of sleep medication, we do not suspect this person to be a true narcoleptic. RLS and OSAS also correlated significantly with each other (r= 0.411, *P* <0.014). Some correlations between the sleep subscales are shown in Table 2.

Table 2. Correlations between outcomes of the SDL scale (excluding parasomnia scores).

	Insomnia	Narcolepsy	OSAS	RLS
Insomnia	-	0.279 (*P*<0.104)	0.512 (*P*<0.002)*	0.372 (*P*<0.028)
Narcolepsy	0.279 (*P*<0.104)	-	0.286 (*P*<0.095)	0.480 (*P*<0.004)*
OSAS	0.512 (*P*<0.002)*	0.286 (*P*<0.095)	-	0.411 (*P*<0.014)*
RLS	0.372 (*P*<0.028)*	0.480 (*P*<0.004)*	0.411 (*P*<0.014)*	-

SDL= Sleep Diagnosis List, OSAS=obstructive sleep apnea syndrome, RLS= restless legs syndrome
* significant

Multivariate linear regression analysis did not show a significant effect of age, gender, TSC mutation or positive seizure history on the ESS score. On the SDL subscales, female gender did have a significant effect on insomnia (*P*<0.05). SDL subscale scores of parasomnia, OSAS and narcolepsy were not included in the regression analysis because few patients scored high for this type of disorder.

Regression analysis within the group with a positive seizure history did not show a significant association of the seizure frequency, number of AEDs and the presence of interictal spikes on scores of RLS or daytime sleepiness.

Prevalence of subjective sleep disturbance

According to the SDL, there were subjective indications for a sleep disturbance in the past 6 months for 11 patients (31%), with 8 patients (23%) scoring above the threshold for insomnia, five (14%) patients for restless legs syndrome, 2 (6%) for OSAS and 1 (3%)

for narcolepsy. No patients had significant parasomnias. All of the patients with insomnia had a positive seizure history. Of the patients with insomnia, two patients were on 1 AED, two patients were on 2 AED, one patient was on 5 AED. One patient was not on any AEDs. Of the patients scoring ≥ 3 on insomnia, patients suffered relatively frequent seizures, 1 patient having more than daily seizures, 2 patients with daily seizures, 5 patients with weekly seizures, and no patients with less than weekly seizures. Of these patients, 3 had ever suffered a status epilepticus, of the total of seven patients who reported having a status epilepticus. Of note is that the two patients with the highest insomnia scores were women with epilepsy, one using 3 and the other 5 AEDs and ages respectively of 24 and 23 years.

On the ESS, 15 patients (43%) scored ≥ 10, which reflects daytime sleepiness indicating a sleep disorder, and 1 patient (3%) scored 18, suggesting a severe sleep disorder. When the EES scores above and below 10 were compared, no significant differences were found in the number of current AEDs, the presence of interictal spikes on the EEG, nor the score on SDL subscales. Three patients had a high score on the ESS as well as the SDL. Looking at patients with a positive history of epilepsy, half of the patients with a history of intractable epilepsy scored over the ESS threshold, compared with 25% of the patients without this history. These numbers were too low for statistical comparison.

Association with mental health

Fifteen patients (43%) were currently on psychiatric medication, with 9 patients using one, and 6 patients using more than one psychiatric drug. Psychiatric medications noted were SSRI's (8), TCA's or other antidepressants (6), benzodiazepines (4), lamotrigine (4), valproic acid (2), risperidone (1), and olanzapine (1). Of the 17 respondents who answered 'yes' to the SEQ question if they had visited a mental health professional this year, 10 (59%) indicated that sleeping problems had an effect on their daily routines, and 10 (59%) scored ≥ 10 on the ESS. On the SDL, 3 out of these 17 (18%) had scored ≥ 3 on insomnia, and 2 (12%) scored ≥ 3 on RLS.

Of the 15 people who scored high on the ESS, 7 were on psychiatric medication. There was a significant positive correlation between the ESS score and depressive symptoms as well as antisocial symptoms, and a trend towards increased anxiety with higher ESS scores (see Table 3). When focusing on the subgroup using AEDs, depression and anxiety scores were significantly elevated, as well as problems with attention. Notably, the antisocial scores were not significant in the group using AEDs, and the insomnia scores on the SDL scale showed extreme low correlation with any of the psychological outcomes.

Association with medication

Thirteen respondents (40%) had tried medical intervention for sleeping problems, most often lorazepam (5), zolpidem (4), melatonin (2), and flu-medication containing paracetamol and antihistamines (2). Most were considered helpful, although about half of the zolpidem and lorazepam-users reporting grogginess on the next day. Eleven patients (33%) had tried non-medicinal interventions to improve sleep, most often including relaxation techniques, which were reported to be helpful.

Table 3. Correlation between psychological complaint scores and scores on sleep scales, for the total responder group and the subgroup of patients currently using AEDs.

ASR subscales	ESS score total group (n=35)	ESS score subgroup using AED (N=20)	SDL subscore insomnia, total group (N=35)	SDL insomnia score subgroup patients using AED (N=20)
Depressed	0.41 (P<0.014)*	0.37 (P<0.11)	-0.07	0.005
Anxious	0.32 (P<0.061)	0.47 (P<0.04)*	-0.03	0.03
Avoidant	0.193	0.16	-0.149	0.35 (P<0.13)
Somatic complaints	0.10	0.02	-0.29 (P<0.091)	-0.18
Attention deficits	0.27	0.46 (P<0.04)*	-0.12	-0.13
Hyperactivity	0.06	0.24	-0.02	0.11
Antisocial	0.37 (P<0.03)*	-0.11	0.01	0.05

ASR= Adult Self Report scale, ESS= Epworth Sleepiness Scale, SDL= Sleep Diagnosis List; * significant

Of the patients with a positive seizure history, 15 (60%) reported an effect of AEDs on their sleep. Specific medications that were causing longer sleep periods, sleepiness or grogginess were valproic acid (4 patients), carbamazepine (4 patients), phenytoin (2 patients), and diazepam, levetiracetam, lamotrigine, phenobarbital, and clonazepam. AEDs or devices causing disrupted sleep and/or sleeplessness were lamotrigine, lorazepam and the vagal nerve stimulator.

When comparing the groups with and without benzodiazepines with the ESS and narcolepsy scores to investigate a sedative effect, there was a significant effect on narcolepsy (P<0.007) scores and a trend towards higher ESS scores. There was a significant positive correlation between the number of mental health medications used, and the level of anxiety (P<0.0095).

DISCUSSION

To our knowledge, this is the only study of sleep in adults with TSC. Various limitations are inherent to our questionnaire-based approach. The response rate of the questionnaires was 25%, which is relatively low for this study type and resulted in a small sample size. The unequal male to female ratio of responders could be explained by the relatively higher prevalence of daytime sleepiness scores in our female cohort, perhaps resulting in more affinity with this study in women. Of the respondents, 25 (76%) had a positive seizure history, which is relatively lower than the reported prevalence of epilepsy in 90% of TSC patients. This could indicate that the respondents are mildly affected with regard to neurological involvement, which is supported by their relatively high level of education. Apart from this gender and IQ bias, our study group does seem to be representative; many of our results, e.g. female gender did have a significant effect on insomnia (P<0.05) and age approached significant

effect on symptoms of restless legs syndrome ($P<0.08$), are consistent with occurrences in the general population and thus validate our study group. As this was an exploratory study, no power calculations were performed to determine the sample size. Additionally, we have shown many statistical comparisons in order to generate hypotheses for future research. Hence, the power and *P*-values should be interpreted cautiously.

A specific limitation is the lack of objective data such as information on polysomnographic (PSG) studies to confirm results of the subjective patient reports. Due to disparity in timing, the EEG data may not accurately reflect the electrographic nature of the patient's brains at the time of filling out the questionnaires. However, there is evidence demonstrating consistent localization of focal interepileptiform features in patients with TSC over a 10-year period.[20] We did not have matched controls for our study group.

Excessive daytime sleepiness (EDS) is a common sleep/wake complaint among people with epilepsy, typically attributed to the effects of AEDs and seizures. A major finding in our cohort is that 15/35 (43%) had a high score on the ESS, indicating EDS. Notably, 12 (80%) were of the female gender, which cannot solely be explained by the female responder bias. Although the number or patients were too small to perform statistical analysis, there was a trend of more daytime sleepiness in patients with a history of intractable epilepsy. Of the eight patients who used a benzodiazepine, four (50%) had a score above the cut-off on the ESS, which confirms the sedative effect of benzodiazepines, although we cannot rule out other influences such as sleep disturbances or medication. Of the fifteen patients taking mental health medication, 7 (47%) had an ESS score higher than 10, indicating these patients are more at risk for daytime sleepiness.

Relationship epilepsy features and sleep

Epilepsy has been implicated in the disturbance of sleep physiology in a variety of ways, especially in the epilepsies associated with structural brain lesions. Sleep is widely recognized as an activator of interictal epileptiform discharges during electroencephalographic (EEG) recordings and additionally, has a pronounced effect on secondary generalization of partial seizures.[21] The prevalence of a subjective sleep disorder in 31% of the total study group, and in 40% of TSC patients with a positive seizure history, is similar to the significantly high prevalence of 39% previously reported in an adult cohort with partial epilepsy,[22] where the presence of a sleep disturbance adversely affected quality of life. In another controlled study of adult epilepsy patients, higher rates of sleep complaints, insomnia and daytime sleepiness was also reported in the epilepsy patients, although symptoms of RLS were decreased.[23]

Although not all patients with a positive seizure history had insomnia, all patients with insomnia did have a positive seizure history, confirming that in TSC patients with epilepsy, health care providers should be alert for presence of a sleep disorder as well as nocturnal seizures. Frequent awakenings have also been observed in patients with learning disabilities[24] and in autistic patients without TSC[25] and both groups have a higher prevalence of epilepsy, confirming the association between neurodevelopmental disorders, epilepsy, and sleeping problems.

Restless legs syndrome

Of our study group, 5 patients (14%) had indication for RLS as measured by the SDL. Additionally, the RLS score on the SDL correlated positively and significantly with insomnia, narcolepsy and OSAS. RLS is a clinical diagnosis with an estimated 9% to 20% prevalence in the elderly.[26] Half of the patients report a positive family history and it has been associated with age, iron metabolism abnormalities and chronic and end-stage renal disease.[27] In renal disease, no biochemical test value is associated with the presence of RLS. The pathophysiology of RLS has been related to the dopaminergic system and several studies have shown significant improvement with treatment of dopaminergic agents. All of the 5 TSC-patients with a high RLS score had renal involvement. However, as most of TSC patients have renal involvement and this is often symptomatic, we can not make any conclusions about renal pathology and RLS in TSC patients.

In adults with ADHD a significantly increased nocturnal motor activity and an enhanced frequency of arousals associated with periodic leg movements is documented. Vice versa, there is a high comorbidity of ADHD in adult RLS patients[28] as well as in TSC patients.[4] In clinical practice, adults with ADHD often complain about sleep disorders.[28] Thus, clinicians should be aware that ADHD and RLS need to be considered either as differential diagnoses, or might alternatively occur also as comorbidity in TSC.

Psychological functioning

Solely high scores on these self-report scales cannot be used for diagnosis of a psychiatric disorder. However, these questionnaires can assist in detection of problems in a specific area. In a recent questionnaire study,[29] the presence of a subjective sleep disturbance, depression and anxiety had a great effect on the quality of life in adults with epilepsy, even greater than short-term seizure control. In TSC, high rates of depression and anxiety have been reported.[4, 5] In this cohort the correlation between the ESS and depression score was highly significant ($P<0.014$), irrespective of AED use. This phenomenon has previously been observed in adult epilepsy patients[30] and could indicate that daytime sleepiness represents a vital sign of depression, which clinicians should be aware of. The correlation between daytime sleepiness and increased symptoms of anxiety and depression indicates that treatment of the cause of this sleepiness may decrease psychological problems in TSC patients, or vice versa, whether it be adjusting medication, screening for nocturnal seizures or depression, or other considerations. Remarkably, increased scores on the 'insomnia' SDL subscale was not associated with anxiety, suggesting there is no indication that the observed EDS was caused by insomnia in these anxious patients. Although antisocial scores correlated with higher ESS scores, they were not associated with use of AED, suggesting they are not related to epilepsy features in patients with TSC. Possibly, the antisocial scores reflect features of aggression and/or autism, which are known to have a high prevalence in TSC, and often result in the use of mental health medication.

