Identification of epileptogenic tubers in patients with tuberous sclerosis complex

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ISBN: 12978-90-39344620
Cover design: Geertjan Huiskamp, Roy Sanders
Lay out: Roy Sanders
Printed by: Gildeprint drukkerijen B.V.

Identification of epileptogenic tubers in patients with tuberous sclerosis complex

Identificatie van epileptogene tubers bij patiënten met tubereuze sclerose complex

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de Rector Magnificus, Prof. Dr. W.H. Gispen,
ingevolge het besluit van het College voor Promoties in het openbaar te verdedigen
op dinsdag 13 maart 2007 des middags te 4.15 uur

door

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geboren op 21 mei 1971 te Stad Delden

Promotoren: Prof. Dr. O. van Nieuwenhuizen
Prof. Dr. A.C. van Huffelen

The studies described in this thesis were supported by a grant of the Nationaal Epilepsie Fonds (project number 02-13).

Financial support by the Stichting Tubereuze Sclerose Nederland, the Stichting Het Remmert Adriaan Laan Fonds, the Stichting EPOCH, the Michelle Stichting, the Nationaal Epilepsie Fonds, UCB Pharma, Sanofi Aventis, TEVA Pharma, Janssen-Cilag and Biogen Idec for publication of this thesis is gratefully acknowledged

Contents

Chapter 1	Introduction	7
Chapter 2	Historical note: Tuberous sclerosis complex and its founders	13

Part I

Chapter 3	Overlapping neurological and cognitive phenotypes in patients with *TSC1* or *TSC2* mutations	21
Chapter 4	Tuber status is not the only determinant of cognitive function in tuberous sclerosis complex	39

Part II

Chapter 5	Consistent localisation of interictal epileptiform activity on EEGs of patients with tuberous sclerosis complex	59
Chapter 6	Identification of the epileptogenic tuber in patients with tuberous sclerosis complex: A comparison of high-resolution EEG and MEG	71
Chapter 7	Diffusion-weighted MRI identifies the epileptogenic tuber in patients with tuberous sclerosis	85

Part III

Chapter 8	Epilepsy surgery in tuberous sclerosis: A systematic review	97
Chapter 9	Epilepsy surgery in tuberous sclerosis: The Dutch experience	111

Part IV

Chapter 10	Differential localisation of hamartin and tuberin and increased S6 phosphorylation in a tuber	129
Chapter 11	General discussion	139
	Summary	147
	Samenvatting	153
	Publications	159
	Dankwoord	163

1 Introduction and outline of the thesis

Introduction

Tuberous Sclerosis Complex (TSC), also known as Bourneville- Pringle disease, is a neurocutaneous syndrome with an autosomal dominant inheritance. The disease is present in about 1 in 6000 births and has an overall prevalence of 1 in 30.000. TSC is characterised by hamartomas and hamartias that may be present in almost any organ, but are most frequently found in the brain, the skin, the kidneys, the eyes, and the heart. Malignant tumours are seldom found.
TSC is noted for its clinical variability and there is no single clinical or radiographic sign that is absolutely specific for the disease. Therefore, a definite diagnosis requires the presence of two major features or one major plus two minor features.[1] TSC results from a mutation in *TSC1*, a gene on chromosome 9q34,[2] or *TSC2*, a gene on chromosome 16p13.[3] *TSC1* and *TSC2* act as growth suppressor genes, which is supported by the identification of frequent loss of heterozygosity (LOH) in hamartomas, indicating that second somatic mutations may be required to produce the TSC phenotype at the cellular level. However, LOH has not been found in tubers and alternative mechanisms for tuberogenesis have been suggested. Hamartin and tuberin, the protein products of respectively *TSC1* and *TSC2* are widely expressed in foetal tissues. A critical role in early development and maturation has been assigned to these two proteins.
Pathologically, TSC is caused by a disruption of cell proliferation, migration and differentiation. Partly differentiated cells have been found at the cortical plate where they form aggregates of dysplastic cortex, the so-called tubers.[4] A variety of neurological symptoms may occur in patients with TSC, including epilepsy, cognitive impairment and behavioural disturbances.[5]
Epilepsy is the most common neurological symptom, occurring in 80- 90% of cases, and is a possible mediator in the underlying encephalopathy. Thus, seizure control is of utmost importance. Drug treatment is often not sufficient in patients with TSC. One of the non-medical treatment options is epilepsy surgery. Clear identification of the epileptogenic zone and its relation to the tuber(s) is a prerequisite for epilepsy surgery. New developments in clinical neurophysiological techniques and the use of more sophisticated source localisation algorithms have contributed to an increase of localising power. Integration of these techniques opens chances for epilepsy surgery in TSC patients with multi-drug-resistant epilepsy, reducing the problem of relating multifocal tubers to one epileptogenic zone. If a single epileptogenic tuber is identified, in a non-eloquent part of the brain, epilepsy surgery should be considered.
The principal aim of this thesis is to contribute to the process of selection of epilepsy surgery candidates. In **Part I** of the thesis the study sample is described. Risk factors of an aggressive epilepsy syndrome as well as cognitive impairment are explored. **Part II** describes the additional value of advanced neurophysiological and neuroimaging techniques with respect to the selection of patients for epilepsy surgery. **Part III** reports on the outcome of epilepsy surgery in TSC world wide and gives an update on the Dutch experience. In **Part IV** an attempt is made to contribute to the understanding of tuberogenesis. The limitations of this thesis are discussed

and future implementations for the treatment of drug-resistant TSC patients are given.

Outline of the Thesis

Chapter two is an historical overview of the first reports of the disease. A rendition of both founders is given.

Part I

In *Chapter three* the differences in neurological and cognitive phenotype between patients with different *TSC1* and *TSC2* mutations are described. *TSC2* is hypothesised to be associated with more severe neurological and cognitive symptoms. In addition, associations between mutations inactivating the tuberin GTPase activating (GAP) domain and truncating mutations and the neurological and cognitive phenotype are analysed. *Chapter four* outlines the study sample. We analyse the relation between tuber status (expressed as the number of tubers and the proportion of brain volume occupied by tubers; tuber/ brain proportion (TBP)) and both the epilepsy syndrome and cognitive functioning. We hypothesise that the TBP more critically determines cognitive functioning than the number of tubers. We further analyse the relation between epilepsy variables and cognitive functioning. In this chapter we attempt to identify prognostic factors of cognitive functioning.

Part II

Because of the characteristics of tubers which are, multiple and widespread, shifting of epileptogenicity from one tuber to another is feared. However, evidence for shifting foci is lacking. In *Chapter five* we refute the assumption of shifting foci by addressing the consistency of the localisation of interictal epileptiform EEG activity. EEGs of patients who had had epilepsy for at least 10 years are reviewed. Clinical characteristics of patients with consistent and patients with inconsistent localisation of interictal epileptiform activity are explored. In *Chapter six* we analyse the surplus value of magneto-encephalography (MEG) with respect to the identification of the epileptogenic tuber. Results of high-resolution EEG recordings are compared with MEG recordings. Identified epileptogenic sources with at least moderate interobserver agreement are integrated in the MRI scans of the TSC patients and distances to tubers are calculated and compared. *Chapter seven* is a pilot study with diffusion-weighted MR images (DWI) in four TSC patients. We report on the diffusion characteristics, expressed as apparent diffusion coefficient (ADC) of tubers and compare the ADC of visually assessed normal regions of cortex with non-epileptogenic tubers, and with presumed epileptogenic tubers. We aim to differentiate epileptogenic from inert tubers on the basis of DWI, in order to contribute to the selection of patients for epilepsy surgery.

Part III

Chapter eight gives an overview of the current literature of epilepsy surgery in patients with TSC. Results of seizure outcome are reported. Patient characteristics as well as results of ancillary investigation are related to seizure outcome in order to identify possible risk factors of recurrent seizures.
In *Chapter nine* the results of epilepsy surgery in Dutch TSC patients are shown. We report on the results of pre-surgical evaluation, with the attempt to identify the epileptogenic tuber, in 25 patients referred to the Dutch Comprehensive Epilepsy Surgery Programme in the last 5 years. Seizure outcome and cognitive functioning of patients who underwent epilepsy surgery are described.

Part IV

In *Chapter ten* we aim to contribute to the current understanding of the mechanism of tuberogenesis. Molecular biological analysis is performed in an epileptogenic tuber, resected from a patient with drug-resistant epilepsy. In *Chapter eleven* the implications of the results as described in this thesis are discussed. Future perspectives of the treatment of drug-resistant epilepsy in TSC patients are given.

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2

Historical note: Tuberous sclerosis complex and its founders

Floor Jansen, Alexander van Huffelen, and Onno van Nieuwenhuizen

History of the tuberous sclerosis complex (TSC)

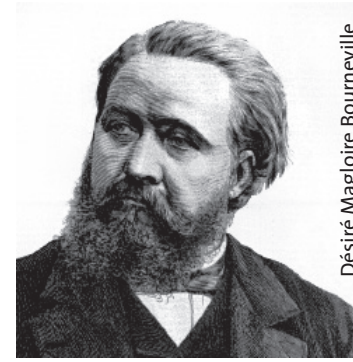
The names of Bourneville and Pringle are associated with the disease, but they were not the first who reported patients with signs later defined as diagnostic criteria. Reports start in the nineteenth century and are based on descriptions of clinical and pathological findings.
The characteristic skin lesions were mentioned for the first time in 1835. Rayer's atlas on skin diseases illustrates the facial 'végétations vasculaires'. These lesions were renamed 'adenoma sebaceum' by Pringle, who is remembered today for his description of a patient with adenoma sebaceum.[1] He also mentioned the associated mental subnormality.
In 1862, Von Recklinghausen, primarily associated with neurofibromatosis, first mentioned the cerebral involvement. On autopsy of a newborn, that died a few minutes after birth, he found cardiac 'myomata' and 'sclerotic' brain lesions, but he did not make the association between the two pathologies. Description of the cerebral pathology and neurological signs is credited, however, to Bourneville. He proposed the term 'sclérose tubéreuse des circonvolutions cérébrales' to characterise the large islets of sclerosis he found in the cortical gyri of two patients who died of epilepsy.[2,3] He found kidney masses in one of the patients as well.
It was in the beginning of the twentieth century that a more complete clinical picture of TSC was obtained. The association between the cerebral, renal, cardiac, and dermal lesions was first recognised in 1905, leading to the clinical diagnostic triad consisting of epilepsy, idiocy and adenoma sebaceum, often called after Vogt. Van der Hoeve, who characterised the retinal hamartomas, introduced the name phakomatosis (phakos means spot), comprising both TSC and neurofibromatosis. After the first descriptions of radiological calcifications, the introduction of the CT and MRI scans greatly improved the diagnostic process. Gomez, the "godfather of TSC research", first published the full clinical and subclinical spectrum of TSC in 1979.[4]
The hereditary basis of TSC was mentioned as early as 1913. Positional cloning studies led to the identification of *TSC1* with the locus on chromosome 9, and *TSC2* with the locus on chromosome 16. Better insight in the putative molecular mechanisms underlying the development of the hamartomatous lesions has yet to be achieved.

The 'founders' of the disease

Désiré Magloire Bourneville (1840-1909)[5]

Bourneville was born on October 21, 1840 in Garencières, Normandy. Although a cholera epidemic prevented him from completing secondary school, he entered the Paris Medical Faculty in 1860, on recommendation of Delasiauve, a family friend, who became one of his mentors. In 1865 he was resident of the Paris hospitals. From 1868 Bourneville became the "unofficial" assistant of Charcot at the Salpêtrière hospital, where he was appointed as head of the department of

Psychiatry, including patients with epilepsy and mental retardation. A remarkable relation between the two men arose.


Désiré Magloire Bourneville

Bourneville's scientific career started in 1861. His doctoral thesis was submitted in 1870. He was the founder of many journals; namely *La Mouvement Medical* (1865), *La Clinique de L'Hôpital Saint- Louis* (1868), *Le Progrès Médical* (1873), and *Archives de Neurologie* (1880). Bourneville's own viewpoints were promoted in *Le Progrès Médical*, which became considerably popular and influential. In the period of the Third Republic, Bourneville, as a medical reformist, was attracted to the political platform. He was elected municipal councillor in 1876. During this period his efforts included upgrading the deteriorated state of the old Paris hospitals and improving training for nurses and midwives. Later he became a member of the Parliament for the extreme left wing party (1883-1889). Being both a physician and a republican was his ideal. His fame started to fall in the early nineties and became irreversible after Charcot's death in 1893. In 1905 Bourneville took compulsory retirement. He died as a poor man in 1909.

John James Pringle (1855-1922)[6]

John James Pringle was born in 1855 in Dumfriesshire, Scotland. In 1876 he qualified in medicine at the Edinburgh University. After a residency at the old Edinburgh Royal Infirmary, he spent four years in Vienna and Paris. His dandy like appearance, excellent knowledge of French and his punctuality resulted in admiration among the foreign dermatologists. From 1883 he worked at the Skin Department at the Middlesex Hospital in London, and took charge in 1888. From 1891 to 1895 he edited the *British Journal of Dermatology*. Due to the long lasting friendships with foreign dermatologists, the journal had an international character from the start. Because of his many ambassadorial skills he was appointed Secretary General to the International Congress of Dermatology in 1896. From 1895 to 1901 he was Secretary of the Dermatological Society. He became a successful president of the Dermatology section of the Royal Society of Medicine. Even during the difficult war times, 1914-1918, Pringle was able to initiate lively discussions during the meetings. He further made a special effort in training his assistants and teaching medical students. He


John James Pringle

From the British Journal of Dermatology of 1923, volume 35

contributed to the Royal Commission of Venereology (1916). A remarkable man who's neat appearance, humour, and affection to dermatology made him a legend of his time. In 1922 he died in New Zealand from tuberculosis, which he had had for 17 years.

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Part I

3

Overlapping neurological and cognitive phenotypes in patients with *TSC1* or *TSC2* mutations

Floor Jansen, Olga Braams, Koen Vincken, Ale Algra, Petronella Anbeek, Aag Jennekens-Schinkel, Dicky Halley, Bernard Zonnenberg, Ans van den Ouweland, Alexander van Huffelen, Onno van Nieuwenhuizen, and Mark Nellist

Abstract

The purpose of this study was to systematically analyse the associations between different *TSC1* and *TSC2* mutations and the neurological and cognitive phenotype in 58 patients with tuberous sclerosis complex (TSC) with comprehensive neuropsychological assessment (determining a cognition index) and a computerised tuber segmentation programme (calculating the number of tubers and the proportion of total brain volume occupied by tubers (tuber/brain proportion; TBP)).

As a group, patients with a *TSC2* mutation had an earlier age at seizure onset, a lower cognition index, more tubers, and a greater TBP than those with a *TSC1* mutation, but the ranges overlapped. Familial cases were older at seizure onset and had a higher cognition index than non-familial cases. Patients with a mutation deleting or directly inactivating the tuberin GTPase activating (GAP) domain had more tubers and a greater TBP than those with an intact GAP domain. Patients with truncating *TSC1* or *TSC2* mutation differed from those with non-truncating mutations in seizure types only.

From these results we concluded that prediction of the neurological and cognitive phenotypes in individuals with TSC should not be based on their particular *TSC1* or *TSC2* mutation.

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterised by the widespread development of hamartomas in a variety of tissues and organs. TSC is noted for its clinical variability and there is no single clinical or radiographic sign that is absolutely specific for the disease. A definite clinical diagnosis requires the presence of two major features or one major plus two minor features.[1] Neurological and neuropsychological manifestations include epilepsy, focal neurological deficits, mental retardation, and behavioural disturbances. Epilepsy is the most common neurological symptom in TSC, occurring in 80 - 90% of cases.[2]
TSC is caused by a mutation in either the *TSC1* gene, with the locus on chromosome 9q34, or the *TSC2* gene located on chromosome 16p13. In two-thirds of the TSC population the family history of TSC is negative. In the majority of these patients *de novo TSC1* or *TSC2* mutations are identified.[3] Seventy percent of the *de novo* mutations are found in the *TSC2* gene. In familial cases, about 50% are associated with a *TSC1* mutation and about 50% with a *TSC2* mutation.
The protein product of *TSC1* is called hamartin, that of *TSC2* tuberin.[4,5] Together, hamartin and tuberin form a GTPase activating protein (GAP) complex that inhibits rheb, the GTPase that activates the mammalian target of rapamycin (mTOR).[6,7] The mTOR pathway regulates a number of important cellular processes including growth, nutrient uptake and protein translation.[8] Mutations to either *TSC1* or *TSC2* disrupt the function of the complex, explaining why mutations to either gene cause the same disease. It has been suggested that a more severe phenotype is to be expected in TSC patients with mutations inactivating the tuberin GAP domain.[9]
Several independent studies of large cohorts of TSC patients have demonstrated that patients with a *TSC2* mutation are more often mentally retarded than patients with a *TSC1* mutation.[3,10-12] In most of these studies, cognitive ability was estimated clinically or through indirect methods such as level of schooling,[13] rather than with standardised measures such as intelligence scales.
In the present study epilepsy, cognitive functioning and tuber status were compared between patients with a *TSC1* or a *TSC2* mutation, between familial and sporadic cases, between patients with a mutation inactivating the tuberin GAP domain (GAP -) and patients with an intact GAP domain (GAP+), and between patients with a truncating or non-truncating mutation.

Methods

Patients

Patients clinically diagnosed with *TSC*[1] in the outpatient Paediatric Neurology, Neurology and Internal Medicine departments of the University Medical Centre Utrecht, The Netherlands,

between 1998 and 2005, were included in the study. Information on the germ-line mutation was a prerequisite. Informed consent was obtained from each patient or parent(s).

Mutation analysis

Mutation analysis of the *TSC1* and *TSC2* genes was carried out at the department of Clinical Genetics, Erasmus Medical Centre, Rotterdam, The Netherlands. DNA was extracted from peripheral blood using standard techniques. Mutation analysis was performed on all *TSC1* and *TSC2* exons and exon boundaries with a combination of single-strand conformational polymorphism analysis, denaturing gradient gel electrophoresis and direct sequencing.[3] To detect large DNA rearrangements, Southern blotting, fluorescence *in situ* hybridisation or multiplex ligation-dependent probe amplification were performed. If possible, the parents of the proband were tested to determine whether they carried the mutation detected in the proband. Uncertainty about the pathogenicity of specific *TSC1* or *TSC2* variants was resolved by assaying the biological activity of the corresponding tuberin or hamartin variant, as described previously.[14]

To investigate whether different mutations on either gene were associated with specific neurological and cognitive phenotypes, the patients were grouped according to mutation type. In addition, patients in whom the tuberin GAP domain was inactivated (GAP-) were compared with patients in whom the tuberin GAP domain was still intact (GAP+). The GAP+ group included all *TSC1* mutations as well as *TSC2* missense mutations outside the GAP domain. Next, patients were grouped into those with truncating mutations (including splice site mutations and large rearrangements) and those with non-truncating mutations (missense or small in-frame insertions and deletions).

Classification of the epilepsy syndrome

Each patient was examined (by FEJ) in the outpatient department. Details on seizure history, including age at seizure onset, history of infantile spasms (IS), seizure semiology, and the variety of antiepileptic drugs taken during the course of the disease, were obtained by taking a history from the patients or their parents, and extracted from medical records and previous correspondence. Seizure semiology was classified on the basis of the definitions proposed by the Commission on Classification and Terminology of the International League Against Epilepsy.[15] Seizure types (including a history of IS) were scored as present or absent. EEGs were performed with electrode positions according to the 10-20 system and epileptiform activity was scored as focal or multifocal.

Neuropsychological assessment

Patients participated in a comprehensive cognitive assessment, as elaborated in the companion paper (*chapter four*). Depending on the widely varying ages and abilities of the patients, additional tests were selected from a fixed battery covering the major domains of cognition. Administration and scoring were according to the test manuals. Every patient underwent at least an intelligence or developmental test; the resulting intelligence quotient and developmental index were called intelligence equivalent (IE).[16]

The test results were either quotient, centiles or standard scores. These were converted to a general scale with the values 1 (extremely below average), 2 (below average), 3 (average) or 4 (above average). In tests with cut-off scores, scale values 1 and 3 were used. We assigned a 'cognition index' to each patient by adding her/his scale values and dividing the sum by her/his number of tests. The cognition index could vary between 1.00 and 4.00.

Structural brain imaging

MRI was performed on a Philips Gyroscan ACS-NT 1.5 T whole body system (Philips Medical Systems, Best, The Netherlands). All patients underwent a transaxial or coronal Fluid Attenuated Inversion Recovery (FLAIR) scan. Slice thickness was 1.5 mm, without slice gap. Scan parameters were: repetition time (TR) = 11000ms, echo time (TE) = 125 ms and inversion time (TI) = 2600 ms. Anaesthetics or sedation were used in children under 7 years of age and in patients with severely impaired cognitive function.

Segmentations were made of the total brain, cerebro-spinal fluid (CSF) and tubers. First a mask was generated with the Brain Extraction Tool.[17] Voxels inside this mask were segmented into brain and CSF by a threshold on the FLAIR image. Tubers were segmented by a K-Nearest Neighbour (KNN) segmentation technique.[18,19] The implementation of this technique is described in more detail in a companion study (*chapter four*). Tuber status was expressed as both the number of tubers and the proportion of total brain volume occupied by tubers (excluding CSF) (tuber/ brain proportion; TBP).

Statistical Analysis

We calculated risk ratios (RR) with corresponding 95% CI of dichotomised predictive variables (genotype) on dichotomised dependent variables (e.g. epilepsy). Analyses with continuous dependent variables (e.g. cognition index, TBP, and number of tubers) were performed with unpaired t-tests yielding mean differences with corresponding 95% CI. Differences in age at seizure onset (determined for patients with epilepsy) were assessed by calculating hazard ratios (HR) with corresponding 95% CI with Cox's proportional hazard model (survival analysis). We considered associations statistically significant for p values < 0.05; p values < 0.1 and ≥ 0.05 were considered trends.

Results

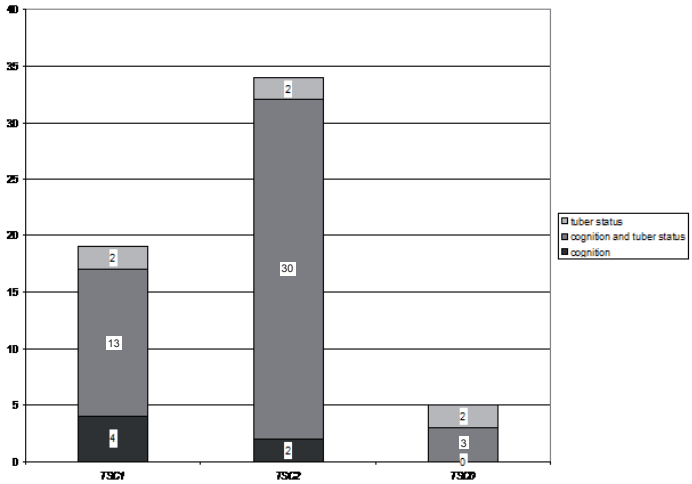
Total sample

Fifty-eight patients, 31 women and 27 men, were included in the study. As shown in *Figure 1*, most patients were subjected to both radiological and cognitive investigations. Mean age at examination was 20.6 years, range 1.6 to 57 years.
Fifty-two patients (90%) had epilepsy, with age at onset between 1 day and 19 years (median 8 months, mean 2 years). Eighteen patients had a history of IS.
The mean cognition index was 1.8 (range 1.0 to 3.8) 48% of patients had a cognition index severely below average).
The number of tubers ranged from 7 to 58, mean 28. The mean TBP was 1.3% (range 0.2% to 5.1%). The relationships between tuber status, epilepsy variables, and cognition index are presented in a companion paper (*chapter four*).

Mutation analysis

Mutation analysis was performed in 53 probands and a pathogenic mutation was identified in 48 (91%); this is in line with previous studies.[7,9-11] In four patients, multiple affected members of a family segregating TSC were investigated. In one case, twins with the same *de novo* mutation were investigated.

Figure 1
Diagram showing in how many patients independent and dependent variables were obtained.



In the majority of patients identification of the pathogenic mutation was straightforward. In two patients the pathogenic nature of the amino acid substitution (*TSC2* 2765T>G (L916R) and *TSC1* 892T>G (M224R)) was confirmed by functional analysis of the tuberin and hamartin variant isoforms (data not shown). In one patient a *TSC2* 1253A>T nucleotide change was identified that co-segregated with TSC in a family. The predicted E412V amino acid substitution did not affect tuberin activity in multiple assays of tuberin-hamartin function. However, RT-PCR analysis revealed that the A>T transversion affected the splicing of the *TSC2* mRNA and therefore the *TSC2* 1253A>T variant was classified as a pathogenic splice site mutation (data not shown).

A *TSC1* mutation was found in 17 probands (32%), including 5 confirmed *de novo* cases and 10 confirmed familial cases. A *TSC2* mutation was found in 31 probands (58%), including 13 confirmed *de novo* cases and 2 confirmed familial cases. In 23 patients mutation analysis of the parents of the proband was not performed. Mutations inactivating the tuberin GAP domain were present in 26 probands and mutations leaving an intact GAP domain were present in 22 probands. Truncating mutations, splice site mutations and large rearrangements were found in 40 probands (16 *TSC1*, 24 *TSC2*) and non-truncating mutations in 8 (1 *TSC1* and 7 *TSC2*). In 5 sporadic cases no mutation could be detected in either gene (TSC0).

Phenotype comparisons

The patients were first split up into 3 groups (*Table 1a*): patients in whom no mutation was identified (TSC0), patients with a *TSC1* mutation and patients with a *TSC2* mutation. Comparisons were limited to the *TSC1* and *TSC2* groups as the TSC0 group was too small for statistical analysis.

Gender and age at examination did not differ statistically significantly between the *TSC1* and *TSC2* groups (*Tables 1a and 2)*.

Age at seizure onset was lower in the *TSC2* mutation group (HR 2.2; 95% CI 1.2 to 4.2) and IS tended to be more common in patients with a *TSC2* mutation (RR 2.5; 95% CI 0.8 to 7.4). None of the other epilepsy variables were associated with either the *TSC1* or *TSC2* mutation group.

Group-wise, patients with a *TSC2* mutation had a lower cognition index than patients with a *TSC1* mutation (mean difference -0.7, 95% CI -1 to -0.2). However, the ranges of the cognition indices in the *TSC1* and *TSC2* groups overlapped considerably (*Table 1a* and *Figure 2*). The major contributor to the cognition index, IE (IQ or DI), showed a clear bimodal distribution only in the *TSC2* group (*Figures 3a and b*). Two patients with a *TSC1* mutation had no history of seizures. One of these patients had an IE below 85 (one standard deviation below the mean). In the *TSC2* group, four patients had no history of seizures, and three of these had an IE lower than 85 and one patient had an IE below 70. There was no difference between the groups in the impact of epilepsy on the cognition index (*Figure 2*).

Table 1a Phenotype variables shown separately for *TSC1*, *TSC2* and TSCo

variable	*TSC1* (19)							*TSC2* (34)							TSCo (5)		
	Total			familial		sporadic		Total			familial		sporadic		Total		
	P/N	%	mean (range)	P/N	%	P/N	%	P/N	%	mean (range)	P/N	%	P/N	%	P/N	%	mean (range)
age at assessment (years)			25 (3- 57)							19 (2- 52)							16 (3- 52)
gender (F)	12/ 19	63						16/ 34	47						3/ 5	60	
epilepsy	17/ 19	89		9/ 11	82	8/ 8	100	30/ 34	88		1/ 2	50	29/ 32	91	5/ 5	100	
age at seizure onset (years)			3.7 (0- 19)							1.2 (0- 10)							1.2 (0.3- 3)
IS	3/ 17	18		1/ 9	11	2/ 8	25	13/ 30	43		0/ 1	0	13/ 29	45	2/ 5	40	
GTCS	7 17	41		3/ 9	33	4/ 8	50	12/ 30	35		0/ 1	0	12/ 29	41	0/ 5	0	
CPS	11/ 17	65		5/ 9	56	6/ 8	75	19/ 30	63		1/ 1	100	18/ 29	62	3/ 5	60	
TS	3/ 17	18		1/ 9	11	2/ 8	25	4/ 30	13		0/ 1	0	4/ 29	14	2/ 5	40	
multiple seizure types	5/ 17	29		1/ 9	22	4/ 8	50	15/ 30	50		0/ 1	0	15/ 29	52	2/ 5	40	
multifocal EEG	8/ 17	47		2/ 9	50	6/ 8	75	15/ 30	50		1/ 1	100	14/ 30	46	4/4	100	
multifocal ictal onset	3/ 6	50		1/ 2	36	2/ 4	50	4/ 7	57		0/ 1	0	4/ 7	57			
cognition index*	8/ 17	47	2.4 (1- 3.7)	4/ 11		4/ 6	67	26/ 31	84	1.6 (1- 3.5)	1/ 2	50	25/ 29	86	4/ 4	100	1.0 (1- 1)
number of tubers	14		22 (10- 53)					30		32 (7- 58)					4		21 (8- 36)
TBP (%)	14		0.6 (0.2- 1.8)					30		1.7 (0.2- 5.1)					4		0.7 (0.2- 1.4)

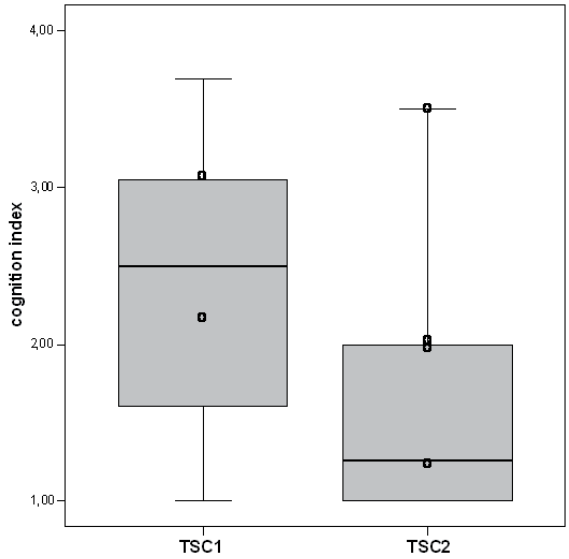
P= number of patients with the symptom, N= number of patients examined, y= year, F= female, IS= infantile spasms, GTCS= generalised tonic clonic seizures, CPS= complex partial seizures, TS= tonic seizures, * here P is the number of patients with below average cognition index, TBP= tuber/ brain proportion

Table 1b Phenotype variables shown separately for mutations deleting the tuberin GAP domain (GAP-) and mutations with retained GAP domain (GAP+); the entries refer to all patients and to patients with *TSC2* only

variable	all patient						*TSC2* only					
	GAP- (28)			GAP+ (25)			GAP- (28)			GAP+ (6)		
	N/ P	%	mean (range)	N/ P	%	mean (range)	N/ P	%	mean (range)	N/ P	%	mean (range)
epilepsy	24/ 28	86		23/ 25	92		24/ 28	86		6/ 6	100	
age at seizure onset (years)			1.3 (0- 10)			2.8 (0- 19)			1.3 (0- 10)			0.4 (0.1- 0.9)
IS	11/ 24	46		5/ 25	20		11/ 24	46		2/ 6	33	
GTCS	7/ 24	29		12/ 23	52		7/ 24	29		5/ 6	83	
CPS	15/ 24	63		15/ 23	65		15/ 24	63		4/ 6	67	
TS	3/ 24	13		4/ 23	17		3/ 24	13		1/ 6	17	
multiple seizure types	9/ 24	38		11/ 23	48		9/ 24	38		6/ 6	100	
multifocal EEG	12/ 27	44		11/ 24	46		12/ 27	44		3/ 5	60	
multifocal ictal onset	3/ 6	50		4/ 7	57		3/ 6	50		1/ 1	100	
cognition index*	21/ 26	81	1.7 (1- 3.5)	13/ 22	59	2.1 (1- 3.7)	21/ 26	81	1.7 (1- 3.5)	5/ 5	100	1.2 (1- 2)
number of tubers	26		31 (7- 58)	18		25 (10- 47)	26		31 (7- 58)	4		34 (28- 47)
TBP (%)	26		1.8 (0.2- 5.1)	18		0.8 (0.2- 2.4)	26		1.8 (0.2- 5.1)	4		1.3 (0.7- 2.4)

P= number of patients with the symptom, N= number of patients examined, y= year, IS= infantile spasms, GTCS= generalised tonic clonic seizures, CPS= complex partial seizures, TS= tonic seizures, * here P is the number of patients with below average cognition index, TBP= tuber/ brain proportion

Figure 2



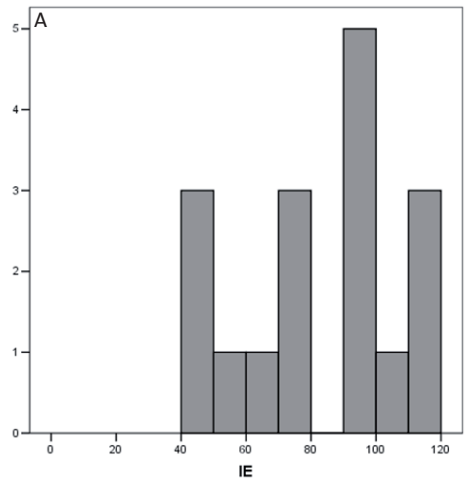
The box plot shows the differences in cognition index between *TSC1* and *TSC2* mutation groups. The bold line represents the mean, the box the standard deviation.
Patients without epilepsy are indicated by a circle.

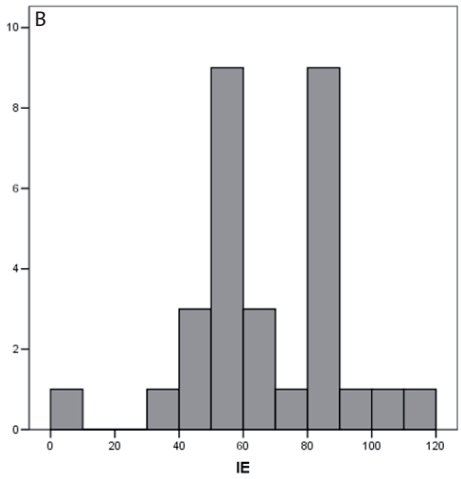
Patients with a *TSC2* mutation had more tubers (mean difference 9; 95% CI: 3.3 to 15.2 *Table 2*) as well as a greater TBP (mean difference 1.1, 95% CI: 0.6 to 1.7) than patients with a *TSC1* mutation, but ranges overlapped here as well.

Comparisons of the 45 sporadic patients (including 18 with a confirmed *de novo* mutation and 5 without a mutation detected), with the 13 familial patients (12 confirmed) revealed a lower age at seizure onset (HR 2.3; 95% CI 1.1 to 4.8; *Table 2*) and a lower cognition index (mean difference 1.0, 95% CI 0.5 to 1.5) in the group with sporadic TSC. Differences in tuber status between familial and sporadic patients were not found. Sporadic patients with a *TSC2* mutation had more tubers (mean difference 10; 95% CI: 1 to 18) and a greater TBP (mean difference 1.1, 95% CI 0.3 to 2) than those with a sporadic *TSC1* mutation (data not shown).

We compared patients with a GAP- mutation with those with a GAP+ mutation once including and once excluding the *TSC1* patients (*Table 1b*). When patients with a *TSC1* mutation were included, patients in the GAP- group tended to have a history of IS more often (RR 2.1; 95% CI 0.9 to 5.1). Further the GAP- group had more tubers (mean difference 6; 95% CI 0.3 to 12.2; *Table 2*) and a greater TBP (mean difference 1.1; 95% CI 0.5- 1.6) than the GAP+ group. When we excluded all the patients with a *TSC1* mutation from the analysis, we found that in the GAP- group secondary generalised tonic clonic seizures (sGTCS) (RR 0.4; 95% CI 0.2 to 0.7, *Table 2*) and multiple types of seizure (RR 0.4; 95% CI 0.2 to 0.6) were less common than in the GAP+ group.

Figure 3





Bar graph showing the distribution of the intelligence equivalent (IE) in 17 patients with a *TSC1* mutation (*A*) and in 31 patients with a *TSC2* mutation (*B*).

When comparing patients with truncating mutations to those with non-truncating mutations (*Table 1*c and *Table 2*) we found that patients with truncating mutations were less likely to have had sGTCS (RR 0.4; 95% CI 0.2 to 0.7) and multiple types of seizure (RR 0.3; 95% CI 0.2 to 0.6) than patients with non-truncating mutations. With respect to the cognition index and tuber status no differences were identified.