The high score on Problems with Attention in the group of TSC patients using AED, reflects previous observations of a high prevalence of ADHD in patients with epilepsy.[31, 32] The reasons for this association are not fully understood, but include effects of seizures,

antiepileptic medications, underlying neurodevelopmental vulnerability, and subclinical epileptiform activity.[31, 33]

Because people with intellectual disabilities have been found to suffer high rates of sleep disturbances which are related to mental health problems and the use of anti-epileptic medication,[34, 35] we expect the prevalence of the above mentioned mental health complaints to be even higher the TSC population with lower IQs.

Genetic effect

Previous studies have demonstrated a more severe neurologic phenotype in patients with TSC2 mutations.[32, 36] The outcomes of this study did not show a significant difference between mutations in TSC1 and TSC2, suggesting that a genetic basis of the sleep disorder in TSC is unlikely.

Therapy

Presumably, improvements of sleep duration and quality will positively affect the daytime behavior of the child,[37] as well as the psychological functioning of adults with TSC. However, in patients with epilepsy, sleep is often fragmented in the absence of seizures or medication, suggesting that sleep instability may be an inherent component of certain forms of epilepsy.[9]

AEDs seem to have variable effects on nocturnal sleep and daytime vigilance,[38] and have different short-term and long-term effects. Carbamazepine, the AED most used by our participants, seems to have a positive effect on sleep with long-term use, as well as newer drugs like lamotrigine, gabapentine, and levetiracetam.[39, 40] Valproate and topamirate do not seem to have that effect. Further studies are needed to determine the long-term effects of AED therapy on sleep and wakefulness, psychological function and whether the anti-epileptic effects vary in patients with different types of epilepsy and TSC. Alterations in the timing or type of AEDs may be helpful.

Carbamazepine, oxcarbamazepine, and topiramate have specific therapeutic efficacy in nocturnal epileptic seizures, possibly by acting on the same mutated acetylcholine receptors,[41] and might be effective in nocturnal seizures in TSC. Contrary to a beneficiary effect in a healthy, non-epileptic population,[40] a study investigating the effects of levetiracetam in patients with TSC reported poor sleep, especially in nonresponders.[42]

In our study, the use of benzodiazepines was significantly related with a high narcolepsy score and also had a clear correlation with daytime sleepiness, confirming the sedating effect of this medication, although there are other explanations such as seizure severity. Traditional sedatives have been found to be less effective in patients with TSC, and they often worsen the situation by increasing hyperactivity.[43] Perhaps, patients in our cohort were suffering of sleepiness and RLS before treatment, resulting in benzodiazepine treatment. General considerations are that sedating AEDs should be minimized during the day but could be useful ante noctem. Activating AEDs should be used as appropriate.

The sedative component of benzodiazepines, measured by the reduction of locomotor activity, has been attributed to neuronal circuits expressing specific GABA-a receptors, the most prevalent receptor subtype in the brain. The development of GABA-b receptor modulators could open up a new era of therapy for troubles like insomnia, epilepsy and

narcolepsy, acting on lower doses and with fewer side-effects.[44] GABA is the main inhibitory neurotransmitter of the CNS. Epileptogenesis in TSC may be related to an impairment of GABAergic transmission, which is supported by the effectiveness of drugs with affinity for these receptors in the treatment of epilepsy in TSC. Additionally, particular neuronal networks defined by respective GABA-a receptor subtypes can now be linked to the regulation of various behavioral patterns.[45] This is of relevance for the pharmacotherapy of sleep and psychological dysfunction in TSC patients. Although one patient in our cohort reported less sleep with gabapentin, in our clinical experience, the GABA-analogue neurontin may improve sleep complaints next to its anti-epileptic qualities. Gabapentin may helpen deepen sleep, positively affecting Stage 4 sleep, and reducing arousals during the night, and could be potentially helpful for both sleep onset and sleep maintenance. Additionally, gabapentin can be effective in the treatment of RLS.[46]

Individuals with TSC often take other medications, increasing the risk of drug interactions. As few significant side effects have been described, melatonin may be an effective and safe treatment option for sleep problems. Melatonin is a chronobiotic drug with hypnotic properties,[47] increases levels of serotonin in the brain, thus raising the seizure threshold,[13] and has been used as adjuvant therapy as AED in children with intractable seizures.[48] Children with intractable epilepsy exhibited significant improvement in various sleep variables including sleep apnea, as well as Epworth sleepiness scores under melatonin therapy.[49] There was also significant reduction in seizure severity. In children with TSC, it has been suggested that the exogenous melatonin does not act by correcting abnormal endogenous melatonin secretion but by a simple sedative effect.[43] As melatonin excretion in children is very similar to that seen in adults, these findings could be extrapolated to the adult TSC population.[43] Another study comparing the effects of 5 mg of melatonin versus placebo in patients with TSC, found increased total sleep time and a trend toward decreased sleep latency, but with varying responses between subjects.[37] In a follow-up study, no evidence of a dosage-effect was found between 5 mg or 10 mg of melatonin.[37] Future studies in the specific TSC population are warranted to confirm this.

It has been reported that VNS treatment may affect respiration during sleep, resulting in stimulation-related apneas and hypopneas, and improved daytime alertness has also been reported.[50] Additional studies with larger numbers of subjects will be necessary to determine the effect of the VNS on daytime sleepiness and overnight sleep, comparing different stimulation intensities. Previous studies have shown that the diagnosis and treatment of OSAS should be considered in patients with poor seizure control or worsening of the epilepsy, and that treatment of OSAS can improve seizure control.[51]

CONCLUSION

This study confirms a high prevalence of sleep disorders in adult patients with TSC and the association with epilepsy features as well as mental health complaints. Although the uncontrolled nature of our data and relatively small and skewed study group limit conclusions, careful history taking and polysomnography (PSG) including EEG should be performed when sleeping problems or daytime sleepiness interfere with daily activities.

Home videos and sleep diaries are an inexpensive and proven method of monitoring sleep patterns.[13] Early detection and treatment of nocturnal epileptiform features and sleep abnormalities will have a positive effect on the quality of life, including mental health, of patients with TSC. Daytime sleepiness could be an indicator of a depressive disorder or other mental health problems and diagnosis and treatment of the causative factor could improve the quality of life.

Future larger and controlled studies should provide more insight into the relationship with epilepsy and the neuroanatomical basis of sleep disorders in TSC patients, as well as the neurophysiologic features of sleep in TSC. Therapeutic trials for epilepsy as well as sleep and mental health disorders in TSC patients can provide more direction in the development and choice for specific pharmacological agents.

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STRESSOR-RELATED DISORDERS IN TUBEROUS SCLEROSIS

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ABSTRACT

Patients with tuberous sclerosis complex (TSC) have high rates of psychiatric comorbidity, such as mood and anxiety disorders. The aim of this study is to identify patients with stressor-related disorders such as post-traumatic stress disorder (PTSD) or adjustment disorder (AD) and to describe their clinical picture in the setting of TSC. A retrospective review was performed of medical charts of TSC patients referred for a stressor-related disorder to a TSC Psychiatric clinic. Seven females and two males were identified (3 PTSD, 6 AD), including four children. The presenting symptom was aggression in two patients with a severe intellectual disability and avoidance in the remaining patients. The mean duration of symptoms at the time of the study was 21 months (range: 7-48 months) and seven of the nine patients were still having symptoms related to the trauma. All patients who received an initial diagnosis of AD changed to another diagnostic category because duration of the symptoms extended beyond 6 months. In most cases, selective serotonin reuptake inhibitors improved the symptoms. Stressor-related disorders in TSC frequently show a chronic course (beyond 6 months) and may appear with triggering events that are not typically viewed as trauma in normal population.

INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder that is due to inactivating mutations in TSC1 or TSC2. It is characterized by benign tumors involving multiple organ systems. Most of the patients have brain involvement, with variable presentations of epilepsy, cognitive deficits, autistic features, depression and anxiety.[1-3]

Post-traumatic stress disorder (PTSD) is a psychiatric disorder characterized by abnormal fear extinction in response to a traumatic event involving actual or threatened death or serious injury of self or others. It lasts for more than a month and it is characterized by several symptoms of re-experiencing, avoidance, negative alterations in cognition and mood and hyperarousal.[4] In the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR), PTSD is classified under the chapter of trauma and stressor-related disorders, next to other categories such as acute stress disorder, when similar symptomatology lasts for less than 4 weeks, and adjustment disorder (AD), which provides a residual diagnosis for individuals who have exhibited marked distress and functional impairment following exposure to stressful, but not traumatic, events. Stressor-related disorders in TSC are understudied and may be underestimated. The aim of this study is to describe the clinical characteristics of these disorders in TSC population.

METHODS

All TSC patients referred to the TSC Psychiatric clinic of the Massachusetts General Hospital between January 2009 and December 2012 with the clinical suspicion of stressor related-disorder were identified. Patients diagnosed with post-traumatic stress disorder (PTSD), acute stress disorder and adjustment disorder (AD), according to the DSM-IV-TR criteria,[4] were reviewed. All the patients were evaluated by the same psychiatrist (PN). Patients with a pre-existent diagnosis of PTSD that were seen in the psychiatric clinic during this period were excluded since the aim of the study was to obtain detailed information on the triggering event, the acute clinical picture and evolution. The following information was noted: age at the time of the event, gender, result of genetic analysis, psychiatric history and treatment, comorbid epilepsy and medication, cognitive phenotype, triggering event, presentation, evolution and duration of the symptoms and response to treatment.

RESULTS

Seven females and two males (mean age 22 years, range: 8-49) were identified. Three patients were adolescents (13-15 years old) and one patient was 8 years old (See Table 1 for clinical characteristics). Three of these patients were diagnosed with PTSD, and six with adjustment disorder (AD) (see Table 2 for symptoms according to DSM-IV-TR criteria). In all of the patients initially diagnosed with AD, this diagnosis was revised to other categories, mainly generalized anxiety disorder (GAD), due to persistence of symptoms beyond 6 months. In the two patients with severe intellectual disability the main presenting symptom was irritability and aggression, while avoidance was the main symptom in patients with normal cognitive function. The triggering event or stressor was easily recalled in all the

Table 1. Characteristics of TSC patients affected by stressor-related disorders.