Table 1c Phenotype variables shown separately for truncating and non-truncating mutations

variable	truncating (43)			non-truncating (10)		
	N/ P	%	mean (range)	N/ P	%	mean (range)
epilepsy	38/ 43	88		9/ 10	90	
age at seizure onset (years)			2.3 (0- 19)			0.9 (0- 5)
IS	14/ 38	37		2/ 9	22	
GTCS	12/ 38	32		7/ 9	77	
CPS	24/ 38	63		6/ 9	67	
TS	5/ 38	13		2/ 9	22	
multiple seizure types	12/ 38	32		6/ 9	89	
multifocal EEG	19/ 42	45		4/ 9	44	
multifocal ictal onset	6/ 12	50		1/ 1	100	
cognition index*	27/ 40	68	2.0 (1- 3.7)	7/ 8	88	1.6 (1-3)
number of tubers	39		28 (7- 58)	5		31 (19- 47)
TBP (%)	39		1.4 (0.2- 5.1)	5		1.1 (0.4- 2.4)

P= number of patients with the symptom, N= number of patients examined, y= year, IS= infantile spasms, GTCS= generalised tonic clonic seizures, CPS= complex partial seizures, TS= tonic seizures, * here P is the number of patients with below average cognition index, TBP= tuber/ brain proportion

Table 2 Relationships between genotype and phenotype characteristics: statistically significant relationships are printed in bold

variable	*TSC2* versus *TSC1*		GAP- versus GAP+		truncating versus non-truncating		sporadic versus familial	
	RR/ HR*	difference	RR/ HR	difference	RR/ HR	difference	RR/ HR	difference
age at assessment (years)		5.6 (-3.1- 14.3)		**8.2 (0.03- 16.4)**		**12.7 (2.5- 23.0)**		12 (2.6-21.4)
gender (F)	0.8 (0.5- 1.2)		0.9 (0.6- 1.6)		0.9 (0.5- 1.5)		0.8 (0.5- 1.4)	
epilepsy	1.0 (0.8- 1.2)		0.7 (0.4- 1.2)		0.7 (0.3- 1.3)		**2.3 (1.1- 4.8)**	
age at seizure onset* (years)	**2.2 (1.2- 4.2)**[1]		0.9 (0.8- 1.1)		1.0 (0.8- 1.2)		1.2 (0.9- 1.7)	
IS	2.5 (0.8- 7.4)		2.1 (0.9- 5.1)		1.7 (0.5- 6.0)		4.6 (0.7- 31.7)	
GTCS	1.0 (0.5- 2.0)		0.6 (0.3- 1.2)		**0.4 (0.2- 0.7)**		1.5 (0.5- 4.5)	
			0.4 (0.2- 0.7)+					
CPS	1.0 (0.6- 1.5)		1.0 (0.6-1.5)		1.0 (0.6- 1.6)		1.3 (0.7- 2.5)	
TS	0.8 (0.2- 3.0)		0.7 (0.2- 2.9)		0.6 (0.2- 2.6)		2.3 (0.3- 16.8)	
multiple seizure types	1.7 (0.8- 3.9)		0.8 (0.4- 1.5)		**0.3 (0.2- 0.6)**		5.0 (0.8- 32.9)	
			0.4 (0.2- 0.6) +					
multifocal EEG	1.1 (0.6- 2.0)		1.0 (0.5- 1.8)		1.0 (0.5- 2.3)		2.5 (0.9- 6.9)	
multifocal ictal onset	1.1 (0.4- 3.2)		0.9 (0.3- 2.4)		nta		1.0 (0.2- 4.5)	
cognition index*		**-0.7 (-1.2- 0.2)**[2]		-0.4 (-0.9- 0.1)		0.3 (-0.3- 1.0)		**1.0 (0.5- 1.5)**
number of tubers		**9 (3.3- 15.2)**[3]		**6 (0.3- 12.2)**		-3 (-12.3- 7.0)		0 (-7.8- 7.6)
TBP (%)		**1.1 (0.6- 1.7)**		**1.0 (0.5- 1.6)**		0.3 (-0.7- 1.2)		-0.4 (-1.1- 0.3)

RR= risk ratio, HR*= hazard ratio, GAP-= mutations deleting the tuberin GAP domain, ( )= 95% confidence interval, IS= infantile spasms, GTCS= generalised tonic clonic seizures, CPS= complex partial seizures, TS= tonic seizures, na= not analysable, TBP= tuber/ brain proportion

[1]Patients with a *TSC2* mutation and epilepsy had a 2.2 higher risk of early epilepsy than those with a *TSC1* mutation and epilepsy

[2]Cognition index was 0.7 lower in patients with a *TSC2* mutation.

[3]Patients with a *TSC2* mutation had a higher number of tubers than those with a *TSC1* mutation; mean difference of 9

+patients with *TSC2* mutations only

Discussion

Several genotype-phenotype studies have reported that, as a group, TSC patients with a *TSC2* mutation have a more severe phenotype than those with a *TSC1* mutation.[3,10-12,20,21] Our analysis is consistent with these findings. In our study, patients with a *TSC2* mutation had an earlier age at seizure onset, a lower mean cognition index, more tubers, and a greater TBP than patients with a *TSC1* mutation. However, the considerable overlap between the cognition indices of the *TSC1* and *TSC2* groups (96%, the cognition index of only two patients from the *TSC1* group exceeded the highest cognition index in the *TSC2* group) means that, in patients with a *TSC2* mutation, cognitive impairments are more frequent but not necessarily more severe than in patients with a *TSC1* mutation.

Sporadic TSC was associated with an earlier age at seizure onset and a lower cognition index than familial TSC. Within the sporadic group, only a more severe tuber status (more tubers and greater TBP) distinguished patients with a *TSC2* mutation from patients with a *TSC1* mutation.

We included 5 affected relatives (4 parents, 1 sister) of probands in our patient sample. In four patients the diagnosis was confirmed by DNA analysis of the affected relative. In one patient no DNA was available for testing. The diagnosis in these 5 relatives was made after the proband presented with neurological symptoms. Only one of them had epilepsy and none of them had a cognitive deficit, which may have contributed to the differences between familial and sporadic cases. However, we decided to include these individuals to maximise the size of our sample.

The over-representation of cognitive impairments in patients with a *TSC2* mutation might be due to a higher frequency of somatic, inactivating mutations at the *TSC2* locus. An alternative possibility is that patients with *TSC1* mutations may still retain some rheb GAP activity since the tuberin GAP domain is still present. Patients from the GAP- group tended to have had a history of IS more often. Further, they had more tubers and a greater TBP than patients from the GAP+ group. IS and a great TBP are associated with a lower cognition index (*chapter four*). Restricting the comparison to patients with a *TSC2* mutation, we did not find any evidence for a greater TBP in the GAP- group compared to the GAP+ group. Furthermore, IS were not more frequent in the *TSC2* GAP- group than in the *TSC2* GAP+ group. Therefore, although the number of patients is very small, our data are consistent with a higher rate of somatic mutation at the *TSC2* locus being responsible for the more severe phenotypes in the *TSC2* mutation group.

When comparing non-truncating with truncating mutations, the only significant differences between the groups were the higher prevalence of secondarily generalised tonic clonic seizures (sGTCS) and multiple types of seizures in the group with non-truncating mutations. One possible explanation for this is that mutant hamartin and tuberin isoforms may have dominant negative effects on neuronal function. Further study is required to clarify this issue.

A relation between age at seizure onset, the presence of IS and cognition has been reported extensively.[22-26] The assumption that patients without epilepsy have a normal cognitive function is not supported by our data. Four of the six patients without a history of seizures had significant cognitive impairment (IE more than one standard deviation below the mean). Joinson et al. found evidence for a bimodal distribution of estimated IQ in TSC (with a high frequency of IQ scores around 30 and a less conspicuous peak around 80).[22] We can now add that only the *TSC2* group showed a bimodal distribution, with a peak around 50 rather than 30 (*Figure 3*). Unfortunately, we were unable to determine how Joinson et al. calculated IQ scores below 50, so the difference between their data and ours may be due to different procedures for extrapolation.

To the best of our knowledge the present study is the first to assess the relationships between mutation types, neurological phenotype, and cognitive functioning on the basis of objective tuber status assessment and standardised measures of intelligence and other cognitive domains. The widely varying ages and cognitive abilities of the patients necessarily resulted in widely varying neuropsychological data sets. Transforming the different measures into a cognition index enabled us to process the data of all patients. A severely below average cognition index in 48% of the patients in our sample concurs with the incidence of mental retardation in the TSC population as reported in the literature.[2]

A couple of methodological objections can be raised. Firstly, in our cohort there was a preponderance of children younger than 5 years (20%). However, as the age distribution was equal for both the *TSC1* and *TSC2* groups, and as only one of these younger children did not have seizures, we believe that the skewed age distribution did not bias our analysis. Secondly, the present study was performed in a tertiary referral centre where intractable epilepsy is a major field of interest. However, as the incidence of seizures in our sample (90%) is in agreement with the reported prevalence in TSC populations, selection bias is unlikely. In two patients we had to rely on historical data to outline the epilepsy syndrome, so an incidental misclassification is possible. We were unable to analyse the relation between genotype and phenotype in patients without epilepsy because only 6 such patients were included in our sample.

In conclusion, in TSC patients an earlier age at seizure onset, a lower cognition index and a more severe tuber status are associated with *TSC2* mutations. However, the phenotypes of the *TSC1* and *TSC2* mutation groups overlap considerably. Therefore multiple factors determine the neurological and cognitive phenotypes in TSC patients. Identification of a mutation is important in confirming a diagnosis of TSC. Yet, neither the onset of seizures nor the degree of cognitive impairment can be predicted on the basis of the mutation type. Whether variables within the phenotype, i.e. tuber status, are better predictors of epilepsy and cognition than the germ-line mutation is addressed in a companion paper (*chapter four*).

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4

Tuber status is not the only determinant of cognitive function in tuberous sclerosis complex

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Abstract

In this study we investigated the relationships between tuber status, seizures and cognitive function in 61 patients with tuberous sclerosis complex (TSC). We hypothesised that the proportion of the total brain volume occupied by tubers (tuber/ brain proportion; TBP) would be a better determinant of seizures and cognitive function than the number of tubers. Tuber status was characterised on 3D FLAIR MRI with a computerised tuber segmentation programme. TBP was inversely related to the age at seizure onset and to the intelligence equivalent and tended to be inversely related to the cognition index. Further, a younger age at seizure onset or a history of infantile spasms was related to lower intelligence and lower cognition index. In a multivariable analysis only age at seizure onset was related to cognition index.
This systematic analysis confirms proposed relationships between tuber status, epilepsy and cognitive function in patients with TSC, but also indicates that TBP is a better predictor of cognitive function than tuber number and that age at seizure onset is the only independent determinant of cognitive function. Prevention of seizure onset is desired, but has not proven possible yet. Therefore, prompt seizure control should be the principal neuro-intervention in patients with TSC.

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder, with a highly variable expression, affecting multiple organs. Tubers are the result of disrupted proliferation, migration and differentiation in early foetal life and can be found throughout the brain. They are characterised by abnormal cortical lamination and the presence of dysmorphic neurons and giant cells.

TSC is associated with a variety of neurological and neuropsychological symptoms including epilepsy and mental retardation. Epilepsy occurs in 80-90% of patients and is often the presenting neurological symptom. A history of infantile spasms (IS) has been shown to be strongly related to the number of tubers.[1]

It is estimated that 50% of TSC patients have some degree of cognitive impairment[2] and several interrelated factors have been suggested to account for cognitive dysfunction in TSC. First, a molecular genetic basis has been proposed, since patients with *TSC2* mutations are more likely to be mentally retarded than patients with *TSC1* mutations.[3-7] We performed an extensive analysis of the impact of genotype on epilepsy, cognitive function, and tuber status (as a characteristic of neurological phenotype) and found that the genotype only accounts for a small fraction of the variation in neurological and cognitive symptoms (*chapter three*). Second, a history of epilepsy, especially IS, is critically associated with impaired cognitive functioning in TSC patients. Moderate or severe mental retardation has been reported in 85% to 100% of patients with a history of IS.[8-11] Although mental retardation in TSC patients without epilepsy is rare, neuropsychological deficits have been described in patients without epilepsy and with normal intelligence,[12,13] thus other factors may also be effective. Finally, anatomical pathology appears to have an impact on intelligence. A quantitative meta-analysis[14] confirmed the findings of previous retrospective studies that described relationships between intelligence and the number of tubers.[1,13,15-17] However, in these studies, sample sizes were small and cognitive ability was estimated clinically or indirectly (e.g. from level of schooling), rather than with standardised measures, such as intelligence scales. Most importantly, the divergence in estimated tuber status was not only a result of clinical heterogeneity, but also due to differences in the scanning sensitivity, lesion identification protocols, and the imaging modalities used. Estimates of tuber status were often subjective and non-reproducible. Moreover, with respect to the relationships between tuber status and neurological and cognitive functioning, simply counting the number of tubers is likely to underestimate the impact of large lesions and overestimate the impact of smaller ones. Thus, the evidence of the association between the number of tubers and intelligence is not entirely unequivocal, and it is possible that tuber volume is a more critical determinant of seizures and cognitive function in TSC patients. As far as we are aware, the impact of tuber volume on neurological symptoms has only been addressed once.[18] In order to estimate the proportions of each cortical and subcortical region

occupied by a lesion, Ridler et al. constructed lesion density maps of the brains of TSC patients with normal intelligence. Whole brain lesion density was increased in patients with a life-long history of epilepsy, but was not related to intelligence.
In the present study, we use objective measurements to investigate whether the proportion of total brain volume occupied by tubers (TBP) rather than the number of tubers determines cognitive function. In addition, we analyse the associations between epilepsy variables and cognitive function in order to identify determinants of cognitive function in patients with TSC.

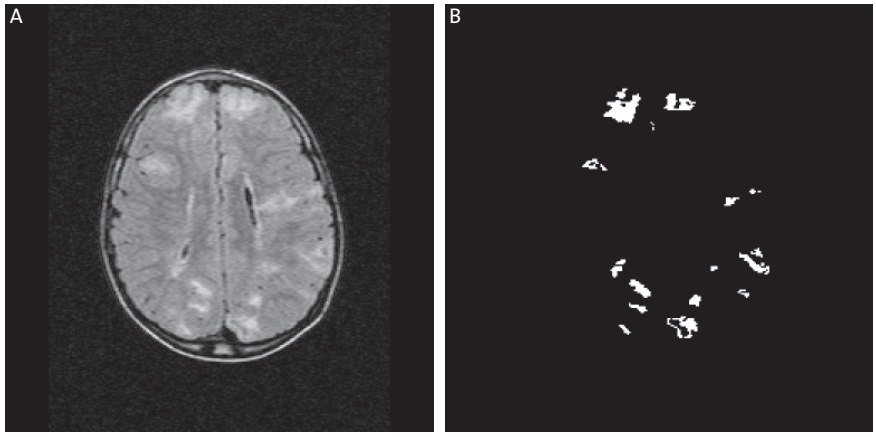
Patients and Methods

Between 1998 and 2005 patients clinically diagnosed with TSC[18] in the outpatient Paediatric Neurology, Neurology and Internal Medicine departments of the University Medical Centre Utrecht, The Netherlands were included in the study. Sufficient MRI data to allow a reliable assessment of tuber status was a prerequisite.
Mutation analysis of the *TSC1* and *TSC2* genes was carried out at the department of Clinical Genetics, Erasmus Medical Centre Rotterdam, The Netherlands. Informed consent was obtained from each patient or parent(s).

Tuber status

MRI was performed on a Philips Gyroscan ACS-NT 1.5 T whole body system (Philips Medical Systems, Best, The Netherlands). All patients underwent a transaxial or coronal Fluid Attenuated Inversion Recovery (FLAIR) scan (*Figure 1A*). Slice thickness was 1.5 mm without slice gap. Scan parameters were: repetition time (11000 ms), echo time (125 ms), and inversion

Figure 1 shows a transversal FLAIR image (A) with multiple tubers, visible as hyperintense subcortical lesions and the corresponding tuber segmentation map (B).



time (2600 ms). Anaesthetics or sedation were used in children under 7 years of age and in mentally retarded patients.

Segmentations were made of the total brain, cerebro-spinal fluid (CSF) and tubers. The total brain was segmented using the Brain Extraction Tool.[18] The result of this segmentation was used as a mask for segmentation of the other tissues (CSF and tubers). To achieve spatial similarity between coronal and axial scans, the coronal scans were rotated approximately 300 degrees. Visible differences in proportion, i.e. relative distances in the x-, y- and z- axes, that occur due to this procedure did not influence the result of the K-Nearest Neighbour (KNN)-segmentation[20,21] or further processing. KNN segmentation is a probabilistic segmentation technique originally developed for the separation of the brain into grey matter, white matter, ventricle system, remaining CSF and both deep and periventricular cerebral white matter lesions. For the purpose of tuber segmentation a new learning set was created, consisting of manual segmentations of tubers on the FLAIR images of 10 patients. Manual segmentations were based on lesions affecting the cortical grey matter and adjacent white matter with an inner core hyperintense to grey and white matter on the FLAIR images.[22] To reduce bias, two independent observers performed each manual segmentation twice. Kappa values of inter- and intraobserver variability exceeded 0.8. Only (parts of) tubers that were segmented by both observers were used in the learning set. Scans of new patients were segmented in a probablistic way, i.e. the probabilities of the voxels being tuber were determined by comparison with voxels in the learning set which had the maximal similarity in signal intensity in the FLAIR scan and in the x-, y- and z-coordinates in the FLAIR image. All features were scaled into a standard value range with a mean of 0 and a variance of 1. The value of K in the KNN-classification was 100. The resulting probability image of a KNN segmentation contained small and large blobs of tubers. Significant lesions, both in size and in probability, were extracted on the basis of "minimum blob segmentation". A single threshold step on the probability image of T% was followed by a blob segmentation to discard lesions smaller than N voxels. Evaluating a series of values for T and N using the automated segmented tubers resulted in an optimal setting of T=20 and N=40 (*Figure 1B*). This corresponds to a minimum volume of 0.064 ml (64 mm^3) per tuber. CSF segmentation was carried out using a histogram-based threshold method. A histogram of all voxels inside the mask was composed, and the optimal threshold was defined automatically as the first local minimum after the first local maximum in the histogram. All voxels with an intensity value lower than this threshold were considered to be CSF. Total brain volume was calculated by multiplication of the number of voxels inside the brain mask (without the CSF voxels) by the voxel size. Tuber volume was calculated by multiplication of all tuber voxels by the voxel size. Tuber status was expressed as the total number of tubers and the proportion of total brain volume (excluding CSF) occupied by tubers (tuber/ brain proportion; TBP). The number of tubers and the TBP were calculated for the whole brain and both hemispheres separately.

Classification of the epilepsy syndrome

Each patient was examined (by FEJ) in the outpatient clinic. Details on seizure history, including age at seizure onset and seizure semiology during the course of the disease, were obtained by taking the history from the patients or their parents and by extraction from medical records and previous correspondence. Seizure semiology was classified according to the system proposed by the Commission on Classification and Terminology of the International League against Epilepsy,[23] and seizure type was scored as present or absent. EEGs were performed with electrode positions according to the 10-20 system and epileptiform activity was scored as focal or multifocal.