P (G)	Age at event (years)	TSC mut	Psychiatric history	History of Epilepsy (a) (treatment)	Cognitive status	Triggering event
1 (F)	15	TSC2	No	Refractory (OXC,GBP, LVT)	Normal IQ	Seizure with conserved consciousness and sensation of imminent death in a boat (intense acute response)
2 (F)	8	TSC1	No	No	Normal IQ and behavior	Streptococcus upper respiratory infection at the beginning of school-year Paternal unemployment
3 (F)	13	TSC2	Generalized anxiety disorder Specific phobia	Refractory (LEV,OXC)	Borderline IQ (77) Emotional lability	Impression that coach made sexual gesture and remark
4 (F)	14	TSC2	No	In remission without treatment	Normal IQ and behavior	Viral illness. Starting high-school. Lower performance in initial exams
5 (F)	25	TSC2	MDD at 17 years old GAD	No	Normal IQ and behavior	Dysfunctional family life (recurrent triggering events, every time they meet)
6 (F)	49	ND	MDD (since husband death when she was 45) GAD	In remission without treatment	Normal IQ and behavior	Sexual assault while waiting in the emergency room
7 (M)	32	ND	ASD features Depressed mood previous year	In remission without treatment	Borderline	Incident at work: impression that a law enforcement officer had been staring at him in an accusatory way
8 (F)	22	ND	Severe ID ASD	In remission with treatment (CBZ, VPA)	Severe intellectual disability	Violent assault (hit with a belt by other student at school´s bathroom)
9 (M)	20	TSC2	Severe ID ASD	Refractory (LEV,TPM, LMT)	Severe intellectual disability	Hospitalization for resection of a pancreatic neuroendocrine tumor

ASD: autism spectrum disorder; CBT: cognitive behavioral therapy; F: female; GAD: generalized anxiety disorder; GBP: gabapentine; ID: intellectual disability; IQ: intellectual quotient; LEV: levetiracetam; LMT: lamotrigine; M: male; MDD: major depressive disorder; mut: mutation; ND: not determined; OXC: oxcarbacepine; P(G): patient and gender; TPM: topiramate; VPA: valproic acid; y:years

(a) As defined by the International League Against Epilepsy (Kwan et al., 2010), epilepsy was defined as:

- refractory: after failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drugs (AEDs)
- in remission without treatment: no seizures for 5 years or greater and not receiving AEDs at the time of ascertainment
- in remission with treatment: no seizures for 5 years or greater and receiving AEDs at the time of ascertainment

Presenting main symptoms	Duration (b)	Treatment (c) and response
Avoidance of boat Panic attack when exposed	10 months	Citalopram (15 mg), good response Supportive therapy
Avoidance of school Somatic complaints (throat and abdominal pain) Generalized anxiety	1 year (resolved)	Citalopram (15 mg) and CBT, good response
Avoidance of men (generalized to all men, even father)	2 years (resolved)	Citalopram (15 mg), good response Supportive therapy
Avoidance of school Generalized anxiety	7 months	Sertraline (100 mg), and starting on-line school, good response Supportive therapy
Depressed mood; Worsening of generalized anxiety; Hyperarousal	9 months	Sertraline (100 mg), good response Supportive therapy
Reexperience: fear when exposed to man resembling the assailant or hospital settings Depressed mood; Hyperarousal Avoidance	4 years	Quetiapine improved sleep initially. Limited response to citalopram, mirtazapine, aripiprazol and clonazepam CBT
Paranoia about others at work giving him accusatory glances and about getting arrested Avoidance of work place	2 years	Citalopram (30mg), good response Supportive therapy
PTSD Irritability Aggression Vomiting	3 y and 7 m (better after 22 months)	No response to risperidone. Improvement after school change and getting a pet. Supportive therapy
Irritability Aggression Destructive behavior	15 months	No response to aripiprazol, duloxetine, asenapine and guanfacine. Mirtazapine helped with sleep and appetite. Good response to olanzapine (7,5 mg) Supportive therapy

(b) If symptoms related to the triggering event have disappeared completely is indicated as resolved. In the rest of cases all or some symptoms persist.
(c) Dosage used for maximum effect with minimal secondary effects.

Table 2. Diagnostic criteria for PTSD or AD according to the DSM-IV-TR in identified patients.

		Symptoms						
P	Traumatic event (1)	Reexperience (2)	Avoidance (3)	Cognition and mood changes (4)	Increased arousal (5)	Duration >1 month	Significant impairment in functioning	Diagnosis
1	yes	yes	yes	yes	Yes	yes	yes	PTSD
2	no	no	yes	yes	no	yes	yes	Initial AD Diagnostic change to GAD
3	no	no	yes	yes	no	yes	yes	Initial AD Diagnostic change to specific phobia
4	no	no	Yes	yes	no	yes	yes	Initial AD Diagnostic change to GAD
5	no	no	yes	yes	yes	yes	yes	Initial AD Diagnostic change to GAD
6	yes	yes	yes	yes	yes	yes	yes	PTSD
7	no	no	yes	yes	yes	yes	yes	Initial AD Diagnostic change to GAD
8	yes	impossible recall	yes	yes	yes	yes	yes	PTSD
9	no	impossible recall	yes	yes	yes	yes	yes	Initial AD Diagnostic change to anxiety disorder NOS

AD: adjustment disorder; GAD: generalized anxiety disorder; NOS: not otherwise specified; P: patient; PTSD: post-traumatic stress disorder
In bold-type: predominant symptoms in each patient
PTSD clinical criteria (DSM-IV-RT)(4):
(1) Traumatic event: involving actual or threatened death or serious injury of self or others and the person's response involved intense fear, helplessness or horror. Otherwise, considered stressful but not traumatic.
(2) Reexperiencing: in the form of intrusion symptoms such as spontaneous or cued distressing memories, flashbacks or nightmares about the trauma
(3) Avoidance of the reminders of the experience
(4) Negative alterations in cognitions and mood: such as dissociative amnesia, pervasive negative emotional state, diminished interest in significant activities or socially withdrawn behavior
(5) Hyperarousal: feeling easily startled, tense, having angry outbursts or having difficulty sleeping or with concentration

cases but it was not intense enough to be considered traumatic in 6 of the cases, as did not involved actual or threatened death or serious injury of self or others.[4] In the PTSD group, the stressor was physical aggression in two cases and a seizure in the other. As the duration of symptoms was longer than six months in all the patients initially diagnosed as AD, their diagnosis was changed to generalized anxiety disorder or specific phobia. The mean duration of the symptoms was 21 months (range: 7-48 months) but seven of the nine patients were still having symptoms related to the trauma at the time the study ended. Citalopram or other selective serotonin reuptake inhibitors (SSRIs) ameliorated the symptoms in most of the cases. All the patients received also non-pharmacological supportive therapy, which consisted of elements of cognitive behavioral therapy (CBT) and caregivers guidance and education. Two patients received formal CBT, which ameliorated the symptoms in both cases.

DISCUSSION

Our study describes the main clinical characteristics of PTSD and other stressor-related disorders in a TSC cohort. Although anxiety disorders are frequent in TSC patients,[2, 3] frequency or clinical features of stressor-related disorders have not previously been reported, suggesting that stressor-related disorders may be underdiagnosed in patients with TSC. The pathophysiology of PTSD is related to dysfunction of the neural circuitry of the fear response, where learned fear due to a traumatic event becomes generalized to safe situations.[5,6] Trauma is necessary but not sufficient for the precipitation of PTSD. Some trauma victims develop PTSD (between 5 and 30%)[7,8] while others seem resilient when experiencing the same trauma. A variety of factors may contribute to this, including the genetic background, abnormal fear brain-circuitry or stressful early life experiences.[9]

Anxiety disorders have a prevalence between 28 and 56% in TSC patients.[2,3] Factors contributing to anxiety and stressor-related disorders in TSC patients may be related to a dysfunctional circuitry of fear response, due to anatomical disruption of neuronal tracts or to functional synaptic disturbance of the main implicated areas (such as amygdala, hippocampus and prefrontal cortex)[6,10] but also to the psychological burden of having a chronic and severe disease. In our group of patients there was a predominance of women (7:2). In the general population, women suffer from anxiety disorders more frequently than men. In a study of psychiatric comorbidity of 43 TSC patients, ADs were diagnosed in 21% of the cases.[2] Most of the large epidemiological surveys of mental health in general population lack prevalence data for ADs, in part because there is no clinical interview used in large epidemiological surveys sufficiently robust in diagnosing it.[11]

PTSD clinical criteria in the DSM-IV-TR are usually applied to normal adults suffering a traumatic event. In other cases, such as children or cognitively impaired people, the diagnosis of this disorder is especially challenging. In children, PTSD may be expressed by disorganized or agitated behavior, which is also predominant in individuals with intellectual disability (ID). An improvement in the future DSM-V[12] will be specific PTSD criteria in children less than 6 years old, as they may present with disorganized or agitated behavior and express intrusive memories as repetitive play. Nonetheless, a similar initiative

has not been undertaken yet in the DSM-V for individuals with ID. Severe intellectual disability was present in two of our patients (cases 8 and 9) and their symptoms consisted primarily in irritability and aggression. Patient 8 suffered a clear traumatic event but due to her intellectual disability collecting clinical details such as symptoms of re-experiencing needed for a PTSD diagnosis was not possible, so it was made from what could be inferred from her behavior. Individuals with ID have been found to be more likely to experience traumatic events and early life stressors such as hospital admissions, more invasive health procedures and high levels of parental stress and they may have different interpretation of distressing experiences such as being exposed to multiple caregivers and changes of house or school. Practitioners often attribute behavioral disturbances as part of the ID itself, a phenomenon known as "diagnostic overshadowing"[13] that may preclude the detection of stressor-related disturbances. The Diagnostic Manual-Intellectual disability,[14] developed in association with the American Psychiatric Association, provides some help identifying the psychiatric symptoms that are often expressed differently in persons with ID. In the case of stressor-related disorders they present mainly with affective disorders and aggressive or destructive behaviors.[13] The predominant symptom in our cases with normal or borderline cognitive function was avoidance and in the cases with intellectual disability was irritability and aggressiveness. This agrees with previous studies suggesting that the severity of PTSD is negatively associated with the level of intelligence. Interestingly, patients 2 and 8 presented somatic symptoms that could not be attributed to other processes, the former throat and abdominal pain and the latter vomiting. It is important to recognize this as a possible part of the clinical picture in order to avoid added unnecessary work-up of physical symptoms.

The intensity of the immediate response (i.e., extreme withdrawal, panic-like response) to the traumatic event is associated with an increased risk of developing PTSD.[15] Case number 1 experienced a very intense initial response to the traumatic event, which consisted in a seizure with preserved consciousness that made her feel completely helpless. Data about seizures as the potential trigger of PTSD in epilepsy are scarce but a recent study suggests that PTSD may be very frequent in this setting, with 51% of patients meeting the diagnostic criteria for full-PTSD and 30% for partial-PTSD.[16] These results suggest that in patients with epilepsy, it is important to consider seizures as a potential triggering event of PTSD, mainly if it causes an intense panic response in the patient.

The observation that all our patients with an initial diagnosis of AD experienced persistent symptoms beyond 6 months raises some doubts about the clinical utility of this diagnostic category in TSC population. In fact, the clinical value of the current DSM-IV-TR diagnosis of AD is also controversial in the general population because is not characterized by sufficiently specific clinical, psychometric or prognostic features, and mainly overlaps with other mood and anxiety disorders.[17] Also, the DSM-IV-TR classifies ADs into subtypes according to the predominant symptoms: depressed mood, anxiety, disturbance of conduct or unspecified. The addition of a new subtype: with PTSD-like symptoms is undergoing further consideration for inclusion in the DSM-V.[12] Our experience agrees with some authors who propose AD as a stress-response syndrome similar to PTSD sharing a common pathophysiology.[18]

Selective serotonin reuptake inhibitors (SSRIs) are the first line pharmacologic interventions for PTSD and their use is supported by several randomized controlled trials.[19] In our cases, citalopram and sertraline were useful in ameliorating some of the symptoms, but only two of the patients had a complete remission, both treated with citalopram. Mirtazapine is a noradrenergic and serotonergic antidepressant that may be useful as a second line of treatment.[20] It was prescribed in two of our patients and one of them showed amelioration of sleep and appetite disturbances. Antiadrenergic medications, such as prazosin or guanfacine, are occasionally used for lowering the sympathetic tone and the associated hyperarousal, impulsivity and intrusive symptoms, such as nightmares.[21,22] Guanfacine was prescribed for one of our patients but was not effective. The antipsychotic quetiapine was useful in controlling nightmares in one of our patients but other prominent PTSD symptoms persisted. A meta-analysis of studies in adults[23] suggests that antipsychotics may be particularly effective in reducing intrusive symptoms. Mood stabilizers such as lamotrigine, valproic acid, topiramate, carbamazepine and levetiracetam can also be useful and overall, most studies have demonstrated a modest improvement.[22]

Trauma-focused cognitive behavior therapy (CBT) and trauma-focused cognitive therapy (without exposure) (CT) are first line treatments and have been used with success in the treatment of PTSD in general population and seem also useful in the case of patients with ID.[13] Two patients received CBT, which was very useful in one case and partially effective in the other.

Interestingly, benzodiazepines (BZDs), that are often used to treat anxiety, are not useful in PTSD treatment or prevention[22] and may even be harmful. Some studies[24] suggest that early administration of BZDs, which is associated with enhancing access to emotional memories, might interfere with the normal spontaneous recovery. Also, BZDs abolish the expected activation of the hypothalamic-pituitary-adrenal (HPA) axis in stress.[25] This consideration is important also in the setting of TSC patients with epilepsy because some of them use BZDs as antiepileptic drugs (AEDs). Only one of our patients was receiving BZDs at the time of the event.

Recent research in PTSD focus on the preventing role of some medications such as glucocorticoids,[26] estrogens[27] and omega-3 fatty acids,[28] that administered in the first hours after the traumatic event can prevent the appearance of symptoms.

Our study presents some limitations inherent to its retrospective nature, such as lack of details about acute behavioral response at the trauma event, presence or not of specific symptoms related to PTSD and detailed response to treatments. Nonetheless, it is the first study to date depicting the specific clinical characteristics of stressor-related disorders in TSC patients. More reports are needed in order to better understand these disorders in TSC and allow an earlier diagnosis and treatment for these patients.

CONCLUSION

TSC patients may have underlying vulnerability to anxiety and stressor-related disorders, even in the setting of a triggering event considered not traumatic for the majority of population. In TSC patients, these disorders usually have a chronic course (more than

6 months) and are quite resistant to standard treatments. Raising awareness about stressor-related disorders in TSC patients will allow an earlier diagnosis and treatment. Additional studies are needed regarding treatment of these disorders in TSC population and the possible role of prophylactic medications.

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CLOBAZAM THERAPY OF REFRACTORY EPILEPSY IN TUBEROUS SCLEROSIS COMPLEX

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ABSTRACT

Clobazam (CLB) was recently approved by the FDA, but has not been evaluated in Tuberous Sclerosis Complex (TSC). We retrospectively reviewed a cohort of patients with TSC and refractory epilepsy who started CLB over a five-year period. Clinical characteristics and number of tubers on MRI were assessed. Duration of therapy, therapeutic response and adverse events were recorded. CLB was prescribed in 29 adults and children of whom 72% were cognitively impaired, with a median age at seizure onset of five months. Mean duration of CLB therapy was 17.3 months with a 12 and 24-month estimated retention rate of 82% and 68%, respectively. Twenty patients (69%) reported a good response (>50% seizure reduction) at the end of the titration, and six patients (21%) remained good responders after 12 months of CLB therapy. Adverse events occurred in 13 patients, predominantly somnolence and behavioral disorders. One quarter of the responders reported improvement in behavior. No predictive factor for a good response could be identified. CLB appears to be a well-tolerated and valuable option for treatment of refractory epilepsy in TSC.

INTRODUCTION

Tuberous Sclerosis Complex (TSC) is a genetic disorder characterized by the presence of hamartomas that affect most organ systems including the brain. Epilepsy is the most frequent neurological manifestation affecting 80 to 90% of individuals, with epilepsy onset during the first year of life in 70%.[26] Two thirds of patients show refractory epilepsy and most have multiple seizure types requiring a combination of antiepileptic drugs (AEDs).[6]

Clobazam (CLB) has been widely used in other countries for years and has been consistently proven effective as an adjunctive therapy in refractory epilepsy in adults and children in four randomized controlled studies summarized in a Cochrane review.[16] Additionally, CLB can be used as monotherapy for non-refractory focal and generalized childhood epilepsies,[3, 9] and has been shown effective in various epilepsy types.[5, 8, 25] CLB has been approved in the US by the Food and Drug Administration in October 2011 for use as adjunctive therapy for the treatment of seizures associated with Lennox-Gastaut syndrome.

CLB is a 1,5-benzodiazepine with nitrogen atoms in the 1 and 5 positions and a keto group in the 4 position, in opposition to classical 1,4-benzodiazepines. It has been initially used as anxiolytic, then as anticonvulsant after Gastaut described its antiepileptic properties in 1979.[12] CLB enhances the gamma-aminobutyric acid (GABA) inhibition by allosteric binding to the GABAa receptor and upregulation of the GABA transporters 1 and 3. CLB is well absorbed, has a rapid onset of action and belongs to the long-acting (>24h) benzodiazepines.[21] Its spectrum of antiepileptic activity is broad including focal and generalized seizures, tonic, clonic and myoclonic seizures, or absences.[3] CLB has the advantage over the 1.4 benzodiazepines in causing less sedation.[27]

No data on the efficacy of CLB specifically in TSC are available. The aim of the present study was to evaluate the efficacy and tolerability of CLB therapy in epilepsy in the setting of TSC and to identify potential predictive factors for a good response.

METHODS

Patient selection

Among 307 patients with a definite diagnosis of TSC,[22] followed at the Herscot Center for TSC at Massachusetts General Hospital, we identified the first use of CLB in January 2007 and reviewed all patients who were prescribed CLB from this date to January 2012.

Data collection

Charts were reviewed to collect information about gender, age at time of the study, age at seizure onset, seizure types, history of infantile spasms, results of genetic mutation analysis and number and types of AEDs used currently and previously. Available MRIs were reviewed for the number of cerebral and cerebellar tubers by one of the authors experienced in the evaluation of MRIs of patients with TSC (A.v.E) after achieving a inter-rater agreement with an experienced neuroradiologist (P.C) in ten cases (Pearson test, $P<0.007$). Cognitive impairment was defined by an intellectual quotient (IQ) <70 estimated from psychometric tests when available or from clinical reports. The diagnosis of autism spectrum disorders (ASD) was made based on DSM-IV criteria. Epilepsy was considered refractory as defined

by the International League Against Epilepsy, after failure of adequate trials of two tolerated, appropriately chosen and used AEDs.[15] CLB dosing, titration schedule and dose of CLB at the time of the last documented visit were noted. Adverse events were classified as mild (not interfering with daily activities and no change in dosage required), moderate (interfering with daily activities requiring decreased doses) and severe (treatment discontinued). Rate and reason for discontinuation were recorded.

The baseline period used to assess efficacy corresponded to the interval between the last visit prior to the one where it was decided to introduce CLB, ranging from 3 to 6 months. The percentage of seizure reduction at the end of the CLB titration with the minimally effective or maximally tolerated dose was assessed based on reports from both patients and parents during outpatient clinic visits and/or phone calls with the clinic nurse. The length of the period to complete the CLB titration was variable and determined by patient response, ranging from one week to three months. Patients were divided in two groups: 1) responders subdivided in patients with a >90% seizure reduction from baseline or >50% seizure reduction and 2) non-responders (<50% seizure reduction, no change or increase in seizure frequency). Furthermore, we assessed the number of patients in whom good response to CLB lasted for more than 12 months, without changing or starting other antiepileptic therapy.

CLB was used to treat both epileptic spasms and partial seizures in TSC patients who did not respond to first-line therapies. The first CLB dose ranged from 0.05 to 0.1 mg/kg/day in the evening and titration (5 mg/day at one week interval) was determined by the clinical response to obtain the minimal effective dose. If needed, the dose was increased until a maximum level of 3 mg/kg/day in children, or was decreased if side effects occurred. If possible, previous medications were gradually reduced and discontinued. No fixed titration or withdrawal schemes were set and titration rates were adapted to each patient. If CLB was not effective or not tolerated at the maximal doses used, the drug was typically tapered over 2 to 3 months, without additional drugs used during the withdrawal period.

Statistical analysis

SPSS version 11.5 (Statistical Package for the Social Science, Chicago) was used for all analyses. Responders and non-responders were compared using chi-square and student test, with significance set a $P<0.05$. A Kaplan-Meier curve was performed for retention of CLB.

The institutional review board of the Massachusetts General Hospital approved this study.

RESULTS

Of the total of 307 patients with TSC and epilepsy, 31 (10%) were treated with CLB. Two patients were excluded due to incomplete clinical information. Twenty-nine patients (14 males, 23 patients <16 years) were included and their characteristics are detailed in Tables 1 and 2. All patients had refractory epilepsy and 25 (86%) experienced daily seizures. Eight underwent resective epilepsy surgery and four had a vagus nerve stimulator before CLB therapy. None fulfilled the diagnostic criteria of Lennox-Gastaut syndrome. All

patients but one had been started on CLB in our institution. Twenty-five (86.2%) patients started CLB as at least the third AED and one patient received CLB as monotherapy.

Twenty (69%) patients showed a good response at the end of the titration including five (17.3%) with >90% reduction in seizure frequency. Nine (31%) patients did not respond as they experienced less than 50% reduction (n=6), no change in seizure frequency (n=2) or paradoxical increase in seizure frequency (n=1). Of the 20 initial good responders, six were identified as being still good responders at 12 months, including two patients who remained with >90% seizure reduction. Figure 1 shows the Kaplan-Meier curve with a CLB retention rate of 82% at 12 months and of 68% at 24 months.

Table 1. Clobazam response and patient characteristics.

	Responders		Non-responders
	>90% reduction n=5	**>50% reduction n=15**	**<50% reduction n=9**
Male, n	3	6	5
Mean age* (SD)	15.2 (+/-9.2)	8.2 (+/-6.9)	10.7 (+/-8.3)
Cognitive impairment, n	4	13	4
History of autism, n	2	5	1
Behavioral disorders, n	3	6	2
Median age (years) at epilepsy onset (range)	0.5 (0.3-2)	0.3 (0.1-1.6)	0.4(0.2-5)
History of IS, n	2	13	6
Seizure types			
PCS only, n	2	6	5
Multiple types of seizures, n	3	8	4
Spasms, n (%)	0	4	2
Median age at CLB therapy (years, range)	12 (1.3-27)	3.5 (0.3-24)	8 (0.9-24)
Median duration of epilepsy before CLB (years, range)	11.5 (0.8-26.5)	63.2 (0.1-23.6)	3 (0.4-23.8)
Mean number of AEDs concomitantly prescribed (SD)	2.6 (+/-1.5)	1.9 (+/-1.2)	2.7 (+/-1.4)
Mean number of AEDs previously withdrawn (SD)	7.6 (+/-4.5)	6.5 (+/-2.5)	5.7 (+/-3.4)
Mean of maintenance dose (mg/kg/day, SD)	1.2 (+/-0.5)	1.7 (+/-0.8)	1.5 (+/-0.9)
Mean of maximal dose (mg/kg/day, SD)	1.5 (+/-0.9)	2.1(+/-0.8)	1.9 (+/-0.8)
Mean duration of CLB therapy (months, SD)	22.2 (+/-8.0)	18.8 (+/-9.7)	14 (2-26)

* at the time of the study, IS=Infantile Spasms, PCS=Partial Complex Seizure, GTC=Generalized Tonic-Clonic seizure, AED=antiepileptic drug

Table 2. Clobazam response and TSC genetic and radiologic features.

	All patients	Responders		Non responders
	n=29	>90% reduction n=5	>50% reduction n=15	<50% reduction n=9
TSC1	3	0	1	2
TSC2	18	3	9	6
No mutation identified	1	0	1	0
No genetic analysis	6	1	4	1
Mean number of tubers, SD	37 (+/-16.9)	27 (+/-12.0)	42 (+/-17.8)	34.7 (+/-16.5)

Responders tended to be less likely to have cognitive impairment but this did not reach significance (P<0.07). Responders and non-responders did not differ with regard to history of infantile spasms, type of seizures, age at CLB therapy, duration of epilepsy prior to CLB, or history of autism. The maximum and maintenance doses used in children were higher than those used in adults (respectively 2.2 vs 0.9 mg/kg/d and 1.8 vs 0.8mg/kg/d). No difference was found in average CLB doses at the end of the titration between responders and non-responders. Patients with >90% seizure reduction tended to have less tubers than partial responders (mean 27 vs 42 respectively, P<0.10).