Neuropsychological assessment

Depending on the ages and abilities of the patients, tests were selected from a fixed battery covering the major domains of cognition. Administration and scoring were according to the test manuals. Every patient underwent at least an intelligence or developmental test. The following tests were used:

Standard and Coloured Progressive Matrices (SPM/CPM) are multiple choice tests of general cognition that require evaluation of logical relations in visual displays.[24,25] The dependent variable was the Intelligence Equivalence score (IE) (mean = 100, s.d. = 15). In 9 cases a full-scale intelligence quotient (IQ) (Dutch versions of Wechsler Adult Intelligence Test (WAIS), Wechsler Intelligence Scales for Children – Revised (WISC-RN)[24,25] or Wechsler Preschool and Primary Scales of Intelligence (WPPSI-R))[23,25] was determined, and in 2 cases a General Cognitive Test score (GCT) (McCarthy Scales of Children's Abilities)[24,25] was determined. If age-appropriate tests did not fit the cognitive abilities of the children being tested, an IE was estimated using the Mental Measurement of Preschool Children.[24,26] For infants, the Bayley Scales of Infant Development (BSID-II-NL)[24,27] provided a Mental Developmental Index (DI) (mean = 100 sd = 15) and a Developmental Age (DA). Raw scores outside the normal limiting values were extended by extrapolation or, for DA, were divided by chronological age and multiplied by 100.

Vocabulary, a subtest of the Wechsler Adult Intelligence Scale (WAIS) (Dutch adaptation)[24,25] was used to estimate verbal intelligence in adults. The dependent variable was the standard score (mean = 10, SD = 3). Verbal communication was judged clinically and classified into one of three classes: normal, below age but conform general cognitive functioning, or specifically disturbed.

The Wechsler Memory Scale (WMS)[24] (unpublished Dutch adaptation: UMCU 2000) was used to measure aspects of memory and learning. The dependent variable was the Memory Quotient (MQ, mean = 100, sd = 15).

The Visual Retention Test (VRT)[24,25] required patients to reproduce line drawings to which they had been exposed immediately before. The dependent variable was the number of correct

reproductions (max. score = 12).

The California Verbal Learning Test (CVLT)[25,28] required patients to memorise a shopping list (four items drawn from four semantic categories). The dependent variables were the standard scores of immediate and delayed recall.

The Controlled Oral Word Production (COWP) required the patient to produce as many words as he or she could think of that began with U, N, K or A in one minute each.[29] The dependent variable was the total number of correct words.

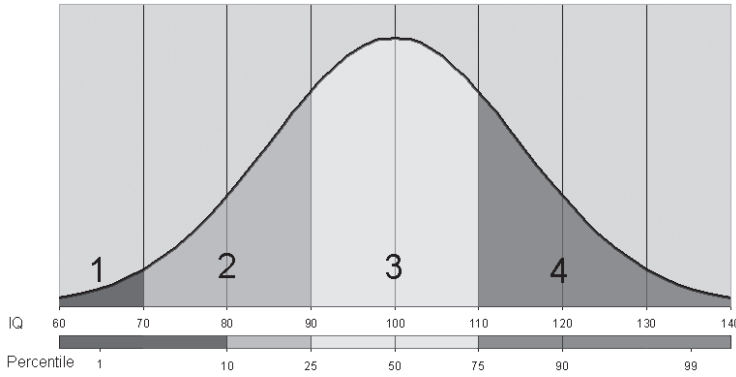
Two tests were used to assess attention or executive function. The Wisconsin modified Card Sorting Test (WmCST)[24,30] required patients to sort a deck of cards according to rules that they had to derive from feedback given by the examiner. The dependent variable was the number of correctly applied sorting categories (range 0-6). Trail Making required visual scanning of a sequence of digits (Part A) and alternating between two series of successive letters of the alphabet and digits 1 to 25 (Part B). The dependent variables were the execution times (sec) converted to centile scores for both parts.[24,25]

Reaction times (RT) were measured under simple conditions (visual and auditory stimuli) and in a go-no go task in which the patient had to respond to combinations of a visual and an auditory stimulus and to ignore all other stimuli.[31] The dependent variables were the Mean Simple and Choice RT (msec).

Manual tapping (with a hand-held stylus on a sensitive plate) provided a measure of motor speed.[32] The dependent variable was the number of taps per time period converted to centile scores.

The test results were either quotient, centile or standard scores. These were converted to a general scale with the values 1 (extremely below average), 2 (below average), 3 (average) or 4 (above average) (*Figure 2*). For cut-off scores, scale values 1 and 3 were used. We assigned a 'cognition index' to each patient by adding the scale values and dividing the sum by the number of tests. When calculating the cognition index from the scaled values, differences

Figure 2 Classification of quotient, centile or standard scores.



in the number of tests used to assess the domains were allowed for by granting a factor 3 to intelligence scores and a factor 0.5 to both CVLT scores. The cognition index could vary between 1.00 and 4.00.

Statistical Analysis

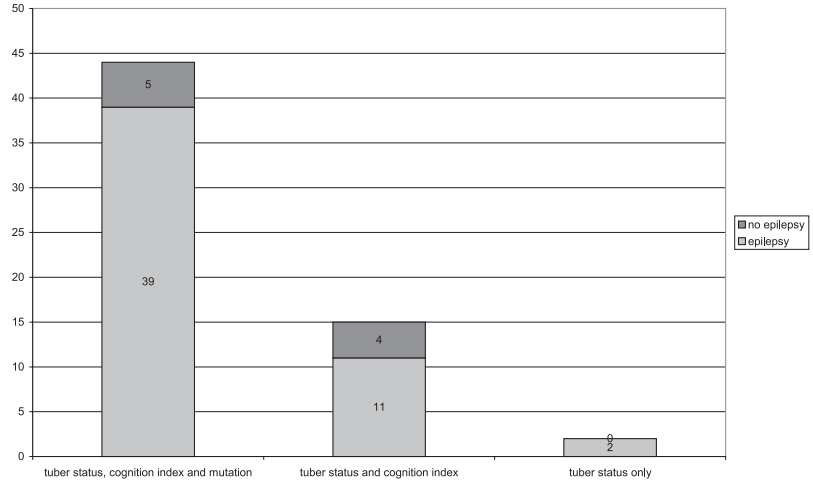
Epilepsy variables were dichotomised into present and absent. With respect to tuber status, mean differences between patients with or without the variable were calculated with corresponding 95% CI and were compared with an unpaired t-test. A linear regression model was used for continuous variables. The relationship between tuber status and age at seizure onset was expressed as a hazard ratio with Cox's regression analysis. We considered associations statistically significant for p values < 0.05; p values < 0.1 and ≥ 0.05 were considered trends. Predictors of the cognition index were analysed further in a multivariable linear regression model. Predictors with a p value < 0.15, as a result of the univariable analysis, were included.

Results

Patient sample

Initially, 65 patients were included in the study. However, in 4 patients the MR images were of insufficient quality. The remaining 61 patients (33 women and 28 men) had a mean age at examination of 17.9 (range 1.6 to 59) years, with 20% of the patients aged 5 years or less. In 44 patients mutation analysis confirmed the diagnosis.

Figure 3 Diagram showing the number of patients in whom independent and dependent variables were obtained.



A *TSC1* mutation was found in 14 and a *TSC2* mutation in 30 patients. DNA analysis did not reveal a mutation in 5 patients and was not performed in 12 patients. As shown in *Figure 3*, all patients were subjected to radiological and many to neuropsychological assessment.

Tuber status

Tubers were detected in all patients. The total number of tubers ranged from 7 to 58, mean 28. The mean number of tubers in the left hemisphere was 13, range 2 to 25, and in the right hemisphere it was 15, range 3 to 33. The total tuber volume ranged from 1.9 to 71.5 cm^3, mean 16.5 cm^3. The mean TBP was 1.3%, range 0.2 to 5.1%. In the left hemisphere, the mean tuber volume was 7.5 cm^3, range 0.8 to 33.2 cm^3, and the mean TBP was 1.2%, range 0.1 to 4.9%. In the right hemisphere, the mean tuber volume was 9.0 cm^3, range 1.1 to 38.3 cm^3, and the mean TBP was 1.4%, range 0.2 to 5.1%. In 39 of the 61 patients (64%) the TBP was greater in the right hemisphere.

Epilepsy

Among the 61 patients, 51 had seizures (85%), including 21 patients with a history of IS (40%). Age at seizure onset ranged from 1 day to 37 years, mean 2.2 years, median 7 months. EEG recordings were performed in 58 patients, including 7 patients without a history of seizures. Epileptiform activity was detected in 46 patients (79%) and was unifocal in 16 and multifocal in 30. Seizure origin was assessed in 14 patients and was unifocal in 6 patients and multifocal in 8.

Cognitive function

The cognition index, assessed in 59 patients, had a mean of 1.7, range 1.0 to 3.7, and was below average in 46 patients (78%). The intelligence equivalent (IE) ranged from 7 to 119, mean 69. Below average scores (IE< 90) were found in 48 patients (81%), and severely below average scores (IE< 70) in 33 patients (56%). With respect to non-cognitive determinants of the responses to the cognitive tests, there was conspicuous motor slowness in 73% (19/26 patients tested).

Associations between tuber status, epilepsy and cognition

Although fewer tubers were detected in patients with multifocal interictal epileptiform activity than in patients with focal interictal epileptiform activity (mean difference -5.6, 95% CI -11.1 to -0.07, *table*), the number of tubers was not related to age at seizure onset, seizure types (including a history of IS) or any of the variables of cognitive function (*Figure 4A*).
In contrast, TBP was inversely related to the age at seizure onset (HR 1.6, 95% CI 1.2 to 2.1, analysed in patients with epilepsy; data not shown in table) and cognitive function (*Figure 4B*). Overall, patients with severely below average results in any of the cognitive tests (scale

Table Differences of tuber status between patients with and without epilepsy variables.

variable	present (%)	absent	difference (95% CI) mean number of tubers	difference (95% CI) mean TBP
epilepsy	52 (85)	9	-4.7 (-11.7- 2.3)	0.09 (-0.6- 0.8)
IS	22 (40)	31	0.1 (-5.6- 5.8)	0.3 (-0.3- 0.8)
GTCS	18 (35)	34	-0.9 (-6.8- 5.0)	-0.1 (-0.7- 0.5)
CPS	33 (64)	29	2.5 (-3.3- 8.3)	0.4 (-0.2- 1.0)
TS	6 (12)	46	-7.4 (-15.9- 1.1)	-0.4 (-1.3- 0.5)
multiple seizure types	21 (40)	31	0.8 (-4.9- 6.6)	0.4 (-0.2- 0.9)
multifocal EEG	30 (52)	28	-5.6 (-11.1- -0.07)	-0.05 (-0.6- 0.5)
multifocal ictal onset	8 (57)	6	-2.8 (-17.3- 11.6)	0.1 (-0.6- 0.8)

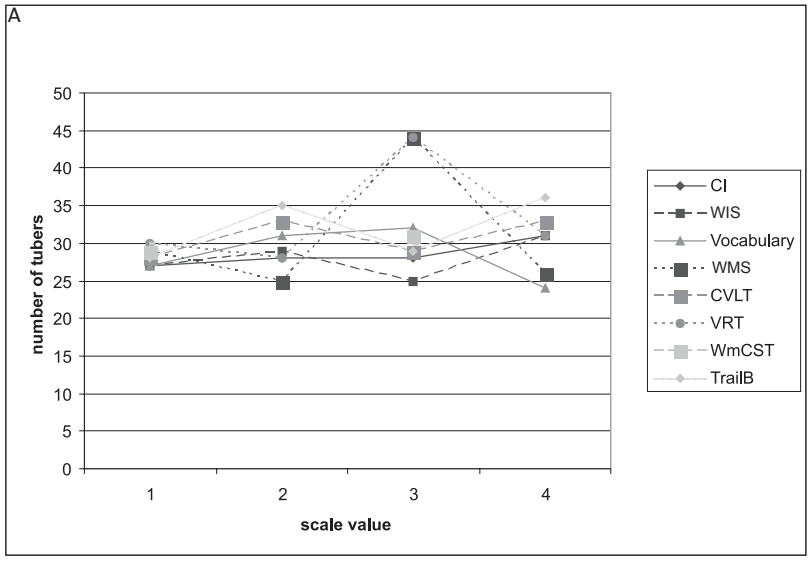
Presence and absence of seizure types has only been analysed in patients with epilepsy (52). EEG was analysed in 58 patients (including patients with epilepsy); ictal onset was determined in 14 patients. IS= infantile spasms, GTCS= generalised tonic clonic seizures, CPS= complex partial seizures, TS= tonic seizures, CI= confidence interval, TPB= tuber/ brain proportion. Statistically significant relationships are printed in bold.

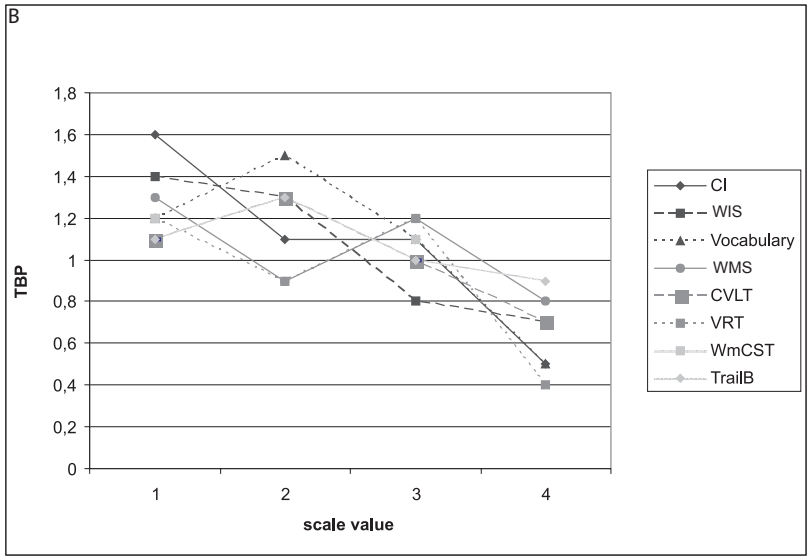
value 1) had a TBP $\geq$ 1% and patients with above average cognitive test results (scale value 4) had a TBP $\leq$ 1% (*Figure 4*B). As a group, patients with a greater TBP had a lower IE (regression coefficient -7.6, 95% CI -14.8 to -0.4; i.e each 1% increase in TBP resulted in a 7.6 point decrease in IE) and tended to have a lower cognition index (regression coefficient -0.21, 95% CI – 0.4 to 0.003). When the analysis was repeated separately for the two cerebral hemispheres, a greater TBP was related to a lower IE (regression coefficient -7.4, 95% CI – 14.5 to -0.4) and to a lower cognition index (regression coefficient -0.23, 95% CI -0.4 to -0.009) in the left hemisphere only.

Associations between epilepsy and cognitive function

Patients with epilepsy had both a lower IE (mean difference 19, 95% CI 3.1 to 34.7) and a lower cognition index (mean difference 0.9, 95% CI 0.3 to 1.4) than those without epilepsy. In the group of patients with epilepsy, earlier seizure onset was associated with a lower IE (regression coefficient 1.2, 95% CI 0.2 to 2.3, i.e. for each year that seizure onset was delayed the IE was 1.2 points higher), a lower cognition index (regression coefficient 0.08, 95% CI 0.01 to 0.08) and increased Trails A execution time (mean difference 10, 95% CI 3.9 to 16.7). In univariable analyses, patients with and without IS differed with respect to IE (mean difference 18, 95% CI 5.8 to 30.7) and the cognition index (mean difference 0.5, 95% CI 0.08 to 0.9). However, in a multivariable linear regression model, IS was not independently related to either IE or the cognition index.

Figure 4 Relationship between the number of tubers (A) and tuber/ brain proportion= TBP (B) and cognitive functioning.





1= severely below average, 2= below average, 3= average, and 4= above average. CI= cognition index, WIS= Wechsler Intelligence Scales, WMS= Wechsler Memory Scales, CVLT= California Verbal Learning Test, VRT= visual retention test, WmCST= Wisconsin modified card sorting test, Trail B= Trail making part B

Predictors of the cognition index

The univariable regression analysis revealed that a younger age at seizure onset, a history of IS and a *TSC2* mutation (*chapter three*) were associated with a lower cognition index. Further, the cognition index tended to be lower in patients with a greater TBP. We identified ten patients without infantile spasms, seizure onset after the first year of life, a *TSC1* mutation and TBP less than the median of the whole sample. Of these 10 patients only one had a below average cognition index. In this particular patient, we cannot exclude the possibility that the cognition index underestimated the actual cognitive ability, since this patient is Flemish while the tests were administered in Dutch, albeit using Flemish standards. The patient in question receives mainstream education. Only one of the 21 patients with either a history of IS, or seizure onset in the first year of life, had a normal cognition index. In this patient we had to rely on historical data (the patient's own account) to define the epilepsy syndrome. It is possible that this was misclassified.

To analyse the contributions of TBP, age at seizure onset, IS, and germ-line mutation to the cognition index, multivariable linear regression analysis was performed on the group of 36 TSC patients with epilepsy and a known mutation (60% of the total sample). TBP showed the weakest association with the cognition index (regression coefficient 0.02, 95% CI -0.3 to 0.2). After excluding TBP, IS was no longer independently associated with the cognition index (regression coefficient -0.16, 95% CI -0.7 to 0.3). Repeating the analysis with age at seizure onset and mutation type only, patients with a *TSC2* mutation tended to have a lower cognition index (regression coefficient -0.46, 95% CI -1.0 to 0.05). The only factor that independently influenced the cognition index to a statistically significant degree was the age at seizure onset (regression coefficient 0.1, 95% CI 0.05 to 0.2).

Discussion

To determine whether tuber status is a critical determinant of epilepsy and cognitive function in TSC, we used a novel automated tuber detection and segmentation technique to allow reliable and reproducible assessment of tuber status, and we calculated a cognition index for each individual on the basis of a comprehensive neuropsychological test battery. We found that patients with a greater TBP were younger when they had their first seizure, had a lower IE, and tended to have a lower cognition index. In contrast, the number of tubers was neither related to epilepsy (apart from the finding that patients with multifocal interictal epileptiform activity had more tubers than patients with unifocal activity) nor to cognitive function. These findings support our hypothesis that TBP is a better marker than the number of tubers for seizures and cognitive functioning in patients with TSC.

Several critical determinants of neurodevelopmental difficulties in TSC have been described,

including age at seizure onset, IS, tuber status and genotype.[1,12,14,21,33-38] In our patient sample, the number of tubers proved an inappropriate predictor of cognitive function since we did not identify an association between these two variables. Despite the significant relation between TBP and intelligence (the problem solving aspect of cognition), patients with normal intelligence and a large TBP were present in our patient sample. Thus, although TBP is an important determinant of cognitive function it is not a completely reliable marker. Furthermore, in our sample, patients with different *TSC1* and *TSC2* mutations could not be distinguished by their neurological and cognitive phenotypes (*chapter three*). Although multivariable analysis was limited to only 60% of the total sample, we identified age at seizure onset as the only independent contributor to the cognition index. Seizure onset in the first year was strongly associated with a low cognition index. Although the occurrence of IS was not an independent predictor, only one patient in our sample had both a history of (presumed) IS and an average cognition index. This suggests that normal cognitive function (average or above average cognition index) is very unlikely in TSC patients with early seizure onset and IS.