Six children had epileptic spasms at the time when CLB was introduced and responded as follows: two patients reported >50% spasm reduction and four did not report improvement

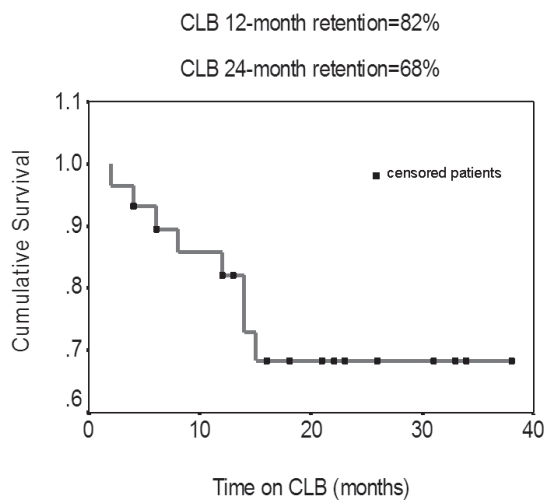

Figure 1. Kaplan-Meier curve showing the retention of CLB (percentage of patients still taking CLB) over a 38-month period. Patients were censored if they were lost of follow-up (n=1) or if they were still on CLB at the time of the study (n=21).

in spasms although CLB had dramatically reduced other types of seizures concomitantly present. Of the CLB responders, 12 had been treated with vigabatrin for infantile spasms in the past and CLB was later started for partial seizures. Of the 9 patients for whom clinical information was available, 6 (66%) became spasm free with vigabatrin.

Adverse effects were seen in 13 (44.8%) patients with the following distribution: mild (n=2), moderate (n=8) and severe (n=3) (see Table 3). There was no significant difference in terms of therapeutic response, number of concomitant AEDs or maximal doses of CLB between patients who reported side effects and those who did not.

Seven patients (24.1%) discontinued CLB. The reasons for withdrawal from treatment were: poor efficacy despite high doses (n=4), adverse events and poor efficacy (n=2) and adverse events only (n=1). Review of records did not suggest increased seizure frequency during the withdrawal period. Seven (24.1%) patients reported behavioral improvement after CLB therapy, characterized by better mood, more interactivity or increased verbal skills, and three patients reported also better quality of sleep. Figure 2 shows the antiepileptic therapies taken in conjunction with CLB in responders and non responders; no specific effective combination could be identified in responders.

DISCUSSION

To our knowledge, this is the first study describing efficacy of CLB in patients with TSC. Our results suggest that use of CLB in this population is safe and effective, with a prolonged good response at 12 months in 21% of patients, consistent with other studies of CLB therapy in refractory epilepsies.[3, 13, 17, 18, 19, 24] There is no consensus on the most effective AED in TSC except for vigabatrin in infantile spasms.[20] Efficacy of other AEDs

Table 3. Adverse events.

Patients with adverse events*	**13***
Requiring dose adjustment only	9
Requiring drug discontinuation	3
Specific type of adverse events	
Behavior disorders, aggressiveness	7
Sleepiness/sedation	7
Mood disorders**	3
Bad quality of sleep	3
Ataxia	2
Drooling	2
Garbled speech	1
Muscle weakness	1

* 7 patients had 2 or more adverse events
** irritability, intolerance to frustration, emotional lability

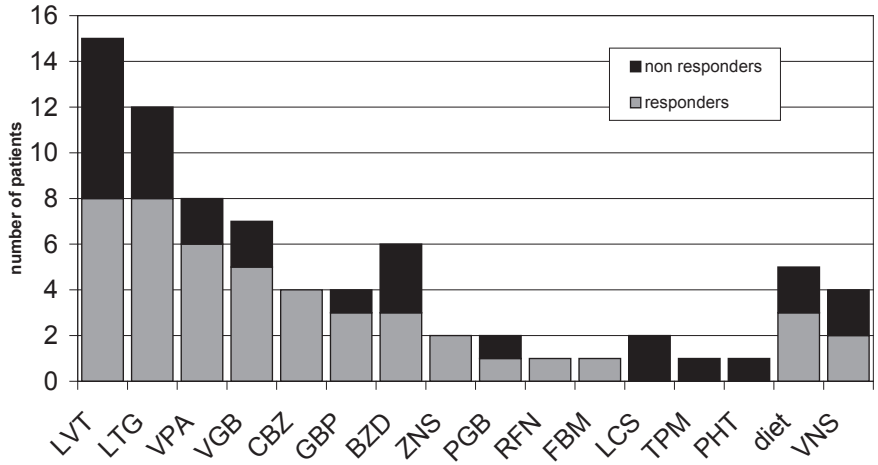

Figure 2. Antiepileptic drugs taken in conjunction with CLB. LVT indicates levetiracetam; LTG, lamotrigine; VPA, valproate; VGB, vigabatrin; CBZ, carbamazepine; GBP, gabapentin; BZD, benzodiazepines (other than CLB); ZNS, zonisamide; PGB, pregabalin; RFN, rufinamide; FBM, felbamate; LCS, lacosamide; TPM, topiramate; PHT, phenytoine; diet, dieteray therapy (ketogenic diet or low glycemic index therapy); VNS, vagal nerve stimulator

in small populations of patients with TSC and epilepsy have been reported regarding topiramate, lamotrigine and levetiracetam, and the CLB response rate in our group is similar or superior to that reported in these studies.[7, 10, 11] Similar to another report, no predictive factor of a good response could be identified in our study.[18]

The majority of TSC2 genotype found in our cohort can be explained by the recognized association between TSC2 mutations and a more severe phenotype.[2] Most of patients experienced multiple types of seizures with a predominance of partial complex seizures. Prior studies have suggested that CLB may be helpful for individual patients with refractory spasms.[13, 25] Despite the small number of patients, response to CLB in refractory partial seizures seemed better than in refractory spasms. Shimizu et al. reported a better response with CLB when more than one generalized tonic-clonic seizure per year occurred with PCS.[24]

The response rate at 12 months was lower compared to the initial response (69% vs 21%), possibly explained by the high frequency of seizures requiring other antiepileptic therapies after CLB, despite a good response. It may also be explained by the potential development of tolerance should also be mentioned as previously described for CLB and other benzodiazepines as well, with an incidence ranging from less than 10% to more than 70%.[1, 3] These variations may be due to more or less restrictive definitions of tolerance and its difficult assessment in retrospective studies. Despite potential tolerance, our results suggest that CLB can be considered as long-term therapy as reported in prior studies.[3] (Remy, 1994).

With regard to dosage, half of patients who responded to CLB did so with dosages > 1.45 mg/kg/day (range 0.5-3 mg/kg/day) and seven required a high dosage > 2 mg/kg/day. Although prior study showed efficacy of CLB with lower doses,[14] our experience suggests

individual variability in necessary doses and that high dosage > 1mg/kg/day is often required. In the current study, frequency and types of adverse events are similar to other reports: most of side effects were mild with sedation and behavioral disorders being the most common without apparent relation to dosage.[13, 18] We did not observe serious toxicity which remains uncommon with CLB, as reported in one study where 11% of the cohort required discontinuation of the medication because of serious behavioral deterioration such as aggressive agitation or self injurious behavior.[23] Furthermore, we reported an improvement in behavior in about one quarter of patients who were all responders, suggesting that this improvement may be related to a better seizure control as previously reported by Munn et al.[19]

Several limitations are inherent to our study. This was not a prospective study with allocation of treatment. The retrospective, observational nature of this study of local practice without any fixed-protocol and the small number of patients implies some weaknesses. It is more difficult to assess accurately the frequency of seizures and tolerability. Side effects may be confounded by the lack of systematic checklist, the use of other drug effects and the high incidence of neurological and behavioral comorbidities in our population. The tolerance rate was impossible to assess accurately in this small and time limited retrospective study. CLB serum concentration was not measured as this has not been routinely available in the United States during the study period. As CLB was only available via importation from Canada and paid for out-of-pocket by patients, its use at the time of the study was limited to patients with refractory epilepsy who have already tried several other AEDs, which may have underestimated the CLB response in TSC related epilepsy.

CONCLUSION

CLB appears to be a well-tolerated and effective option for treatment of refractory epilepsy in TSC, showing an initial good response rate in 69% of patients and 21% at 12 months. Although tolerance may possibly develop, seizure freedom for sustained periods can occur. Additional clinical experience is necessary to demonstrate its long-term efficacy and define the best use of CLB in TSC patients.

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GENERAL DISCUSSION AND FUTURE PROSPECTS

10

TSC is a genetic multi-system disorder characterized by specific brain abnormalities and neurobehavioral manifestations, which are often severe. Although promising therapies for these brain manifestations are currently under study, the factors contributing to the neurobehavioral morbidity of TSC remain poorly understood. In this thesis, the neuroanatomical and neurobehavioral phenotypes, as well as underlying genotypes, of TSC are explored. By providing an updated clinical and theoretical framework, these studies provide insight into the mechanisms associated with neurobehavioral morbidity and into potential treatment targets.

This research was made possible by the contributions of many individuals with TSC and other neurodevelopmental disorders, and their families, to the databases of the Carol and James Herscot Center for TSC at Massachusetts General Hospital (MGH), the Autism Consortium and the Tuberous Sclerosis Alliance. Prospective and retrospective data collection was greatly facilitated by the presence of specialized clinics for patients with rare disorders and by supportive collaborators at MGH, the Erasmus Medical Center and elsewhere. The studies described in this thesis were mostly carried out on a cohort of TSC patients between 4 and 62 years old, who attend the Carol and James Herscot Center for TSC at MGH, the largest TSC referral center in New England. Other pediatric and adult patients were recruited at the outpatient Neurofibromatosis Type 1 clinic and outpatient epilepsy clinics. Active recruitment of patients without cognitive impairment, non-response analyses and comparisons to historical data in the literature showed that the findings in the selected patient cohorts are applicable to the general TSC population.

SEARCHING FOR BIOMARKERS

Recent pre-clinical studies of TSC and related disorders present exciting possibilities for rescue, and perhaps even prevention, of neurocognitive morbidity.[1-4] Because neurocognitive morbidity in TSC is variable and can be very mild, intervention is not always necessary or justifiable, and biomarkers for patient stratification are needed. The identification of practical biomarkers that are predictive for cognitive severity, at a time point early enough for intervention, is an ongoing challenge for clinical research. In **Chapter 1**, **Chapter 2** and **Chapter 4**, the use of genotypic and neuroanatomical biomarkers for neurocognitive morbidity is discussed. Other parameters, such as electrophysiological measures,[5] are equally interesting but beyond the scope of this thesis.

Genotype-phenotype associations

It is well-established that, as a group, *TSC2* mutations are associated with a relatively severe phenotype compared with *TSC1* mutations.[6-10] Patients in whom no mutation can be identified (NMI) also seem to display a distinct, relatively mild phenotype.[11] However, the phenotypes of these three mutation groups overlap greatly, limiting their value as predictors of the neurocognitive morbidity in individual patients. Distinguishing solely between the *TSC1*, *TSC2* and NMI subgroups may be an over-simplification, and the type and location of the mutation may also play a role. For example, *TSC2* deletions

that extend into the adjacent *PKD1* gene are associated with early-onset severe polycystic kidney disease[12] and other reports indicate that some *TSC2* missense mutations are associated with a less severe epilepsy phenotype.[13-16] In **Chapter 2** and **Chapter 3**, we show associations between specific germline mutation types and neurocognitive outcomes. In general, *TSC2* missense mutations were associated with a milder neurocognitive phenotype than truncating *TSC2* mutations. These observations are concordant with findings of milder phenotypes associated with missense mutations in other neurogenetic disorders[17-19] and reflect the possibility that the presence of a protein in the cell, albeit unstable and/or with variable remaining function, may be associated with milder brain disruption and a relatively less severe clinical phenotype.[20-22] *TSC1* missense mutations are rare. Therefore, we could not draw conclusions on these as a group. Nonetheless, it would be useful to establish whether this class of mutation is also associated with a less severe TSC phenotype.

In **Chapter 2**, the previously reported bimodal intelligence distribution[10] is refined to only the *TSC2* population, and it is shown how the phenotypic differences between individuals with truncating and non-truncating mutations could, at least partly, explain this bimodal distribution. Data presented in **Chapter 3** and **Chapter 6** indicate that similar mechanisms are likely to play a role in the occurrence of infantile spasms and in the severity of autistic features.

In addition to the importance of mutation *type* (protein-truncating versus non-truncating mutation), findings in **Chapter 2** also show that another factor contributing to the severity of the disease may be the *position* of the mutation. Centrally located *TSC2* missense mutations were associated with a distinctly milder intellectual phenotype compared with proximal or distal non-truncating mutations, consistent with previous case reports[14-16] and possibly due to retention of TSC1-TSC2 binding and/or GAP activity. Taken together, these findings suggest that different types of genetic mutation have a differential effect on TSC1-TSC2 function, and thus on the TSC neurocognitive phenotype. As it is sometimes difficult to evaluate the pathogenicity of *TSC1* and *TSC2* missense variants, genetic mutation analysis and genotype-phenotype investigations should be supported by functional studies.[20, 23] Furthermore, more detailed knowledge of the structure of the TSC1-TSC2 complex will help us to predict how specific mutations affect TSC1-TSC2 function and contribute to TSC pathogenesis.[24, 25]

Unfortunately, for now, even the detailed genotype-phenotype associations reported in **Chapter 2**, **Chapter 3** and **Chapter 6** were too variable to predict neurocognitive outcome for individual patients. This variable expressivity suggests that other genetic, epigenetic and environmental factors modulate the cognitive phenotype. These factors are discussed further below. The use of genetic predictors of disease severity in TSC should be viewed in the context of a larger equation, including other types of probabilistic prognostic indicators. However, at the present time, genetic mutations are the only biomarkers that can be interrogated prior to the development of the brain malformations in TSC.

UPDATING THE NEURORADIOLOGICAL PHENOTYPE

Tubers are considered the hallmark brain feature of TSC and have been relatively well-characterized. Tuber number and/or burden is significantly associated with neurocognitive morbidity.[26-29] During brain development, tuber formation is presumed to be preceded by proliferation and migration abnormalities in the periventricular zone and white matter.[30] With magnetic resonance imaging (MRI), white matter migration abnormalities are visible as radial migration lines (RMLs), although they are not always seen in relation to a tuber.[31, 32] The presence of migration abnormalities may underlie the regional white matter abnormalities in TSC patients.[33-35]

Recently, the notion that tubers are responsible for neurocognitive morbidity in TSC was challenged by findings in mouse models that suggested possible additional mechanisms such as cell-autonomous synaptic dysfunction[2, 36] or axon guidance abnormalities.[37, 38] So far, little is known on the 'white matter phenotype' of patients with TSC, or how to distinguish RMLs, which are often subtle, from normal white matter. The contribution of white matter migration deficits to the neurocognitive phenotype of TSC also remained unclear.[7, 39, 40]

Imaging techniques such as diffusion tensor imaging (DTI) and thin-section fluid-attenuated inversion recovery (FLAIR) imaging allow the increasingly detailed characterization of white matter. By combining DTI analysis with a detailed morphological evaluation, **Chapter 4** provides an updated neuroanatomical phenotype for TSC, including qualification and quantification of white matter migration deficits viewed as RMLs. For each patient, RMLs were the most frequent neuroanatomical lesion and greatly outnumbered tubers. Comparisons of DTI parameters revealed that normal-appearing white matter could *not* be distinguished from control patients, and confirmed that RMLs were associated with DTI abnormalities. These findings re-identify TSC as a multifocal disorder.[41, 42] As RMLs, tubers and sub-ependymal nodules (SENs) all arise from the periventricular zone and have similar histological characteristics,[30] these lesions are probably very closely related and reflect the same basic deficit in neuronal cell proliferation, differentiation and migration. This idea is supported by the observations in **Chapter 4** that the frequencies of RMLs, tubers and SEN are strongly associated with each other, and with neurocognitive outcomes. By interfering with the development of neural circuitry, these focal dysplasias can cause the seizure phenotype, and affect the widely distributed networks responsible for intelligence, social behavior and communication, as reported in **Chapter 6**.

Findings in mouse models of TSC suggest that activation of mTORC1 is sufficient to cause seizures without the presence of gross neuroanatomical defects, and that these seizures respond to therapy with mTOR inhibitors.[1] This may indicate that mTORC1 dysfunction in the focal lesions is responsive to therapy with mTOR-inhibitors. Postnatal administration of mTORC1-inhibitors cannot completely reverse the widespread neuroanatomical malformations in mouse models of TSC,[43-45] suggesting that interventions are necessary prior to the development of gross pathology, in order to prevent these.[46] It will be important to develop and investigate animal models which most closely resemble the focal phenotype[47, 48] to find out which neurocognitive and neuroanatomical manifestations of TSC can be rescued, and at which neurodevelopmental stage.

EXPANDING THE NEUROPSYCHIATRIC PHENOTYPE

Expression of the phenotype over time

Although the natural history of epilepsy in TSC has been described,[49] little is known about the longitudinal development of intelligence, adaptive functioning and autistic features. As described in **Chapter 5**, most individuals with TSC continued to progress and the level of intelligence was relatively stable for many patients. However, a significant decline in adaptive functioning was observed for the whole cohort, indicating that individuals with TSC fall increasingly behind the general population. The great variability in cognitive function appeared to be mostly determined by seizure variables, confirming previous observations in infants with TSC.[50] Observations suggestive of an epileptic encephalopathy in a subgroup of TSC patients should encourage pro-active treatment of seizures, and perhaps even suppression of interictal epileptiform discharges,[51] regardless of the age of the individual. For example, as described in **Chapter 9**, clobazam was a reasonably tolerated and valuable option in the treatment of refractory epilepsy in TSC, even in adulthood.

The cross-sectional results in **Chapter 6** showed that, similar to intelligence outcomes, the level of autistic features is stable throughout life, and strongly associated with seizure variables. The high rate of subjective sleep disorders in adults with TSC, as described in **Chapter 7**, is similar to observations in children with TSC,[52] suggesting that these are pervasive throughout life and more frequent in patients with epilepsy. In contrast, the stressor-related anxiety disorders, as described in **Chapter 8**, show a more complex pattern. They could develop at any age in patients without pre-existing anxiety disorders, but once arisen, often assumed a chronic course. The pervasive but complex neurobehavioral aspects of TSC are largely unfamiliar to psychiatrists outside specialized centers, and most primary mental health services cannot meet the particular needs of these patients. The holistic approach, including mental health expertise, of physicians for the intellectually disabled (in The Netherlands called 'arts voor verstandelijk gehandicapten' (AVG)), would be beneficial for adolescents and adults with a multisystem disorder such as TSC, regardless of the age or intelligence of the affected individual.

Lastly, as expected for a developmental brain malformation, the frequency of neuroanatomical lesions associated with TSC, as derived from the study group aged four years and older described in **Chapter 4**, was not influenced by age, although cystic degeneration and calcification of tubers over time has been described previously.[53] The compelling observations noted in **Chapter 5**, such as the influence of gender, neurosurgery and genetic mutation on longitudinal cognitive and adaptive development, may provide further clues for research.

Epilepsy-intelligence–autism triad

In **Chapter 6**, we reveal relationships between various neurocognitive manifestations of TSC. In patients with TSC, epilepsy severity, intellectual outcomes and the level of autistic features were closely linked, such that those with an earlier age of seizure onset and lower IQ show a greater level of autistic features as measured by the Social Responsiveness Scale.

In other words, the more severe and generalized the seizure phenotype and cognitive impairment, the more likely it was that the underlying brain disruption also affected the widely distributed networks responsible for social behavior, communication and cognitive flexibility.

Autism spectrum disorders (ASD) are reported to occur with a greater prevalence in TSC patients than in patients with Neurofibromatosis type 1 (NF1).[7] Thus *TSC1* and *TSC2* mutations are presumed to be associated with a high risk of autism.[7, 54] However, the comparisons in Chapter 6, between TSC and related disorders such as NF1 and childhood-onset epilepsy, suggested that the severity, as well as the type of autistic features, were more strongly associated with the severity of the neurocognitive comorbidity, than with the etiological diagnosis. This could indicate that patients with comparable epilepsy and intelligence profiles show similar autistic endophenotypes, regardless of the etiology. Furthermore, these observations suggests that the factors underlying the great variability in the neurocognitive phenotype of TSC and NF1, which are not yet understood, might better explain the severity of autistic features than the underlying genetic mutation. This was further emphasized by observations that TSC patients without seizures or intellectual disabilities showed a normal mean level of autistic features, indicating that the germline mutation alone is not sufficient to cause autism spectrum disorders (ASD), but that additional factors are necessary to lead to the epilepsy-intelligence-autism triad. The study in **Chapter 6** could not provide causal relationships, and future research will hopefully distinguish the role of epilepsy, focal migration abnormalities, or other mechanisms in the development of these outcomes.

Although it has been suggested that aberrant mTORC1 signaling may be a shared feature of syndromic and high-functioning idiopathic ASD,[55] genetic linkage and genome-wide association studies are contradicting, and often underpowered and/or lacking in clinical information.[56-58] The observations in **Chapter 6** suggest that autism is *not* an isolated disorder in TSC. Nonetheless, TSC research may serve to understand the epilepsy-intelligence-autism triad, as mTORC1 might be a common mediator in the interaction between early life seizures, cognitive dysfunction and neurodevelopmental comorbidities.[59] Studies on patients manifesting this triad, which is about a third of the total ASD population[60] might provide more insight.

Sleep and stressor-related disorders

As described above, research in TSC is often focused on neurobehavioral outcomes. It is therefore surprising that very little is known about the predisposing factors, phenomenology, and treatment of the sleep and anxiety disorders that are highly prevalent in TSC. In **Chapter 7** and **Chapter 8**, selected sleep and anxiety disorders are described in pediatric and adult patients with TSC, using a questionnaire-based approach and a retrospective investigation, respectively.

Previous research on sleep in TSC has been limited to children.[52, 61, 62] The questionnaire study discussed in **Chapter 7** showed a subjective sleep disorder in 31% of adults with TSC, that was significantly associated with daytime sleepiness, psychological complaints, and the use of psychotropic medication. As a group, responders showed relatively mild

neurocognitive deficits, and it is possible that sleep abnormalities are even more frequent and severe in adult TSC patients with more significant intellectual disabilities, due to the greater underlying brain disruption in these individuals, as described in **Chapter 4**. The reported sleep disruption and daytime sleepiness are especially interesting with regards to recent suggestions that mTOR signaling may contribute to distinct aspects of the circadian rhythm.[63] Future studies should further investigate the underlying pathophysiology of sleep disorders in TSC.

In **Chapter 8**, the phenomenology of post-traumatic stress disorder (PTSD) and adjustment disorder (AD) is described in nine TSC patients. Importantly, PTSD and AD arose in settings that for the general population would not usually be considered traumatic. Presenting symptoms, consisting of aggression and/or avoidance, were of long duration and refractory to standard treatments. Anxiety is a frequent and debilitating symptom in patients with TSC, but the risk factors are less clear than those found for seizures, intelligence and autistic features. As in the general population, anxiety disorders may also follow a different developmental course than ASD or attention-deficit-hyperactivity-disorder (ADHD).[64] Raising awareness about the vulnerability of TSC patients to stressor-related disorders will facilitate early diagnosis and treatment. Further exploration of the cause, expression and treatment of anxiety disorders in TSC is warranted.