Our results extend those of previous studies[1,14,21,35,39] to further disentangle the associations between tuber status, epilepsy variables and cognitive function. The implementation of an automated tuber detection and segmentation technique enabled us to objectively and reproducibly assess tuber status in patients with TSC. In comparison to previous studies, we found a high number of tubers in most of the patients, most likely due to the sensitive detection method we used. We performed 3D scans with slices of only 1.5 mm, without a slice gap, which enabled us to detect multiple small tubers. Although the number of detected tubers was high, the TBP was relatively small, <6%. Taking into account the often severe neurological and cognitive symptoms of TSC, we suggest that the detectable lesion volumes may just be the visible peak of a more extensive underlying, diffuse structural disorganisation.

Deficits of learning and memory appeared to be consistent with the general intelligence level and did not suggest specific cognitive impairments although in some patients motor slowness may have affected timed cognitive task performances. It is also important to mention that, although the majority of patients in our cohort had a below average cognition index, 22% had normal scores for all the cognitive domains tested.

A couple of methodological issues can be raised. First, we performed a cross-sectional study of a TSC sample containing a preponderance of children under 5 years of age (20% of the sample). In all but one of these children, seizures and cognitive impairment were present, if at all, from early childhood. Although tubers are dynamic lesions,[40] there is no MRI-based longitudinal study reporting tuber growth or changing effects on cognitive function. Data to exclude cognitive worsening with age was not available for our patients.

Second, the widely varying ages and cognitive abilities of the patients necessarily resulted in widely varying neuropsychological data sets. The number of tests that could be administered per patient and the outcome measures of the tests differed. However, by using standardised

tests and converting results to well-defined scale values, we surmounted the disadvantages of different types of outcome measures. We calculated a cognition index for each participant. This enabled us to enter patients of all ages who were assessed by different tools, and for whom we had obtained different numbers of test results. For some patients the cognition index was calculated solely on the basis of the intelligence test or developmental scale. In these cases, cognitive abilities were so limited that these were the only tests that could be performed, and the resulting cognition index was a realistic account of cognitive abilities.
The incidence of seizures and of below average IE in our sample was 85% and 81% respectively. Most studies of large TSC populations find a prevalence of ~80% for seizures and ~50% for mental retardation.[2] In these studies mental retardation is often defined as an IQ below 70. On the basis of this definition, mental retardation was present in 56% of the patients in our sample, rendering the sample representative for a TSC population, although patients were identified through a tertiary referral centre.
In two currently seizure-free patients a history of epilepsy was reported. In both cases we had to rely on the patient's own recollections to classify the epilepsy syndrome. Consequently, it is possible that there was some misclassification in these patients.

In conclusion, TBP is a better predictor of seizures and cognitive function in TSC than the number of tubers. However, a large TBP is neither necessary nor sufficient for early seizure onset or cognitive impairment. Epilepsy at an early age is the only independent risk factor of cognitive impairment and therefore the prevention of seizure onset is vital to ensure normal cognitive development in patients with TSC. However, in order to be able to prevent seizure onset, the nature and timing of the epileptogenic trigger needs to be known. Until then, adequate treatment should be started as soon as possible after seizures occur. The wide range of cognitive functioning in TSC renders standard measures precarious. Hence, early assessment of cognitive problems is necessary for multidisciplinary counselling and interventions.

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Part II

5

Consistent localisation of interictal epileptiform activity on EEGs of patients with tuberous sclerosis complex

Floor Jansen, Alexander van Huffelen, Mireille Bourez-Swart, and Onno van Nieuwenhuizen

Abstract

We addressed consistent localisation of interictal epileptiform activity on EEGs of 21 patients with tuberous sclerosis complex (TSC), in whom three or more EEG recordings were obtained during a 10-year history of epilepsy. Local maxima of interictal epileptiform activity were measured from 76 EEG traces and 33 EEG reports.

In eight patients interictal epileptiform activity was consistently detected in one or two regions (group 1) and in 13 patients epileptiform activity was detected in three or more regions (group 2). The number of foci increased throughout the disease in both groups, but in 19 of the 21 patients one localisation was consistently present (94% of the EEGs). Age at seizure onset, seizure types and IQ differed between the groups. Patients with one or two regions of epileptiform activity were characterised by later onset of often complex partial seizures and normal intelligence or mild impairment. These patients may be considered candidates for epilepsy surgery.

Introduction

Tuberous sclerosis complex (TSC) is a neurocutaneous syndrome with an autosomal dominant inheritance. Although the disease may manifest in any organ, hamartomas most often lead to clinical symptoms in the skin, the heart, the kidney, and the brain.

Excessive neuronal proliferation and abnormal migration and differentiation underlie the development of the characteristic brain lesions, such as subependymal nodules, giant cell tumours, and tubers. Tubers are located (sub)cortically and are characterised by a disruption of cortical lamination.[1] They are associated with neurological symptoms, such as epilepsy, mental retardation, focal neurological deficit, and autistic spectrum disorder. Epilepsy is often the presenting symptom of TSC and occurs in more than 80% of patients.[2] The epileptic manifestations are age related and most of the patients with seizure onset in the first year of life present with infantile spasms. The course, especially after presentation with infantile spasms, is often unfavourable. Seizures are resistant to antiepileptic drugs in more than 50% of patients and relapse is common.[4] Moreover, an early onset of seizures adversely affects psychomotor development.[5-7] In adolescence and adulthood, most patients experience complex partial seizures or secondary generalised seizures. Patients who develop secondary generalised seizures following infantile spasms more often have an unfavourable outcome, whereas seizures are usually well controlled in patients who develop partial epilepsy subsequent to infantile spasms.[8]

Epilepsy surgery is an option for patients with TSC and intractable epilepsy but results are variable.[9-12] The success of surgery depends on adequate identification of the epileptogenic zone and its correspondence to a single tuber, the so-called "epileptogenic tuber". Most patients who underwent epilepsy surgery showed localised ictal epileptiform activity, whereas interictal epileptiform activity was often multifocal. Most resections were tuberectomies or lobectomies, but a few patients underwent hemispherectomy or multilobar resection.[13] No standard preoperative clinical work-up exists for patients with TSC. Ictal EEG is most often performed to localise the "epileptogenic tuber". Subdural grids or strip electrode recordings are used in some patients. Promising results regarding localisation of epileptogenic tubers have been reported for alpha [^{11}C] methyl-L-tryptophan (AMT) positron emission tomography (PET),[14] magneto-encephalography,[15] and diffusion-weighted magnetic resonance imaging (MRI) (DWI).[16] However, only limited series of patients have been studied. Fluid Attenuated Inversion Recovery (FLAIR) MRI has proven sensitive in the detection of small subcortical tubers but does not differentiate between epileptogenic and non-epileptogenic lesions.

A major concern is that epileptogenic foci may shift from one epileptogenic tuber to another during the course of the disease, which could constitute a reason not to perform surgery. There are very few data on changes in the epileptogenic focus during disease progression in the literature. In one long-term follow-up study of patients with TSC, seizure type changed in

almost half of the patients.[17] However, in another study the epileptogenic foci changed with increasing age in only 2 of 15 patients with partial epilepsy.[18]
In this retrospective study we evaluated the consistency of localised interictal epileptiform EEG activity over time in 21 patients with TSC and epilepsy, by retrospective analysis, with a view to improving the selection of patients eligible for epilepsy surgery. Patients with a consistent focus of epileptiform activity may be candidates for surgery.

Patients and methods

Between April 1999 and April 2004, 70 patients with TSC visited the Neurology and Paediatric Neurology outpatient department of the University Medical Centre of Utrecht (UMCU), The Netherlands. All patients fulfilled the revised diagnostic criteria for TSC.[19] Patients with minimally a 10-year history of epilepsy who showed interictal epileptiform abnormalities on three or more EEG recordings (interval of at least 3 years) were included. Initial evaluation started with investigation at the outpatient clinic of the UMCU and was considered the starting point of the retrospective analysis.
In all but the final EEG, the technique of EEG recording was the same: 21-channel recordings were made with electrode positions according to the 10-20 system. The final EEG in all patients was an 85-channel high-resolution (HR) EEG, with electrode positions according to the 10% system, performed in the UMCU. This final EEG was considered to provide the most precise information about focus localisation. In these HR-EEGs, epileptiform spikes were averaged and maps of the potentials of these averaged spikes were plotted. These maps were then used to localise the spike maxima. For comparison with the previous EEG recordings, the maxima in the 85-channel recording were attributed to the most appropriate electrode of the 10-20 system or to an intermediate position if the maxima were equal for two adjacent 10-20 electrode positions. In the UMCU and most of the referring hospitals, the EEG traces from the last 10 years were available for assessment (digital recordings were available from 1996 on). For earlier times, we obtained information about the EEG characteristics from relevant clinical and EEG reports. Ictal EEG recordings were available for seven patients but were not included in this study.
One clinical neurophysiologist (ACvH) reviewed all EEGs. If the assessment did not correspond to the original assessment, a second clinical neurophysiologist (MBS) reviewed the recordings. Both were blinded for the previous EEG, information about seizure semiology, and MRI findings. A final consensus reading was arranged to identify the location of epileptiform abnormalities. Consistency was defined as the presence of interictal epileptiform activity at the same location in all EEGs (actual and reports) reviewed.
Clinical data were obtained from the medical records. Seizure semiology was classified on the basis of the definitions proposed by the Commission on Classification and Terminology

of the International League Against Epilepsy.[20] None of the patients had undergone epilepsy surgery. Seizures were considered controlled if the patient was seizure free for at least 1 year before the initial evaluation.
At the start of the study, most patients were administered neuropsychological tests to determine a full-scale IQ (FSIQ). FLAIR MR images were obtained in all patients.
Consistency was expressed in two ways. First, as the percentage of presence of foci at the same location in all reviewed EEGs. Second, as the ratio obtained by dividing the number of foci in the final EEG reviewed by the number of foci in the first EEG reviewed.
The patients were split up into two groups: patients with one or two foci (group 1) and patients with three or more foci (group 2), because the first two foci appeared to be rather consistent whereas the third and fourth foci were not. Patient characteristics were compared between the two groups. Data are expressed as medians. Statistical analysis was performed with the Mann-Whitney U-test. Differences were considered to be statistically significant at a p value < 0.05.

Results

Patient characteristics (*Table 1*)

Twenty-one patients fulfilled inclusion criteria (8 women and 13 men). DNA analysis confirmed the clinical diagnosis of TSC in 14 of the 21 patients. Data on seizure history were available for the last 10 to 55 years. The median age at seizure onset was 0.8 years (range 0.1 to 10 years). Eleven patients had infantile spasms as initial seizure type, six patients had complex partial seizures, and four patients had secondary generalised seizures. Infantile spasms transformed into other types of seizures in all patients. Eight of the 11 patients with infantile spasms subsequently developed complex partial seizures, five of whom also had secondary generalised seizures. The six patients who had complex partial seizures at onset continued to have this type of seizures. Three of the four patients who first had secondary generalised seizures developed partial seizures; the other patient had multiple seizure types (including atonic and myoclonic seizures). One of the patients had become seizure free at the start of the retrospective analysis.
Multiple tubers were detected by MRI in all patients. The median FSIQ was 76 (range from <48 to 119; n= 16).

EEG analysis

Seventy-six EEG traces (17 on paper and 59 digital recordings) and 33 EEG reports were available for analysis. The number of EEGs reviewed per patient ranged from three to 12. The median interval between the first and last EEG was 9 years (range 45 to 3 years). Focal or multifocal

Table 1 Clinical data of 21 patients with tuberous sclerosis complex

patient	age (y)	onset (y)	seizure type at onset	seizure type at follow-up	seizure frequency	FSIQ	foci	mutation
1	55	1.5	CPS	CPS	1	94	1	*TSC1*
2	29	0.9	CPS	CPS/ SPS	1	78	1	*TSC2*
3	27	9	sGC	SPS	2	117	1	*TSC1*
4	20	0.5	IS	CPS	1	78	1	*TSC2*
5	17	5	CPS	CPS	2	119	2	*TSC1*
6	18	1.5	CPS	CPS	2	93	2	*TSC1*
7	27	10	CPS	CPS	1	97	2	*TSC1*
8	20	0.7	IS	CPS/ sGS	2	73	2	*TSC1*
9	12	0.9	sGS	CPS	2	80	3	*TSC2*
10	29	2	IS	CPS	1		3	*TSC1*
11	13	0.7	IS	CPS/ sGS	2	48	3	*TSC1*
12	32	0.8	IS	CPS/ sGS	2		3	*TSC2*
13	22	0.6	IS	CPS/ MS	2		3	
14	18	0.3	sGS	CPS/ sGS	2	<48	3	
15	17	0.9	IS	CPS	seizure free	<48	4	
16	21	0.7	IS	CPS	2		4	*TSC2*
17	12	0.8	IS	sGS	2		4	
18	12	0.1	sGS	CPS	3	<48	4	*TSC1*
19	12	0.1	IS	sGS/ CPS	3	<48	4	
20	10	0.3	IS	CPS/ sGS	2	<48	>4	*TSC2*
21	13	0.1	IS	sGS	3	<48	>4	

y= years, FSIQ= full scale intelligence quotient, CPS= complex partial seizures, IS= infantile spasms, sGS= secondary generalised seizures, MS= myoclonic seizures, 1= monthly, 2= weekly, 3= daily seizures

spikes, spike and wave complexes, sharp waves, and sharp and slow wave complexes were detected in the EEGs of all patients. In eight patients (38%), the review revealed different conclusions than those originally drawn. In most of these cases, multiple EEG foci were detected on review, whereas single or double foci were detected previously.

Consistency of EEG foci

One region of interictal epileptiform activity was consistent in the EEGs of four patients during the period covered by the retrospective analysis (*Table 2*). These patients had partial seizures with consistent semiology.

Two foci were detected in four patients; one of these foci was consistent in all recordings.

Table 2 Consistency of interictal epileptiform foci (defined as % of presence in all EEGs)

Patient	EEGs	epileptiform focus (%)				ratio[1]	ratio[2]
		1	2	3	4		
1	4	100				1	1
2	3	100				1	1
3	3	100				1	1
4	1	100				1	1
5	5	100	50			2	1
6	4	100	75			1	0.5
7	3	100	75			1	1
8	2	100	100			1	1
9	7	100	50	17		1.5	1
10	5	100	100	20		1.5	1.5
11	4	100	33	33		0.5	1
12	3	100	100	33		1.5	1
13	2	100	67	33		2	3
14	2	100	67	33		1	1
15	6	60	20	20	20	2	1
16	3	100	66	66	66	0.5	0.75
17	3	100	100	50	50	2	2
18	3	100	100	66	66	1	1
19	3	100	100	66	33	1	1
20	6	66	50	33	33	1	0.5
21	5	100	75	50	50	1	1.5
average		96	72	43	45	1.25	1.19

Ratio[1] = number of foci in final reviewed EEG / number of foci in 1st reviewed EEG
Ratio[2] = number of foci in penultimate reviewed EEG / number of foci in 1st reviewed EEG
EEGs= number of EEGs (EEG reports) reviewed

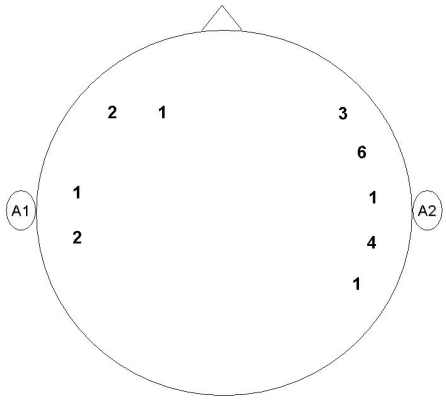
The second focus was consistent in 50- 100% of EEGs. Three of these four patients had partial seizures, the other patient first experienced infantile spasms but developed secondary generalised seizures later in life.

Three foci were detected in six patients. Again, one of these foci was consistently found in all EEGs of these patients. A second focus was consistently found in 33- 100% and a third in 17- 33% of the EEGs. One patient started with complex partial seizures, one with secondary generalised seizures, and four patients with infantile spasms.

Four or more foci were detected in seven patients. One focus was consistently found in the EEGs of five of the seven patients. A second focus was detected in 20- 100% of the EEGs, and

the third and fourth foci were detected in 20- 66% of the EEGs.
A ratio has been calculated to express the change in number of foci (*Table 2*). In 12 patients the number of foci did not change, in seven patients the number of foci increased and in two patients the number decreased. The average ratio in all 21 patients was 1.25. The increase in the number of foci in the final EEG was thought to be partly due to the higher resolution of the final EEG recording. To test this, we performed the same calculation with the information from the last EEG recorded with the 10-20 montage. This time the number of foci increased in only four patients (average ratio 1.19).
Consistent interictal epileptiform activity (19 of the 21 patients) was most frequently localised to the fronto-temporal region (*Figure*) and only sporadic to the frontopolar, central, parietal, or occipital region.

Figure Localisation of the most consistent regions of interictal epileptiform activity on EEGs of 19 patients.



Statistical analysis of patient characteristics and consistency of EEG foci (*Table 3*)

The median age at seizure onset was significantly higher in patients from group 1 compared with patients from group 2 (1.5 versus 0.7 years). Seizure types both at onset and during the course of the disease differed between the two groups. In group 1, five of the eight patients had complex partial seizures at onset, whereas in group 2 infantile spasms was the most common initial seizure type. During the course of the disease secondary generalised seizures occurred more often in patients from group 2 than in patients from group 1. The median FSIQ was lower in patients from group 2 than in patients from group 1. Group 1 and group 2 were not distinguished by mutation type or the ratios for changes in foci.

Table 3 Comparisons of patient characteristics and EEG foci between group 1 and group 2

group	patients	median age at onset (y)	seizure type at onset	seizure type at follow-up	FSIQ	*TSC1/2*	ratio[1]	ratio[2]
1	8	1.5	IS (2/8)	sGS (1/8)	94	6/2	1.13	1.0
2	13	0.7	IS (9/13)	sGS (8/13)	48	3/4	1.38	1.33
P		0.01	0.02	0.03	0.001	0.3	0.4	0.4

Y= years, IS= infantile spasms, sGS= secondary generalised seizures, *TSC1/2*= number of patients with a *TSC1* mutation/ number of patients with a *TSC2* mutation, ratio[1] and ratio[2]: see table 2

Discussion

Most patients with TSC have frequent seizures, often starting in the first year of life, and seizure remission is rare.[21] The seizures are medically intractable in 50% of these patients. Although structural and functional imaging techniques (MRI, DWI, functional MRI, single photon emission computed tomography, and PET) have improved considerably in the last decade, the neurophysiological techniques for recording epileptiform activity have shown a more modest improvement. We evaluated the EEG and clinical records of patients with TSC to determine whether certain EEG findings identify patients eligible for epilepsy surgery.