Neuroradiological and electrophysiological biomarkers

In **Chapter 4**, the frequency of radial migration lines (RMLs) was shown to be strongly associated with age at seizure onset, intelligence outcomes and the level of autistic features. Although this suggests that RMLs may be suitable imaging biomarkers in TSC, quantification of RMLs is extremely laborious, as optimal detection depends on evaluating MRI images at various angles and in sufficiently thin sections. Furthermore, the imaging features of focal white matter deficits *in utero* are not yet known. The finding that RMLs are responsible for the microstructural white matter abnormalities detected with diffusion tensor imaging (DTI), as presented in **Chapter 4**, provides a theoretical model for the use of connectivity parameters as biomarkers.[35] DTI may detect brain abnormalities before conventional MRI, which is particularly critical in the context of the administration of neuroprotective therapies. Other automated imaging methods for evaluating white matter abnormalities, such as white matter hyperintensity indices,[65] may also be useful. The strong brain-behavior relationships found in this thesis, combined with the insights on the inextricability of the autism-intelligence-epilepsy triad reported in **Chapter 6**, suggest that a valid biomarker specific for one domain of neurocognition, could predict global neurocognitive morbidity.

Lastly, it should be noted that DTI and lesion characteristics may serve as biomarkers for treatment stratification and response. For example, while patients with refractory epilepsy, as described in **Chapter 9**, are in most dire need of therapy, the results of clobazam treatment suggest that patients with relatively less tubers respond better. Thusfar, little is known about efficacy predictors and the side-effect profiles of anti-epileptic drugs and psychotropic medications in patients with TSC. Further research should elucidate these important issues.

CROSS-DISORDER COMPARISONS

As shown in **Chapter 1** and **Chapter 2**, genotype-phenotype predictions in TSC are limited by the variability of the TSC phenotype. This variability occurs in most neurodevelopmental disorders, as was observed in the cross-disorder explorations in **Chapter 6**. Shared mechanisms, such as additional genetic or epigenetic and environmental factors may modulate these phenotypes, but for now, it is unclear which of these 'second events' are random or syndrome-specific. To facilitate the identification of factors uniquely associated with specific neurobehavioral outcomes, a cross-disorder approach is required. For example, genetic, epigenetic and environmental modifiers of the neurobehavioral phenotype, such as have previously been suggested for viral infection during gestation in TSC,[66] maternal autoimmune disorders in Fragile-X,[67] and paternal age in autism spectrum disorders,[68] could be more easy to identify when patients with all of these neurodevelopmental disorders are combined into one cohort. Investigators should aim to distinguish large cohorts of individuals with neurodevelopmental disorders based on behavioral homogeneity, only enabling syndrome heterogeneity when necessary.[69] Subgrouping into pathophysiologically similar subgroups, such as individuals with *TSC1*, *TSC2*, *NF1* and *DEPDC5* mutations which have all been associated with focal seizures, oncogenic processes and neuropsychiatric disorders, could assist in identifying the pathways leading to epileptogenesis as well as other comorbidities.

The exploration of syndrome-specific behavioral manifestations is limited by overlaps between neurodevelopmental disorders, which are the rule, rather than the exception.[70] For example, the observations in **Chapter 6** indicate that the rate of autistic features is closely associated with the level of global cognitive deficits. To acknowledge a common underlying biological construct, terms such as 'complex autism', 'multiple complex developmental disorder', and 'syndromal autism' have been proposed[71-73] and this approach may also be applicable to other psychiatric classifications.

Anxiety disorders are frequent in pediatric and adult patients with TSC,[74, 75] and in many other neurodevelopmental disorders syndromes such as Fragile X syndrome, Williams Syndrome, Neurofibromatosis type 1, and idiopathic autism spectrum disorders. Only rarely, potential causes for anxiety have been identified, such as hyperacusis leading to phobias in Williams Syndrome.[76] In TSC, predisposing factors for anxiety may include brain disruption involving fear circuitry, anticipatory fear for seizures or tumor growth, anxiety associated with medical interventions and cosmetic appearance, an overall life course that increases vulnerability to anxiety-evoking events. In **Chapter 8** this complexity was underlined in a cohort of TSC patients with post-traumatic stress syndrome (PTSD) or adjustment disorder (AD). These disorders could arise at any time point, even without a clear precipitating event, with various and complex symptomatology, often taking on a refractory course. Distinguishing 'primary' psychiatric disorders from those that originate directly from the disruption of brain structure and/or function by a genetic mutation, can be difficult for the clinician. It may be that anxiety associated with genetic syndromes such as TSC is less genetically determined and under great influence of other factors, as has been proposed for idiopathic anxiety.[64, 77] Cross-disorder identification of genetic and

non-genetic causes of anxiety may assist in prevention, early detection and appropriate treatment of anxiety disorders in individuals with neurodevelopmental disorders.

Quantitative data, as described in **Chapters 1, 4**, **5** and **6**, generally provided significant insight into gene-brain-behavior relationships, whereas dichotomous variables, such as 'history of infantile spasms' in **Chapter 3**, proved to be less informative. This, and the overlap between neurodevelopmental disorders as described above, support previous calls that neurobehavioral traits such as intelligence or autistic-like features should be assessed using quantitative measures rather than as a qualitative, dichotomous trait.[78] For example, for TSC, age at seizure onset is a consistent and independent determinant of cognitive function[9] and is easily retrieved from clinical databases. The longitudinal TSC database at the Herscot Center proved invaluable for providing information on the natural history of the manifestations of TSC. Similar databases should be established for all neurodevelopmental disorders, preferably combined. In addition to providing information for research, a repository for both the clinical data and the quantitative measurements of neuropsychiatric functioning will provide useful historical data for the care provider. Experience suggests that adult patients with intellectual disabilities often become 'known well by no one',[79] and a database would help prevent this problem.

In conclusion, the studies addressed in this thesis provide new insight into the clinical spectrum of TSC, particularly in adolescents and adults. Increased awareness of the variable, pervasive and treatment-refractory phenotype of TSC should be translated into improved health care for TSC patients. Joint pediatric/adult neurodevelopmental clinics run by pediatricians, child neurologists, clinical geneticists and physicians for the intellectually disabled (AVG) may provide such a supportive environment.[79, 80] Specialized AVGs could consider dedicating themselves to improving health and quality of life for individuals with neurodevelopmental disorders across the spectrum of neurocognitive morbidity and in all age groups, and not limit their care to adults or the intellectually disabled population only.

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11

SUMMARY

SAMENVATTING

LIST OF PUBLICATIONS

CURRICULUM VITAE

PHD PORTFOLIO

ACKNOWLEDGEMENTS

LIST OF ABBREVIATIONS

SUMMARY

Tuberous Sclerosis Complex (TSC) is a genetic disorder which is caused by mutations in the *TSC1* or *TSC2* genes. Mutations in these genes can result in growth of benign tumors in virtually every organ system, including the brain. Characteristic brain abnormalities are tubers, white matter abnormalities, sub-ependymal nodules and sub-ependymal giant cell astrocytomas. These brain abnormalities can result in severe neurobehavioral comorbidity such as refractory seizures, learning problems and intellectual disability, and behavioral symptoms such as autistic features, hyperactivity, aggression, anxiety and sleep disorders. The neuropsychological problems are experienced as the greatest burden of TSC by patients as well as caregivers, and the major cause for seeking help from health care professionals.

Thusfar, little is known on the cause and treatment of the epilepsy and psychological disorders in TSC, or the expression of the broad neurobehavioral phenotype over time. In addition, the *TSC1/TSC2* mutation groups, or the tuber burden, seem inadequate as predictors of the neurobehavioral phenotype. The purpose of this thesis was to further explore the neurobehavioral phenotype in children and adults with TSC, as well as the underlying genetic and neuroanatomical factors.

In **Chapter 2**, **Chapter 3** and **Chapter 6**, the most recent genetic insights were applied in large patient cohorts, using quantitative outcomes where possible. This revealed that the *location*, as well as the *type* of mutation, are predictive factors for the level of intelligence, the occurrence of infantile spasms, and the rate of autistic features. Mutations that did not result in truncating of the protein, such as missense mutations, were associated with a milder neurocognitive phenotype. The same was the case for mutations in the central part of the *TSC2* gene, which is less involved in the binding and GAP-function. Results in **Chapter 2** also divulged that the bimodal distribution of intelligence in *TSC2* patients may, for a large part, be explained by these differential effects of mutations that do, or do not, lead to truncation of the TSC2 protein. Unfortunately, even when applying these detailed mutation classifications, the genotype-phenotype associations remained variable, limiting their use in a clinical setting.

The elusive 'white matter phenotype' in TSC inspired the detailed and complete radiological evaluation of the neuroanatomical phenotype, including white matter abnormalities called 'radial migration lines' (RMLs). Findings in **Chapter 4** suggest that, when RMLs are taken into account, the normal-appearing white matter does not show microstructural abnormalities. RMLs were the most frequently occurring neuroanatomical abnormality, and were strongly associated with the number of sub-ependymal nodules and tubers. Furthermore, the number of RMLs as well as other lesion types were strongly associated with the age of seizure onset, level of intelligence, and the rate of autistic features. These findings imply that these neurobehavioral manifestations of TSC can be explained by the presence of focal abnormalities rather than a global synaptic white matter deficit.

Since many patients with TSC receive the diagnosis 'autism', and the cause of idiopathic autism spectrum disorder remains unclear, TSC is often used as a study model for idiopathic autism. In **Chapter 6**, the relationship between autistic features, epilepsy

variables and intelligence is described, showing that these variables are inextricable in TSC. This epilepsy-intelligence-autism triad seemed to express itself similarly in related disorders such as childhood-onset epilepsy of unknown cause and Neurofibromatosis type 1. When comparing these disorders, the underlying disorder was a weaker predictor for the severity of autistic features than the severity of the neurocognitive comorbidity. In other words, the level and expression of autistic features is more similar between patients with comparable age of seizure onset and intelligence, than between patients with the same disorder. Such observations imply that autism is not an isolated disorder in TSC, which is important for clinical care as well as research. Although TSC-research could help elucidate the pathophysiology of autistic features in combination with epilepsy and cognitive impairment, it may be an inadequate model to study isolated autism spectrum disorders.

Although the natural history of epilepsy in patients with TSC has been studied, the trajectory of intelligence, and a functional part called 'adaptive functioning' remained unclear. In **Chapter 5**, results are presented of a longitudinal study on these important outcomes, showing that adaptive functioning declined significantly for the whole patient cohort. Intelligence seemed relatively more stable, but could be very variable, mainly under the influence of the epilepsy severity. This chapter emphasized the importance of aggressive detection and treatment of seizures, and perhaps interictal epileptogenic discharges, even at a later age. For example, the study described in **Chapter 9** shows that clobazam could be an acceptable treatment option for refractory seizures, even in older patients with TSC.

In **Chapter 7**, sleep disorders in adults with TSC are addressed, revealing that these occur frequently and are associated with psychological complaints and the use of psychotropic drugs and anti-epileptic medications. The report on stressor-related disorders in **Chapter 8** clarified that these are also frequent, and refractory to treatment. These chapters underline the complex symptomatology of TSC and that many questions remain on the etiology, pathophysiology and treatment of neurobehavioral disorders in patients with TSC.

This thesis offers an update on the neurobehavioral phenotype of children and adults with TSC, as a starting point for future research as well as clinical care. All of the included chapters underlined that the TSC phenotype is complex and pervasive. Insights in germline- and later risk factors that lead to the neuroanatomical and neurobehavioral phenotype in children and adults, will hopefully generate effective and preventative therapies for this severe disorder. Until then, the complex and treatment-refractory symptoms of TSC require specialized and holistic care, such as provided by physicians for the intellectually disabled.