In general, we found no characteristic interictal EEG pattern in the patients with TSC, consistent with an earlier study.[22] In 19 of the 21 patients, one region of interictal epileptiform activity was consistently detected in all the EEG traces reviewed. Overall, the number of foci increased over the period of investigation (ratio 1.25), although part of this increase could be explained by the fact that the final EEG was an 85-channel HR recording, which is considered to provide the most accurate information. Although not statistically significant, the ratio was higher in patients from group 2 (ratio 1.33) than in patients from group 1 (ratio 1.0). Further, patients with one or two EEG foci (group 1) differed from patients with three or more EEG foci (group 2) in age at seizure onset, seizure types and IQ.

Interictal epileptiform activity seemed to occur preferentially in the fronto-temporal region. This is potentially important because the fronto-temporal region is typically more accessible to a surgical approach.

There are certain limitations to our study. We included only patients with a minimum of 10 years of seizures in whom three or more EEGs had been recorded. These criteria limited the number of patients included. Unfortunately, paper EEG traces are kept for only 10 years, and a number of reports of EEG recordings had to be used to evaluate changes in foci. Because review of actual EEG traces yielded different conclusions from those drawn originally in 8 of the 21 patients, the information included in the written reports of the EEGs should be used with caution because it cannot be checked retrospectively. Therefore, we did not use the information from the paper EEGs when analysing the ratios. With data from actual EEG traces only, we found that the number

of epileptic foci increased with time in a minority of the patients.
To the best of our knowledge, this is the first study to analyse the consistency of EEG foci and clinical long-term history of epilepsy in patients with TSC. Although the number of patients included was too small to draw firm conclusions, we suggest that it is possible to identify a group of patients with consistent interictal epileptiform EEG abnormalities during their long-term history of epilepsy. If one or two consistent interictal EEG foci are found, in TSC patients with drug resistant seizures, ictal recordings should be performed and epilepsy surgery should be considered. Clinically, candidates for epilepsy surgery may be characterised by onset of complex partial seizures after the first year of life, the development of complex partial seizures during follow-up, and normal or mildly impaired cognitive function. However, epilepsy surgery should also be considered in TSC patients with two or more interictal EEG foci, especially if one is most abundantly firing and seizure semiology has not changed. In these patients ictal recordings should be performed as well. A prospective long-term follow-up study to analyse the consistency of EEG foci, preferably with the 85- channel EEG technique and applying source localisation to the data, is warranted. A study to analyse whether these regions of interictal EEG abnormalities are concordant with the location of seizure onset (obtained with long-term video EEG recordings) is currently being undertaken.

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6

Identification of the epileptogenic tuber in patients with tuberous sclerosis: A comparison of high-resolution EEG and MEG

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Abstract

High-resolution (HR) EEG recording was compared to magneto-ecephalography (MEG) in 19 patients with tuberous sclerosis complex (TSC) and epilepsy.
Epileptiform activity, i.e. spikes, were identified in HR EEG and MEG recordings offline by three observers. Source localisation with CURRYV 3.0 software was applied to spikes with moderate or good interobserver agreement (consensus spikes). MUSIC analysis was performed. The distance between EEG and MEG sources and the border of the closest tuber was calculated and compared.
Consensus spikes on EEG recording were identified in 12 patients and on MEG recording in 14 patients. MEG sources were closer to tubers in all but one patient. Three patients underwent epilepsy surgery, two of whom are seizure free after complete resection of the tuber. MEG contributes to accurate identification of the epileptogenic zone creating opportunities for epilepsy surgery in patients with TSC and drug-resistant epilepsy.

Introduction

Tuberous sclerosis complex (TSC) is a neurocutaneous syndrome, involving multiple organs. The characteristic hamartomas are most commonly found in the skin, retina, heart, kidney, and brain. TSC is an autosomal dominant disorder with linkage to chromosome 9q34 (*TSC1*)[1] and chromosome 16p13 (*TSC2*).[2]

In the central nervous system of patients with TSC subependymal noduli, giant-cell astrocytomas, and cortical tubers are found as a result of disordered proliferation, migration, and differentiation.[3] Cortical tubers are associated with neurological symptoms, such as epilepsy, mental retardation, and focal neurological deficit. Although the phenotypic expression of TSC is extremely variable, seizures are common, occurring in 80-90% of cases, and are often the presenting symptom. Furthermore, they are medically intractable in 50% of the patients. Surgery should be considered in patients with TSC and drug-resistant epilepsy, but it may be difficult to identify the epileptogenic tuber if there are several tubers distributed throughout the cerebral cortex. To date, the outcome of surgery has been variable in patients with TSC.[4-10]

Although structural and functional imaging techniques (MRI, functional MRI (fMRI), single photon emission computed tomography (SPECT), positron emission tomography (PET)) are being increasingly used in the pre-surgical evaluation, epileptiform activity can only be recorded with neurophysiological techniques. With standard EEG it is often impossible to delineate the irritative zone and establish its relation to the tuber(s) with sufficient precision. For this reason high-resolution (HR) EEG and magneto-encephalography (MEG) are used to increase the accuracy of functional localisation.[11] Source localisation with EEG requires modelling of the skin, skull, and brain, which in turn requires knowledge of their shape and their conductivity. Conductivity values in particular can only be approximated. Because MEG records the magnetic field around the head, and magnetic fields are not attenuated by volume conductors, MEG is more accurate than EEG for source localisation. Results from the literature based on realistic phantom models, with the same methods (MUSIC) as described in this paper and for comparable numbers of measurement channels, indicate that localisation errors for true dipolar sources on average are 7-8 mm for EEG and 3 mm for MEG.[12] However, because a radial dipole does not generate a magnetic field outside the head, MEG selectively detects tangential sources (e.g. sources on a sulcus). Moreover, measurements are affected by movement of the head, which means that cooperation is essential. The shortcomings of EEG and MEG can largely be overcome by combining the two techniques, by recording MEG and EEG simultaneously. The sources computed on the basis of HR EEG and MEG findings can be visualised by plotting the equivalent current dipole representation on the MRI of the patient's brain with volume reconstruction. This technique, termed magnetic source imaging (MSI), has been successfully applied to reduce the need for invasive monitoring in candidates for surgery.[11,13,14] Experience with MEG in patients with TSC and epilepsy is limited.[11,15,16]

In this study we determined the correspondence between epileptiform activity recorded with HR EEG and MEG in patients with TSC and epilepsy and assessed to what extent the sources differed after mapping of the relevant dipoles on MR images.

Patients and Methods

Patients

In the period 1998 to 2003 19 patients with TSC who fulfilled the revised TSC diagnostic criteria and who were admitted to the outpatient clinic of the Departments of Paediatric Neurology and Neurology, University Medical Centre, Utrecht, The Netherlands could be included in this study. All but one patient had active epilepsy and all had relatively frequent interictal spikes on HR EEG. A seizure history was taken and further information was extracted from the medical files.

Neuroimaging

Cerebral MR images were performed in all patients with a 1.5-Tesla magnet (Gyrocsan NT, Powertrak 6000, Philips Medical Systems, Best, The Netherlands). Fluid Attenuated Inversion Recovery (FLAIR) images were generated with a slice thickness of 1.5 mm, no gap. Optimal scan parameters were determined in a pilot study (IR= 2600ms, TE= 125ms, TR= 11000 ms, FOV 256 x 256, RFOV 80); the contrast-to-noise ratio with a slice thickness of 1.5 mm was sufficient to detect small tubers. On the FLAIR images, tubers were semi-automatically segmented using imageXplorer [www.imagexplorer.nl]. The interface requires manual identification of each tuber by a single click inside the tuber. The local maximum (highest intensity value) inside the tuber was used as starting point for a region growing procedure. The lower threshold of the region growing range (that defines the border of each tuber) could be determined automatically based on statistical analysis of previously segmented tubers in the same data set (mean value, standard deviation). Using this learning system, the majority of the bright tubers have been segmented with a single mouse click. The tuber status was expressed as number and volume of tubers. For the construction of the volume conductor models, T1-weighted images, with a resolution of 0.89 x 0.89 x 1.5 mm, were recorded. FLAIR images were matched to these according to a previous published image registration protocol.[17]

EEG and MEG spike acquisition

An EEG (85 channel; BioSemi Mark-6, Brainstar system 4.0) was recorded in 14 patients at a sampling rate of 1024 Hz, using a cap containing Sn electrodes (ElectroCap Inc.). Furthermore, when MEG was performed a 64- or 32- channel EEG was recorded at the same time in all patients. For the purpose of this study, the highest quality EEG recordings with the lowest

number of bad leads and the highest signal-to-noise ratio were analysed. In all cases this was the 85-channel recording. Only in those cases in which such a recording was not available, or where no spikes were detected, the EEG recorded simultaneously with MEG was (also) analysed. Thus, in 14 patients the 85 channel EEG recording was analysed and in 5 patients the 64- or 32- channel EEG recording, performed simultaneous to the MEG recording was analysed. The electrodes of the HR EEGs were positioned according to the 10% system accepted by the American EEG society. Electrode positions were registered with a magnetic tracking device (Polhemus, Colchester, VT, USA). In addition, three anatomical marker points (e.g. nasal and pre-auricular) and head-shape were measured, which allowed matching with MRI and MEG markers. Spontaneous activity was recorded during a 40-minute session.
MEG, using 151 axial gradiometers arranged as a helmet (Omega 151, CTF system Inc., Port Coquitlam, British Columbia, Canada) and simultaneous 32- or 64-channel EEG were recorded, at a sampling rate of 625 Hz, inside a magnetically shielded chamber (Vacuumschmelze GmbH, Hanau, Germany). Head position with respect to the helmet, and after each recording session, electrode positions and head-shape were recorded with four magnetic localising coils.[18] The signals were (software) bandpass filtered between 0.7 and 70 Hz. A recording session took 1.5 to 2 hours. Aside from voluntary sleep deprivation no activation procedures were used. None of the patients needed sedation.

Spike detection and averaging

Interictal epileptiform spikes were identified offline in both EEG and MEG recordings. Epileptiform activity was analysed in two or three selected 10-minute MEG epochs. Epileptiform activity was defined as sharp activity different from background activity and lasting less than 0.1 ms. Sharp activity registered during the QRS complex of the electrocardiography (ECG) was neglected. Three clinical neurophysiologists independently analysed the selected HR EEG and MEG epochs for the 19 patients in order to obtain consensus spikes (spikes selected by two or more observers).[19,20] The observers were not given information on seizure semiology, MRI findings, and spike selection by the other observers. Kappa statistics were calculated between all three combinations of the three observers for both EEG and MEG recordings. Recordings were rejected when the interobserver kappa values were lower than 0.4 for two or more combinations of the three observers. For EEG and MEG recordings with kappa values equal to or greater than 0.4 cluster analysis of the marked consensus spikes was performed.[21] Spikes for each cluster were then averaged. In the case of multiple clusters only the one with the highest number of spikes, resulting in the highest signal-to-noise ratio, was considered for source imaging.

Source imaging

Spherical volume conductor models are inadequate for accurate source localisation, especially in the case of EEG. Therefore, based on the 3D T1 MRI, an accurate volume conductor model including brain tissue (MEG only) and skull and skin (EEG) was constructed with CURRY 3.0 software. The conductivity of the skin, bone, and brain compartments was set at 0.33, 0.0168 and 0.33 m*S/m,* respectively. Measured EEG electrode positions and position of the MEG helmet with respect to the volume conductor model were computed on the basis of the co-registered marker positions and head shape. Averaged spike data (for a period of 30-40 ms from spike onset to spike maximum) were imported into CURRY. A Multiple Signal Classification (MUSIC) analysis for rotating dipoles was then performed at a resolution of 1.5 mm. The rank applied was that suggested by an SVD analysis of the spike selection, assuming a noise level as indicated by the data before spike onset. The distances between the dipole maximum identified by the MUSIC analysis of both EEG and MEG data and the border of the closest tuber were calculated and compared.

Results

Patient characteristics

Table 1 summarises the clinical data of the 19 patients (11 women, 8 men). The diagnosis TSC was confirmed by mutation analysis in 11 patients (six patients with a *TSC1* mutation and five with a *TSC2* mutation). Median age at seizure onset was 1 year (range 1 month to 37 years). Median age at the time of the study was 27 years (range 6 to 54 years). Thirteen patients had complex partial seizures, three had tonic seizures, and six had secondary generalised tonic-clonic seizures. One patient had been seizure free for more than 20 years but had relatively frequent interictal epileptiform activity. Two patients had daily seizures, 11 patients had several seizures per week, and five patients had seizures at least once a month. Four patients had more than one seizure type at the time of investigation. Seizure semiology of the patients with only partial seizures had not changed for many years. Fourteen patients were examined neuropsychologically: IQ ranged from < 48 to 119, median 78. In 18 patients FLAIR images allowed the segmentation of tubers. The median number of tubers was 19, range 5 to 45; tuber volume ranged from 0.1 to 22.2, median 1.0 cm^3.

Table 1 clinical characteristics of 19 TSC patients

Patient	age (years)	age at onset (years)	seizure type	seizure frequency	IQ	mutation	no volume of tubes (cm^3)
1	6	2	CPS	2	83	*TSC1*	21/ 4.9
2	53	0.1	sGTCS	2	<48		24/ 0.7
3	32	0.9	TS, sGTCS	1		*TSC2*	19/ 1.0
4	19	0.7	TS, sGTCS	3	73	*TSC1*	12/ 2.9
5	23	12	CPS	1		*TSC2*	4/ 11.9
6	20	0.7	CPS	3	79	*TSC2*	30/ 0.2
7	54	1	sGTCS	0	57		16/ 0.2
8	51	1.5	CPS	3	94	*TSC1*	10/ 0.1
9	12	0.9	CPS	2	80	*TSC2*	25/ 22.2
10	21	0.8	sGTCS	2			19/ 6.0
11	28	27	CPS	2			25/ 19.8
12	21	10	CPS	2	97	*TSC1*	13/ 0.2
13	17	5	CPS	2	119	*TSC1*	12/ 1.8
14	29	0.9	CPS	2	78	*TSC2*	45/ 1.0
15	27	14	CPS, TS	2			8/ 0.1
16	53	0.5	sGTCS	3	55		
17	30	0.4	CPS	2	67		18/ 0.6
18	27	9	CPS, SPS	3	117	*TSC1*	29/ 15.8
19	60	37	CPS	2	78		11/ 0.3

IQ= intelligent quotient, CPS= complex partial seizures, sGTCS= secondary generalised tonic clonic seizures, TS= tonic seizures, SPS= simple partial seizures. 0= seizure free, 1= monthly, 2= weekly, 3= daily seizures

Spike detection (*Table 2*)

In all 19 patients only interictal spikes were recorded. EEG recording revealed a single localisation of epileptiform activity (spikes) in nine individuals. The interobserver analysis of all HR EEG recordings showed kappa values exceeding 0.40, for at least two combinations of observers, in 12 patients. The number of consensus spikes in the HR EEG recording ranged from 5 to 168, median 38. In those recordings with poor interobserver agreement, we compared the 85-channel EEG with the EEG recorded simultaneously to the MEG. Spike detection and agreement were not better for the simultaneous EEG recording. In two patients EEG spikes were absent although spikes were detected in the MEG recording. MEG recordings showed unifocal epileptiform activity in 12 patients. The kappa values exceeded 0.40 in 14 patients. The median number of consensus spikes was 29 (range 8–163). Kappa values were low for the recordings with few consensus spikes.

Table 2 Spike detection in 19 patients with TSC

Patient	EEG				MEG			
	kappa	spikes	foci	distance (mm)	kappa	spikes	foci	distance (mm)
1	>0.4	140	u	5.5	>0.4	15	u	7.4
2	>0.4	126	m	28.4	>0.4	84	u	12.1
3	>0.4	97	m	15.4	>0.4	122	m	6.7
4	>0.4	92	u	21.0 (26.3)	>0.4	70	u	20.1 (46.2)
5	>0.4	71	m	30.8	>0.4	143	m	22.6
6	>0.4	70	u	22.5 (34.4)	>0.4	34	u	10.4 (32.3)
7	>0.4	38	m	39.3	>0.4	10	u	29.3
8	>0.4	33	u	30.9	>0.4	24	u	13.5
9	>0.4	127	m	19.2	<0.4	12		
10	>0.4	32	m	33.7	<0.4	8		
11	<0.4	36			>0.4	163	u	11.7
12	<0.4	125			>0.4	80	u	13.1
13	<0.4	8			>0.4	29	u	10.8
14	<0.4	14			>0.4	22	u	7.6
15	>0.4	168			>0.4	123		
16	>0.4	29			>0.4	29		
17	<0.4	16			<0.4	11		
18	<0.4	14			<0.4	16		
19	<0.4	5			<0.4	15		
average				24.6 (26.4)				13.8 (21.3)

u = unifocal, m = multifocal, in brackets is the distance between the source and the tuber closest to the other modality.

Distance to tubers

The HR EEG and MEG sources were plotted on the FLAIR images for eight patients, EEG sources only for two patients (9 and 10), and MEG sources only for four patients (11-14). Source modelling could not reliably be performed in two patients even though interobserver agreement was moderate for both EEG and MEG recordings. Too many artefacts were found in the MEG recording of patient 15 because of previous surgery (meningeoma) and FLAIR images were not available in patient 16. The EEG and MEG recordings of three other patients (17-19) were rejected for source imaging analysis because of poor interobserver agreement (kappa <0.40).

Figures 1 and *2* are examples of EEG and MEG sources plotted on the MR images. Overall, MEG

Figure 1 Example of source localisation. Integration of EEG (A) and MEG (B) sources in a 3D FLAIR MR image. The crosshair indicates the tuber. The MUSIC result for epileptiform activity is shown in yellow. Note that the closest tuber is the same tuber in both modalities.

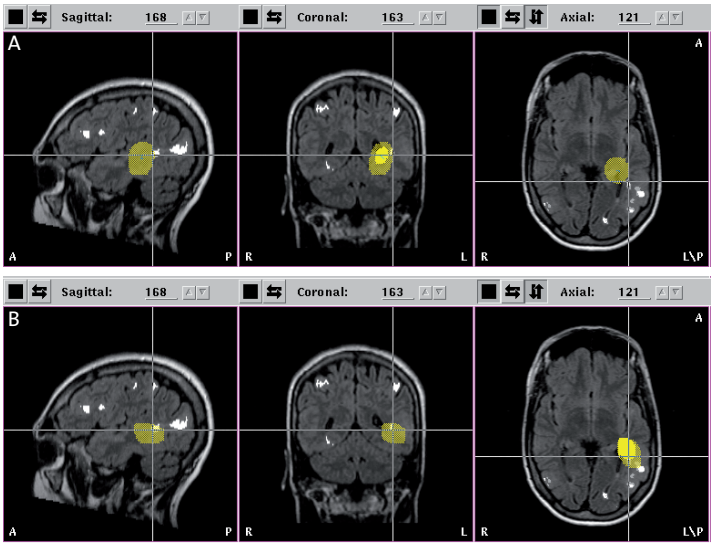


Figure 2 Example of source localisation. Integration of EEG (A) and magneto-encephalography (B) sources in a 3D FLAIR image. The crosshair indicates the tuber. The MUSIC result for epileptiform activity is shown in yellow. Note that the closest tuber is a different tuber in both modalities.

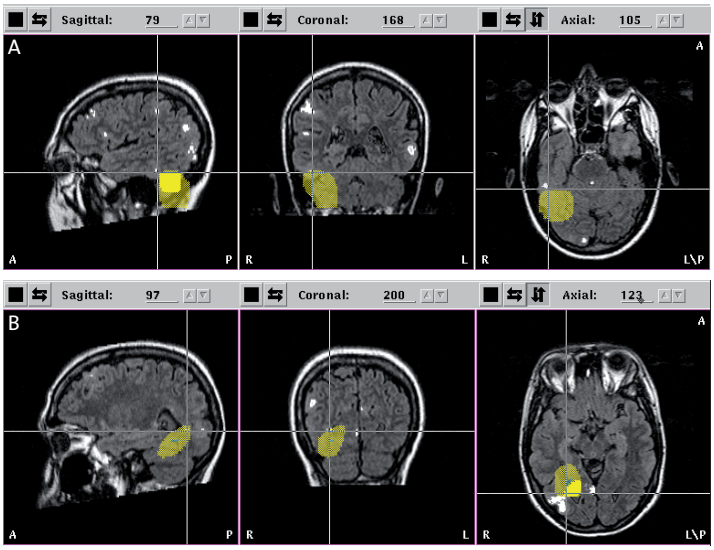
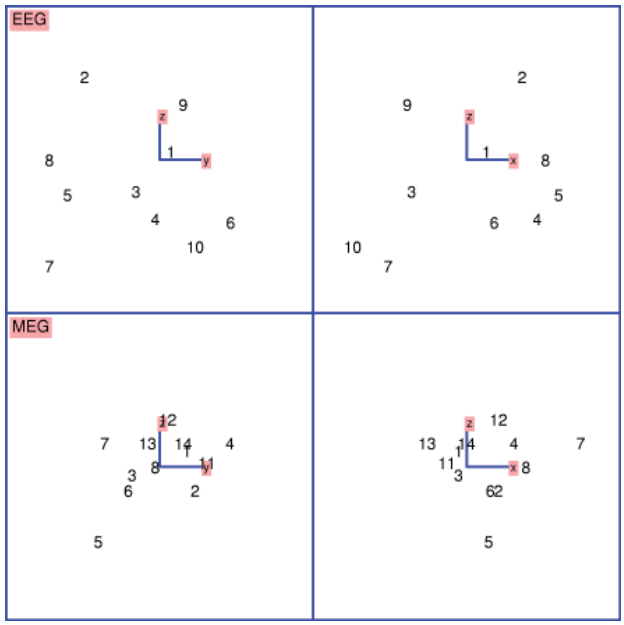


Figure 3 Scatter of 3D distances of the EEG and MEG sources to the closest tuber. Each number indicates a patient.



sources were statistically significantly closer to the tubers (*Table 2*) (t- test; p=0.007). *Figure 3* shows a scatter of 3D distances of the EEG and MEG sources to the closest tuber.

The EEG source was closer to the tuber in only one patient. The mean distance between source and tuber was 13.8 mm (range 6.7- 29.3 mm) for MEG and 24.6 mm (range 5.5- 39.3 mm) for EEG. In patients 4 and 6 with only one source of interictal epileptiform activity on both HR EEG and MEG recordings, the closest tuber to one modality differed from the other modality. The mean distance between tuber and source was still smaller for MEG (21.3 mm) than for EEG (26.4 mm). Interestingly, in patients with multiple localisations of interictal epileptiform activity there was a good agreement between EEG and MEG sources with respect to which tuber was closest. In agreement with a previous study[22] the identified epileptogenic tuber was most commonly (in nine patients) located in the temporal region. In addition, tubers presumed to be epileptogenic were found parietally (3 patients), occipitally (1 patient) and frontally (1 patient). Epileptogenic tubers were located in the right hemisphere in 10 patients and in the left hemisphere in four patients.

Electrocorticography

Three patients underwent epilepsy surgery (patients 1, 6 and 13). In patient 1 the ECoG showed epileptiform activity in the region posterior to the resected tuber. This was in agreement with MEG findings. In patient 6 ECoG recording showed epileptiform activity in the frontopolar and basal frontal region. MEG spikes were found in the basal frontal region. In patient 13 ECoG recorded epileptiform activity in the hippocampus and over the medial temporal gyrus, the latter in correspondence with MEG recording. Peri- or postsurgical complications were not seen. Patient 1 and 13 are seizure free (follow up 3.5 and 5 years). The seizure frequency in patient 6 improved by < 50%. Epilepsy surgery was considered in two other patients but postponed because of low seizure frequency.

Discussion

We found that epileptiform activity detected with MEG is closer to presumed epileptogenic tubers than epileptiform activity detected with HR EEG. The value of MEG in the pre-operative work-up of candidates for epilepsy surgery has been acknowledged worldwide,[23,24] but experience with MEG in TSC patients has been limited. Spike detection with MEG may be difficult and is subjective.[19] For this reason, only sources with at least moderate interobserver agreement were analysed. Interobserver agreement tended to be worse when only a few spikes were detected.

MEG detected a single localisation of epileptiform activity more often than EEG did. However, we cannot exclude the possibility that MEG was unable to detect radial sources whereas EEG did. In most of the patients the analysed traces were not recorded at the same day. Only two patients had significantly more spikes in the MEG recording than in the EEG recording. Although interictal EEG recordings (sometimes HR EEG) are used routinely in the pre-operative work-up of patients, our results show that spike detection is better with MEG recordings.

The characteristics of TSC lesions, e.g. multiple tubers, make it difficult to assess the association between the functional and these structural abnormalities. Tubers are often not far apart and it is not surprising that EEG and MEG sources were not associated with the same tuber in two patients. We found that the distance from the MEG source to the EEG-associated tuber was less than the distance from the EEG source to the MEG-associated tuber. The difference between EEG and MEG recordings may not be as marked as one would expect from data in the literature. However, studies comparing EEG and MEG data often involved standard EEG recording, whereas HR EEG, as used in our study, probably allows more accurate source localisation. This would mean that a better agreement may be found between HR EEG and MEG data.

It could be argued that the tuber closest to the epileptogenic source is not necessarily the epileptogenic tuber. Obviously immediate electrocorticography (ECoG) during epilepsy surgery

or invasive subdural recording is the gold standard. MEG and video-EEG results have been proven to be equivalent in most patients considered for epilepsy surgery, with MEG providing additional information in a significant number of patients.[25] The localisation of interictal epileptiform activity has to be confirmed by ictal recordings before epilepsy surgery is performed. We are currently investigating the relation between interictal and ictal source localisation. If reproducible spikes are not detected during interictal EEG recording, or integration of the EEG source in the MRI does not identify a single tuber, simultaneous MEG recording is helpful to identify the tuber that should be resected. However, during and after resection of the presumed epileptogenic tuber, ECoG recordings should be performed for more accurate delineation of the irritative zone. Other techniques that can be used to identify the epileptogenic tuber include alpha [^{11}C] methyl-L-tryptophan (AMT) PET[26] and diffusion-weighted MRI.[27] Both techniques have proven to distinguish epileptogenic from non-epileptogenic tubers.
Three of the patients underwent epilepsy surgery. In these patients we found a good agreement between interictal HR EEG, interictal MEG, and ictal EEG recordings. Complete tuber resection was performed in two patients, who were seizure free post-operatively. In the third patient only partial resection of the tuber was possible due to its size and extension in the eloquent cortex. Unfortunately in this patient post-operative seizure frequency had not changed in a clinically relevant way.

In conclusion, our results show that the epileptogenic source identified on MEG recordings was closer to the presumed epileptogenic tuber than the source identified on HR EEG recordings from the same patients with TSC. In addition, interobserver agreement was better in MEG recordings than in HR EEG recordings. Especially in patients with numerous tubers in whom interictal and ictal EEG recordings are performed for source localisation, MEG source localisation can help to delineate the epileptogenic source and define its relation to the closest tubers. Hence, MEG is a useful technique and should be applied to TSC patients considered for epilepsy surgery.

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7

Diffusion-weighted MRI identifies the epileptogenic tuber in patients with tuberous sclerosis

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Abstract

In this study we tested whether diffusion-weighted MRI (DWI) enables differentiation between epileptogenic tubers and inert ones in four TSC patients with one localisation of interictal epileptiform activity. Fluid-attenuated inversion recovery MRI and DWI were performed. Apparent diffusion coefficient (ADC) maps were calculated in the identified epileptogenic tubers and compared with those in non-epileptogenic tubers and regions of normal appearing cortex.
We found a significant increase in the ADC of the epileptogenic tubers. Furthermore, the ADC of the non-epileptogenic tubers was significantly higher than the ADC of regions of normal appearing cortex.
The increase of ADC of epileptogenic tubers may be explained by an even higher loss of neurons or by oedema, caused by the seizures themselves. DWI may be of additional diagnostic value in the identification of epileptogenic tubers in patients with TSC and drug-resistant epilepsy.

Introduction

In patients with tuberous sclerosis complex (TSC) cortical tubers may give rise to epilepsy, mental retardation and focal neurological deficits. Epilepsy occurs in 80 to 90% of TSC patients and is refractory to medical treatment in about half of the patients. In these patients surgical treatment should be considered. However, as the success of surgery depends on clear identification of the epileptogenic zone and most TSC patients have multiple – potentially epileptogenic – tubers, this option is often insufficiently envisaged. Studies on the outcome of surgery in TSC have shown variable results.[1-5]

MRI with Fluid Attenuated Inversion Recovery (FLAIR MRI) techniques have been proven successful in the detection of even small tubers,[6] but do not differentiate epileptogenic lesions from 'silent' ones. In patients with localised epileptiform activity, simultaneous high-resolution (HR) EEG and magneto-encephalography (MEG) in combination with MRI has been applied to identify a primary epileptogenic zone matching with a single tuber.[7] Disadvantages of MEG, however, are the required cooperation of the patient and its restricted availability.

In patients with partial epilepsy, diffusion-weighted imaging (DWI) techniques have been employed to identify focal abnormalities when conventional MRI was normal. In some of these patients, areas of increased diffusivity concurred with the focus of epileptiform EEG abnormality.[8] So far, only very limited experience has been obtained with DWI in patients with TSC.[9]

In this study we correlated quantified diffusion trace MR images with FLAIR MR images and data obtained from simultaneous HR EEG and MEG recordings to test whether DWI enables differentiation between epileptogenic tubers and inert ones.

Patients and Methods

Patients

We examined four patients who fulfilled the revised TSC diagnostic criteria.[10] In two patients the clinical diagnosis was confirmed with detection of a nonsense mutation in the *TSC2* gene. All these four patients had active epilepsy. Clear unifocal interictal spikes were identified with MEG, using 151 axial gradiometers arranged as a helmet (Omega 151, CTF system Inc.), and simultaneous 64 channel EEG (sampling rate of 625 Hz). Co-registration of MEG, HR EEG and MRI was obtained by identifying common marker positions. Three interictal recordings of ten minutes were performed in each patient. An average of 20 spikes per recording was found. Volume conductor models were constructed with CURRYV3 software (Neuroscan lab, Inc. Sterling, Virginia, USA). A Multiple Signal Classification (MUSIC) analysis for the localisation of the dipole source of interictal spikes was performed.[11] Data of simultaneous EEG

and MEG sources were superimposed on FLAIR images, to enable the identification of the epileptogenic tuber.

MRI (FLAIR and DWI) scanning protocol and image analysis

MR investigations were repeated on a 1.5-T scanner (Philips Gyroscan NT-Intera, Best, The Netherlands) after informed consent had been given. None of the four patients had a seizure on the day the MRI was performed. FLAIR images were performed with a repetition time / echo time / inversion time of 6000 / 100 / 2000 ms, a section thickness of 4 mm, no intersection gap, 230 x 230 mm field of view, 2 averages, 256 x 256 matrix size and a scan reduction of 85% (85% of the data points acquired). Corresponding DWI scans were performed with a fat suppressed (Spectral Presaturation with Inversion Recovery), multishot, spin echo/echo planar imaging sequence with a repetition time / echo time of 5393 / 87 ms, a section thickness of 4 mm, no intersection gap, 230 x 230 mm field of view, 1 average, 256 x 256 matrix, a scan reduction of 75% and 8 b-values: 0, 240, 480, 720, 960, 1200, 1400 and 1680 s/mm^2. Apparent diffusion coefficient (ADC) maps of the trace of the diffusion tensor were calculated on the basis of the DWIs that were acquired at each b-value, over the three principal axes (trace ADC = [X + Y + Z]/3), thus eliminating diffusion anisotropy effects. On the FLAIR images, tubers were segmented with a previously published protocol.[12] These FLAIR segmentations were registrated manually on the matching slices of the trace DWI and ADC maps. ADC measurements of the epileptogenic tuber, the non-epileptogenic tubers, and the normal appearing cortex, as assessed on FLAIR MRI, were performed. Differences in ADC were calculated and compared with analysis of variances (ANOVA) and Scheffe post hoc tests. ADC values are expressed as mean, standard deviation, and range.

Results

The patient characteristics are given in the *Table*. Seizure semiology corresponded well with the zone of interictal epileptiform activity, e.g. spikes. After integration of HR EEG and MEG with

Table Patient characteristics

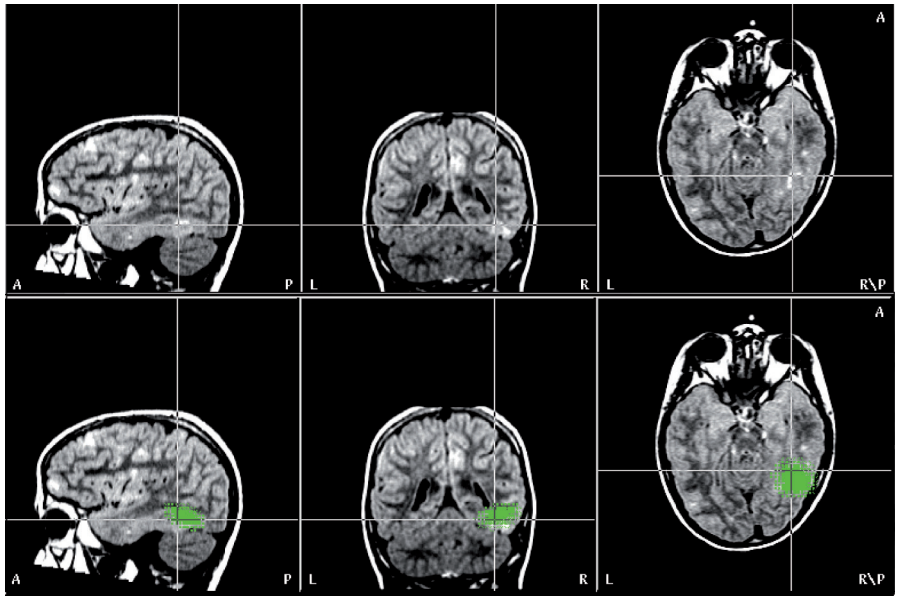
Patient	age (years)	age at onset (years)	type of seizures	IQ	source	number/volume (cm^3) of tubers
1	22	0.3	CPS	67	left central	28/ 22.4
2	24	0.4	CPS	79	right temporo-occipital	18/ 9.4
3	22	0.9	SPS/ sGTCS	78	right frontal	26/ 12.2
4	55	0.1	SPS/ sGTCS	<48	right central	13/ 6.3

IQ= intelligence quotient, y= years CPS= complex partial seizures, SPS= simple partial seizures, GTCS= generalised tonic clonic seizures, L= left, R= right

FLAIR MRI, a single epileptogenic tuber was identified in each of the four patients (example *Figure 1*).[7] The FLAIR images, DWIs and ADC maps showed multiple tubers (*Figure 2* and *3*). On the trace DWIs and ADC maps, tubers were characterised by decreased and increased signal intensity respectively, as compared with the surrounding normal appearing white and gray matter. Although the delineation of tubers on DWIs and ADC maps was in close agreement with that on FLAIR images, small tubers (diameter less than 3 mm.) could not be detected with DWIs.

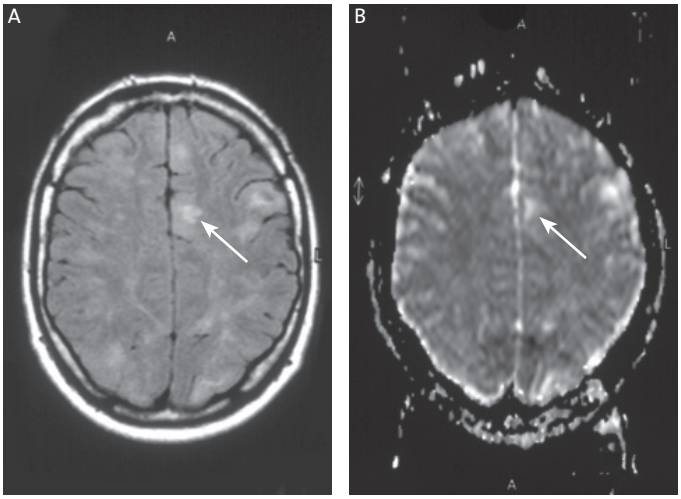
The trace ADC of 18 non-epileptogenic tubers in these patients was statistically significantly higher (mean 926 mm^2/s, SD 69.4, range 828 - 1037 mm^2/s) than the trace ADC of 16 regions of normal appearing cortex (mean 784 mm^2/s, SD 61.7, range 689 - 897 mm^2/s) (p value < 0.001). The trace ADC of the four epileptogenic tubers was higher (mean 1099 mm^2/s, SD 35, range 1049 - 1141 mm^2/s) than that of the 18 non-epileptogenic tubers (p value <0.001) (*Figure 4*). Furthermore, using each patient as its own control, the trace ADC of the epileptogenic tuber was outside the range of the ADC of all other non-epileptogenic tubers with approximately the same size in the same patient. The higher ADC measurement in epileptogenic tubers, as compared with non- epileptogenic tubers, was not always evident on visual inspection.