SAMENVATTING

Tubereuze Sclerose Complex (TSC) is een autosomaal-dominant overervend syndroom dat wordt veroorzaakt door mutaties in de *TSC1* of *TSC2* genen. TSC kan leiden tot groei van goedaardige tumoren in alle orgaansystemen, inclusief de hersenen. Karakteristieke neuroanatomische afwijkingen zijn tubers, witte stof afwijkingen, sub-ependymale nodules en sub-ependymale reusceltumoren. Deze hersenafwijkingen gaan vaak gepaard met ernstige epilepsie, leerproblemen en verstandelijke handicaps, en gedragsverschijnselen zoals autistische kenmerken, hyperactiviteit, agressie, angst-stoornissen en slaapproblemen. Deze neuropsychologische verschijnselen worden als zeer belastend ervaren door zowel patienten als hun verzorgers.

Tot dusver is er nog weinig bekend over de oorzaak en behandeling van de neuropsychologische verschijnselen van TSC, en het verloop hiervan na de kinderleeftijd. Ook voldoen *TSC1/TSC2* mutatie-groepen of tuber-kwantificaties niet als voorspellers van het neuropsychologische fenotype voor individuele patienten. De doel van het onderzoek in dit proefschrift waren het verder verduidelijken van het neuropsychologische fenotype in kinderen en volwassenen met TSC, en de neuroanatomische en genetische factoren die hieraan bijdragen.

In **Hoofdstuk 2**, **Hoofdstuk 3** en **Hoofdstuk 6** werden de meest recente genetische inzichten toegepast in grote patintcohorten, met kwantitatieve uitkomsten waar mogelijk. Hieruit bleek dat zowel de *locatie*, als het *type* van mutaties een rol speelt in het tot stand komen van het niveau van intelligentie, het syndroom van West, en de mate van autistische kenmerken. Mutaties die niet tot truncering van het eiwit leidden, zoals missense mutaties, waren geassocieerd met een milder neurocognitief phenotype. Hetzelfde gold voor mutaties in het centrale gedeelte van het *TSC2* gen, dat niet betrokken is bij de bindings- of GAP-functie. In **Hoofdstuk 2** bleek tevens dat de bimodale distributie van intelligentie in *TSC2* patienten ook grotendeels kon worden veklaard door deze verschillende effecten van mutaties die wél of niet de truncering van de TSC2 eiwit veroorzaken. Ondanks deze gedetailleerde classificatie van mutaties bleef de grote variabiliteit van de genotype-phenotype bevindingen de klinische toepassing hiervan beperken.

Alhoewel het bekend is dat in TSC zowel macrostructurele als microstructurele witte stof afwijkingen voorkomen, was het complete 'witte stof phenotype' van TSC onduidelijk. De gedetailleerde en complete beschrijving van het complete neuroanatomische phenotype in **Hoofdstuk 4** gaven het inzicht dat, als de focale witte stof afwijkingen die zichtbaar zijn als radiale migratie-lijnen, goed in acht worden genomen, de resterende normale witte stof geen microstructurele afwijkingen vertoont. Tevens was het aantal radiale migratie lijnen zowel zeer sterk geassocieerd met het aantal sub-ependymale nodules en tubers, als met de leeftijd van het ontstaan van epilepsie, en het niveau van intelligentie en autistische verschijnselen. Deze bevindingen bekrachtigen dat de neurocognitieve problemen in patienten met TSC verklaard kunnen worden door de aanwezigheid van focale afwijkingen, in tegenstelling tot de globale synaptische afwijkingen die in TSC muis-modellen zijn geobserveerd.

Omdat bij veel patienten met TSC de diagnose 'autisme' word gesteld, en de oorzaak van idiopathisch autisme nog onduidelijk is, wordt TSC vaak als studiemodel voor idiopathisch autisme gebruikt. In **Hoofdstuk 6** werd de relatie tussen autistische kenmerken, epilepsie variabelen, en intelligentie onderzocht, waaruit bleek dat deze drie onlosmakelijk verbonden waren. Deze epilepsie-intelligentie-autisme triade leek ook tot uiting te komen in gerelateerde neuro-ontwikkelingsstoornissen zoals Neurofibromatose type 1 en idiopathische epilepsie op kinderleeftijd. De onderliggende genetische aandoening leek een zwakkere voorspeller van autistische verschijnselen dan de ernst van neurocognitieve comorbiditeit. Met andere woorden, het niveau en expressie van autistische kenmerken kwam sterker overeen tussen groepen met vergelijkbare neurocognitieve morbiditeit, dan tussen etiologische subgroepen. De observaties dat de autistische kenmerken niet uniek leken voor deze aandoeningen, en ook niet in geïsoleerde vorm voorkwamen, is belangrijk voor patientenzorg, maar ook voor toekomstig onderzoek. Alhoewel TSC-onderzoek zou kunnen helpen in het begrijpen van autistische kenmerken in combinatie met epilepsie en cognitieve beperkingen, lijkt het inadequaat als model voor geïsoleerd voorkomende autistische spectrum stoornissen.

Alhoewel er veel bekend is over het verloop van epilepsie in patienten met TSC, was het verloop van het intelligentie-niveau, en een functioneel onderdeel daarvan wat 'adaptief functioneren' word genoemd, onduidelijk. De bevindingen in **Hoofdstuk 5** onthulden dat het adaptief functioneren geleidelijk afnam voor de gehele groep. Het niveau van intelligentie leek relatief meer stabiel, maar bleek ook variabel, wat vooral leek verklaard door de ernst van de epilepsie. Dit hoofdstuk benadrukte het belang van aggressieve behandeling van epilepsie, en wellicht ook interictale ontladingen, op elke leeftijd. In **Hoofdstuk 9** werd bijvoorbeeld het effect van clobazam beschreven bij een groep patienten met TSC en therapie-resistente epilepsie, waaruit bleek dat clobazam een goede behandelingsoptie kan zijn.

In **Hoofdstuk 7** werd de aandacht gevestigd op slaapstoornissen bij volwassen patienten met TSC, waaruit bleek dat deze veel voorkomen en gepaard gaan met psychologische klachten en het gebruik van psychotrope medicijnen en anti-epileptica. In **Hoofdstuk 8** werd duidelijk dat stressor-gerelateerde aandoeningen, zoals post-traumatische stress stoornis, relatief veel voorkomt bij patienten met TSC, en dat deze moeilijk te behandelen zijn. Deze hoofdstukken benadrukken de complexe symptomatiek van TSC en dat er nog veel onduidelijkheden zijn op het gebied van etiologie en behandeling van neuropsychiatrische problemen in patienten met TSC.

Dit proefschrift biedt een 'update' on het gedragsfenotype van kinderen en volwassenen met TSC, als uitgangsbasis voor zowel de wetenschap als klinische zorg. Alle genoemde hoofdstukken droegen bij in het inzicht dat het gedragsfenotype van TSC complex is, en zich uit over de gehele levensduur. Meer inzicht in de kiemcel- en latere gebeurtenissen die leiden die leiden tot de neuroanatomische en neuropsychologische verschijnselen bij kinderen en volwassenen, leidt hopelijk tot preventieve en effectieve therapieën voor deze ernstige aandoening. Tot die tijd vereisen de complexe en therapie-resistente verschijnselen van TSC, gespecialiseerde en holistische zorg, bijvoorbeeld van een arts voor verstandelijk gehandicapten (AVG).

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12/2007-05/2008	Physician for the intellectually disabled at the Hartekamp Groep, Heemstede, the Netherlands.
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10/2001-12/2002	Clinical Genetics at the Clinical Genetics Department at the Vrije Universiteit Hospital in Amsterdam.

Education

1998-2001	Medical Internships at the Academic Medical Centre in Amsterdam, resulting in Medical Diploma, M.D..
1997-1998	Research at the Pediatric and Clinical Genetic Department of the Academic Medical Centre in Amsterdam on the subjects of holoprosencephaly, Costello Syndrome and autism, under mentorship of Prof. R.C.M. Hennekam.
1993-1997	Medicine Studies in Groningen, the Netherlands.
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PHD PORTFOLIO

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PhD/research period:	November 2009-December 2013
Promotor:	Prof.dr. Ype Elgersma

	Year	Workload
Courses		
Collaborative Institutional Training Initiative (CITI) Basic Biomedical or Basic Social & Behavioral Research Course	2010, 2013	2 x 6 hours
'Basic Biostatistics', Harvard Medical School, Boston, USA	2010	6 x 2 hours
'Applied Biostatistics', 4x 2 hours, Harvard Medical School	2011	4 x 2 hours
Seminars		
Child Neurology, Boston, USA	2010	5 days
Advances in diagnosis and treatment of autism spectrum disorders, Boston, USA	2012	1 day
Seminar Complex Trait Genetics, Boston, USA	2010	5 x 2 hours
Conference Autism Consortium Symposium, Boston, USA	2012	1 day
Conference 'Psychopharmacology' Boston, USA	2013	3 days
Seminar 'Epigenetics' 3 x 2 hours, Boston, USA	2010	
International Conferences		
Summit on Drug Discovery in TSC and related disorders, Washington, USA	2011	3 days
Conference American Society of Neuroradiology, San Diego, USA	2013	3 days
Presentations		
Various poster presentations, including: 'Relationship between autism, epilepsy and intelligence; a cross-disorder approach', awarded the MGH Department of Neurology Research Award. 'Association between genotype and cognitive phenotype in TSC', awarded the poster award by the American LAM society.	2012 2011	
Presentation "Relationship between genotype and neurocognitive phenotype in TSC" at AVG symposium "Turen naar Horizon', Rotterdam, the Netherlands.	2012	
Presentation 'The neuroradiological phenotype of TSC: focus on radial migration lines'. San Diego, USA.	2013	
Presentation "Neuroradiological phenotype of TSC: focus on radial migration lines". Boston Children's Hospital, USA	2013	
Teaching activities		
Course 'Neurogenetics' for Harvard neuroscience bachelor students	2011	

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LIST OF ABBREVIATIONS

(in alphabetical order)

ABC	Adaptive Behavior Composite
AD	Adjustment Disorder
ADC	Apparent diffusion coefficient
ADHD	Attention deficit hyperactivity disorder
AED	Anti-epileptic drugs
ASD	Autism spectrum disorder
CBT	Cognitive behavioral therapy
CLB	Clobazam
CT	Computed tomography
DWI	Diffusion weighted imaging
DSM	Diagnostic and statistical manual of mental disorders
DTI	Diffusion tensor imaing
DQ	Developmental Quotient
EEG	Electroencephalogram
EUC	Childhood-onset epilepsy of unknown cause
FA	Fractional anisotropy
FLAIR	Fluid-attenuated inversion recovery
GAD	Generalized anxiety disorder
GAP	GTPase-activating domain
HID	Hamartin interaction domain
ICC	Intraclass correlation coefficient
ID	Intellectual Disability
IED	Interictal epileptiform discharges
IS	Infantile spasms
IQ	Intelligence Quotient
LD	Learning disability
MD	Mean diffusivity
MR	Mental retardation
MRI	Magnetic resonance imaging
NAWM	Normal-appearing white matter
NF1	Neurofibromatosis type I
NMI	No mutation identified
NPA	Neuropsychological assessment
OSAS	Obstructive sleep apnea syndrome
PT	Protein-truncating
PTSD	Posttraumatic stress disorder
RLS	Restless legs syndrome
RML	Radial migration line
SEGA	Sub-ependymal giant cell astrocytoma
SGCT	Sub-ependymal giant cell tumor
SEN	Sub-ependymal nodule
SWI	Susceptibility weighted imaging
TID	Tuberin interaction domain
TSC	Tuberous Sclerosis Complex
WM	White matter