Figure 1 Example of co-registration of EEG, MEG and FLAIR MRI in a patient with TSC



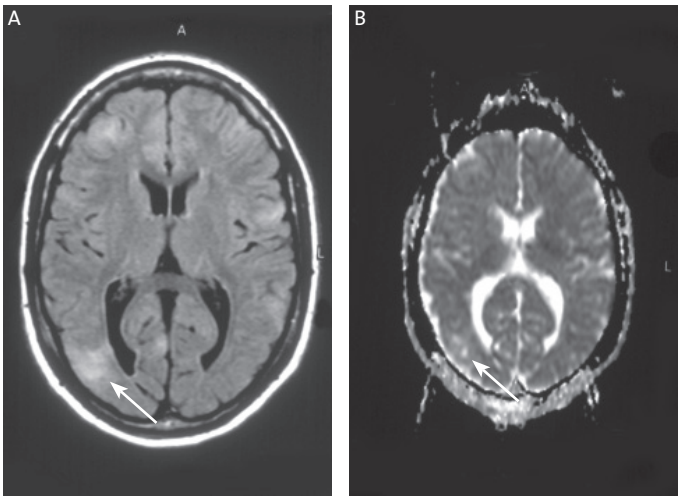
Upper row: FLAIR MRI, the crosshair indicates a tuber
Lower row: the results of combined spatial temporal dipole analysis of averaged HR EEG and MEG spikes superimposed on FLAIR MRI, indicating the epileptogenic tuber. R= right and L= left

Figure 2



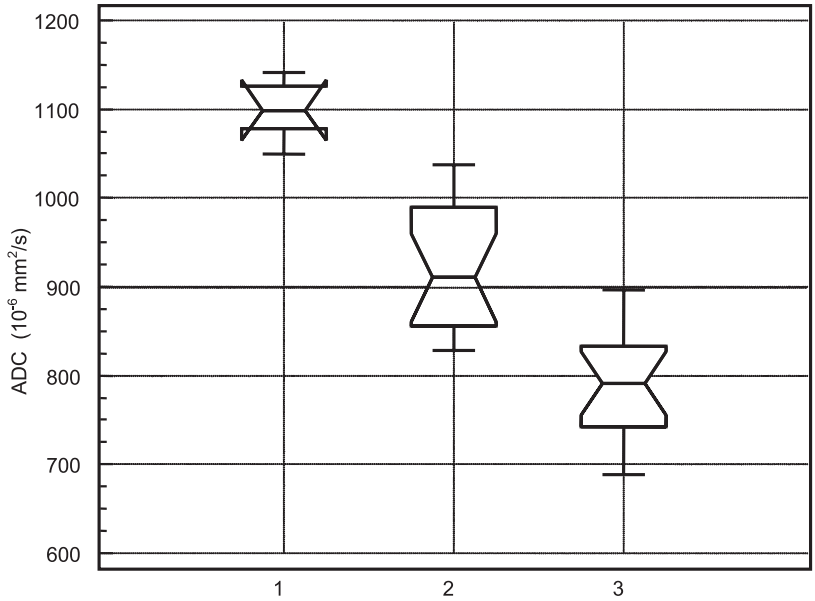
FLAIR image (A) and corresponding ADC map (B) of patient 1 showing multiple tubers as hyperintense regions. The epileptogenic tuber was identified in the left central, parasaggital region (arrow).

Figure 3



FLAIR image (A) and corresponding ADC map (B) of patient 2 showing multiple tubers as hyperintense regions. The epileptogenic tuber was identified in the right temporo-occipital region (arrow).

Figure 4 Box plot of the mean ADC values in four patients with TSC



1. epileptogenic tubers, 2. non-epileptogenic tubers, 3. normal appearing cortex.

Discussion

The most important finding of this study is that DWI may be of additional diagnostic value in the detection of epileptogenic tubers in patients with TSC and epilepsy.

DWI is increasingly acknowledged to be a powerful noninvasive technique that enables both the evaluation of pathophysiological changes in experimental and clinical epilepsy, as well as the identification of epileptogenic zones in patients with partial seizures. Ictal DWI studies have shown a reduction of tissue water ADC, most likely as a result of excitotoxicity-related cell swelling and changes in the intracellular tortuosity, whereas interictal DWI has revealed increases in diffusivity.[13] Recent DWI studies have detected regional decreases in diffusion anisotropy, and increases in diffusivity, in patients with partial epilepsies that were cryptogenic, acquired, or associated with malformations of cortical development, reflecting microstructural and architectural tissue changes.[8,14]

In the present study, tubers were detected by means of DWI. Trace ADC values of tubers were significantly higher than those of the surrounding normal appearing cortex. Similar findings have been reported recently.[9] An increase in diffusivity of tissue water may reflect an increased extracellular space, the loss of structural organisation, and poor myelination.[15] This is in close agreement with the histopathological findings of cerebral lesions in TSC.

Tubers are characterised by a disruption of the normal cortical lamination and by abnormal cell differentiation.[16] The density of myelinated fibers and the number of normal neurons are reduced.[17] Tubers and other cortical malformations are generally detected with MRI (FLAIR images). However, conventional MR techniques do not distinguish epileptogenic lesions from non-epileptogenic lesions. Simultaneous HR EEG and MEG enables identification of the epileptogenic zone, but not all patients fulfill the criteria of MEG paradigms. In four of our patients with multiple tubers, in whom a single epileptogenic tuber was identified with a combination of MRI, HR EEG and MEG, a significant increase in ADC was found in the epileptogenic tubers as compared to the non-epileptogenic tubers. One may speculate that in the epileptogenic tubers the number of neurons is even less and myelination is poorer. Another explanation may be that the seizures themselves have caused more neuronal loss or an increase of the extracellular water compartment due to edema, contributing to the increase of ADC.

In conclusion, this is the first study that demonstrates the potential of DWI to detect epileptogenic tubers in patients with TSC. Identification of the epileptogenic tuber with the single and widely available technique of DWI offers many advantages as compared to identification with a combination of FLAIR MRI, HR EEG and MEG. If the present findings can be reproduced in a larger series of patients with TSC and intractable epilepsy, DWI may become an important tool for the identification of the epileptogenic tuber. This would greatly facilitate the selection of candidates for epilepsy surgery in this patient group.

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Part III

8

Epilepsy surgery in tuberous sclerosis: A systematic review

Floor Jansen, Alexander van Huffelen, Ale Algra, and Onno van Nieuwenhuizen

Abstract

A systematic review of the available literature has been undertaken to assess the overall outcome of epilepsy surgery in patients with tuberous sclerosis complex (TSC) and identify risk factors of seizure recurrence. Twenty five articles, describing post-operative seizure outcome and type of surgery in 177 TSC patients, were included in this study.

Seizure freedom was achieved in 101 patients (57%). Seizure frequency was improved by > 90% in 32 patients (18%). Moderate or severe intellectual disability (or IQ <70) (RR 1.8; 95% CI 1.2- 2.8) and the presence of tonic seizures (RR 1.7; 95 % CI 1.2- 2.4) were related to seizure recurrence.

This systematic review provides evidence that seizure control can be achieved in a large number of patients. This review supports consideration of resective epilepsy surgery in all TSC patients with drug-resistant seizures as a relation between multiple seizure types with early onset, multiple cortical tubers and multifocal epileptogenicity and poor outcome is not found.

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant multisystem disorder. A mutation in either the *TSC1* gene, with the locus on chromosome 9q34, or the *TSC2* gene located on chromosome 16p13 is detected in 90% of the TSC patients.[1,2] Hamartin and tuberin, the protein products of the genes, are suggested to play a role in neuronal differentiation.[3]

Cortical tubers are associated with various neurological symptoms including epilepsy, mental retardation, behavioural disturbances, and focal neurological deficits. Seizures are the most common neurological symptom with the highest incidence in the 4th and 5th month, e.g. in case of infantile spasms. An unfavourable course is found in patients with seizure onset in the first year of life, multiple seizure types, multifocal epileptiform activity and occurrence of new EEG foci.[4,5] Despite the use of all available antiepileptic drugs many patients with TSC have intractable seizures.[6] Current non-medical treatment includes the ketogenic diet, vagal nerve stimulation, and epilepsy surgery. The first report of successful epilepsy surgery in patients with TSC was published in 1964.[7] Subsequently, a number of reports have demonstrated that good seizure outcome after epilepsy surgery in TSC is achieved in approximately 60% of the medically intractable patients. If a single primary epileptogenic tuber can be identified in patients with TSC and seizures, who were previously considered medically intractable, epilepsy surgery may be appropriate. Novel non-invasive neurophysiological and neuroimaging modalities, such as high-resolution (HR) EEG, magneto-encephalography (MEG),[8-11] cerebral magnetic resonance imaging (MRI) including diffusion-weighted MRI (DWI),[12] and proton magnetic resonance spectroscopy (MRS),[13] single photon emission computed tomography (SPECT),[14] and positron emission tomography (PET) have been explored to identify the presumed epileptogenic tuber.[15,16]

Assessment of outcome of epilepsy surgery in TSC is essential to provide evidence of surgical effectiveness and may help to define the essential pre-surgical investigations. The current literature is limited by a variability of data collection methods and analyses.

We performed a systematic review of the published cases from observational studies regarding epilepsy surgery in patients with TSC. The main aim was to give an estimate of the chance of success of surgery. We further analysed the relation between clinical and neurosurgical data, and results of ancillary investigations and recurrent seizures, outlining the characteristics of the clinical condition.

Methods

Data sources

We performed a comprehensive literature search of Medline and Embase. We also searched bibliographies of reviews and book chapters and consulted experts about other studies. Searches were restricted to English articles published from 1960 until June 2006. The keywords used in the search were: "tuberous sclerosis", "epilepsy", "treatment" and "epilepsy surgery", in different combinations.

Study selection

From the literature search studies reporting on epilepsy surgery in TSC were selected by one author (FEJ). Studies were included if they at least reported 1) the diagnostic criteria for TSC,[17] excluding *formes frustes*, 2) quantitative seizure outcome for each case, and 3) a description of the type of surgery. We excluded duplicate publications, i.e. studies with overlapping patient populations from the same centre. The corresponding authors of each study were contacted via e-mail for additional information.

Strategy and data collection

A database was implemented for the identification and inclusion of the suitable articles. The data were extracted following a semi-structured form for each study. The following variables were considered:

1) Clinical characteristics: age at seizure onset, pre-surgical duration of epilepsy, type of seizures (seizure semiology was classified on the basis of the definitions proposed by the Commission on Classification and Terminology of the International League Against Epilepsy),[18] frequency of seizures, intellectual disability (assessed with formal neuropsychological testing or level of schooling), mutation analysis, age at surgery and type of surgery. Patients were categorised by type of surgery into resective (e.g. focal or cortical resection, lobectomy, multilobar resection, or hemispherectomy) and corpus callosotomy.

2) Results from ancillary exams for pre-surgical work-up: interictal EEG, ictal EEG, preoperative invasive monitoring, MEG, CT or MRI, ictal SPECT, and alpha [^{11}C] methyl-L-tryptophan (AMT) PET studies. The results were classified as focal, i.e. one interictal or ictal zone of epileptiform or otherwise abnormal activity, one dominant tuber, one region of abnormal tracer uptake, or multifocal, i.e. multiple interictal or ictal zones of epileptiform or otherwise abnormal activity, no predominant tuber, multiple regions of abnormal tracer uptake (as defined by the authors);

3) Histopathological findings (presence of giant cells and balloon cells);

4) Duration of follow-up and seizure outcome. The outcome of seizures was classified with

Engel's outcome classification[19] or author specific outcome classification: 1= seizure free, 2= infrequent non disabling seizures or seizure reduction by > 90%, 3= significant improvement > 50%, 4= not improved by > 50%.
The primary outcome was seizure freedom as defined by the authors in each study, i.e seizure free status for at least one year at the last reported follow-up. The above mentioned variables were related to seizure outcome in two ways: (1) to seizure freedom (no recurrent seizures versus recurrent seizures) and (2) to good seizure outcome (outcome classification 1 and 2) versus poor seizure outcome (outcome classification 3 and 4). Outcome classification 2 (seizure reduction >90%) may be considered successful in patients with TSC supporting the latter type of analysis.

Data analysis

Statistical significance was only calculated for group sizes exceeding 5. If available, raw data on prognostic factors were pooled into a 2x2 contingency table relating that variable toseizure outcome. We calculated the risk ratio (RR) and its 95% confidence interval (CI). For continuous variables (e.g. age at seizure onset, duration of epilepsy, and age at surgery) mean differences with corresponding 95% CI were calculated and compared between patients with seizure freedom and patients with recurrent seizures. We considered associations statistically significant for p values < 0.05.

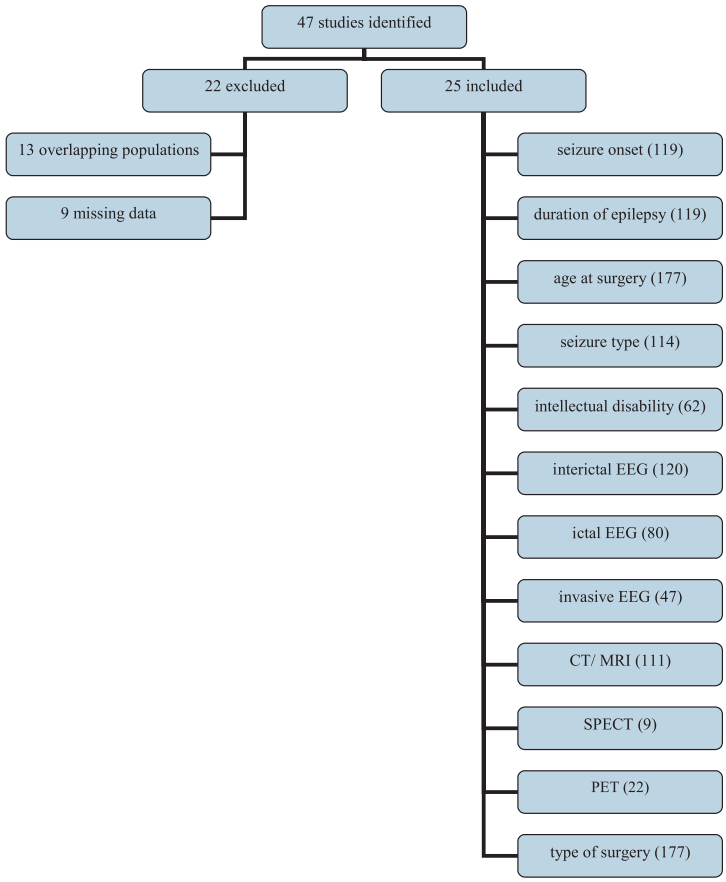
Results

Description of studies and clinical characteristics

The literature search yielded 47 original studies reporting on epilepsy surgery including at least one TSC patient (*Figure*). These 47 studies were all reviewed in full text. Thirteen studies were excluded because overlapping patient populations had been described. Nine additional studies were excluded because seizure outcome and type of epilepsy surgery were not assessed and additional information was not obtained. Thus, 25 studies (*Table 1*) met the prerequisites for eligibility.[15,16,20-42] From these 25 studies three patients had to be excluded because of loss to follow-up. Additional information of 9 studies was obtained from 7 corresponding authors.[15,20,27,29,31,33,37,38,40] Of 4 studies additional data was sought but no longer to be available.[21,25,30,42] Of the other 12 studies no or negative response to the request for additional information was obtained.[16,22-24,26,28,32,34-36,39,41]

The total sample included 177 TSC patients (in only 6 patients confirmation with mutation analysis was reported; two patients had a *TSC1* mutation and four a *TSC2* mutation), whose age at surgery ranged from 3 months to 54 years, although the majority of patients was

Figure Study selection and variables obtained



under 20 years of age, with a mean of 8.6 years (median 6 years). Mean age at seizure onset was 1.4 years (median 0.3 years; range 1 day to 11 years), and mean pre-surgical duration of seizures was 8.1 years (median; 4.8 years; range 3 months to 53.7 years), as assessed in 119 cases. Information on clinically assessed seizure types was available for 114 patients. Seizure type was partial (with and without altered consciousness) in 29 patients, generalised (including tonic clonic seizures, tonic seizures and infantile spasms) in 19 and a variety of seizure types was seen in 66 patients.

Information on intellectual disability (ID) (or IQ score) was available for 62 patients, of these 12 patients had a normal intellect (or IQ> 90), 26 had mild intellectual disability (or 70< IQ< 90), 15 had moderate intellectual disability (or 50< IQ < 70) and 9 severe intellectual disability (or IQ< 50).

Results of interictal EEG recordings were available for 120 patients; focal epileptiform activity

Table 1 Studies included in the analysis

Author, year	cases	prognostic factors studied	type of surgery	seizure free	follow-up (y)
Asano et al. 2000[15]	7	b,d,e,i	FR (3), L (4)	5	0.3- 2.8
Asano et al. 2005[20]	8	a,b,j	FR (3), L (5)	6	0.3- 3.8
Avellino et al. 1997[21]	4	a,b,c,d,g,j	L	2	0.7- 5.3
Baumgartner et al. 1997[22]	4	a,b,c,d,e,f,g,j	L	1	1- 3
Bebin et al. 1993[23]	9	b,c,d,g,j	FR	6	0.9- 6
Bittar et al. 2002[24]	1	a,b,d,e,g,h,j	FR	0	NR
Bye et al. 1989[25]	1	a,b,c,d,e,g,j	L	0	1
Guerreiro et al. 1998[26]	17	a,b,c,d,g,j	FR (11), CC (6)	7	0.1- 47
Kagawa et al. 2005[16]	17	a,d,e,f,g,i,j	FR (13), L (1), H (3)	12	0.4- 4.8
Karenfort et al. 2002[27]	8	a,b,c,d,e,f,g,j	FR (4), L (2), H (2)	3	0.5- 4.4
Koh et al. 2000[28]	11	b,d,e,f,g,h,j	FR	8	NR
Kossoff et al. 2003[29]	2	a,b,d,e,g,j	H	2	8- 10
Lachhwani et al. 2005[30]	17	a,d,e,g,j	L	11	0.5- 15
Leiphart et al. 2001[31]	14	a,b,j	L (8), ML (3), H (2), CC (1)	7	0-5
Mackay et al. 2003[32]	7	a,b,g,j	L	2	3.2- 7.8
O'Connor et al. 2003[33]	2	a,b,c,d,e,f,g,j	L	1	5- 20
Ohta et al. 2001[34]	1	a,b,c,d,g,j	FR	0	2
Perot et al. 1966[35]	7	a,b,c,d,g,j	FR (5), L (2)	3	0.5- 17
Romanelli et al. 2002[36]	2	a,b,c,d,e,f,g,h,j	FR(1), ML (1)	1	2.2- 2.6
Sinclair et al. 2003[37]	4	a,b,d,e,g,j	L	4	2- 8
Sinclair et al. 2005[38]	2	d,e,g,j	L	1	NR
Sugimoto et al. 1999[39]	1	a,b,c,e,g,h,j	FR	0	1.8
Vigliano et al. 2002[40]	4	a,b,c,d,e,g,j	FR	2	3.9- 9.5
Weiner et al. 2006[41]	23*	f,j (a,b,c,d,e,g,h in 2 pt)	L (11), ML (12)	15	0.5- 6.2
Wennberg et al. 1999[42]	4	j	L (3), FR (1)	2	7- 8

a= age at seizure onset and duration of epilepsy, b= seizure types, c= intellectual disability, d= interictal EEG findings, e= ictal EEG findings, f= invasive EEG findings, g= structural imaging (CT/ MRI) findings, h= ictal SPECT findings, i= AMT PET findings, j= age at surgery. FR= focal resection, L= lobectomy, ML= multilobar resection, H= hemispherectomy, CC= corpus callosotomy, NR= not reported, *two of the 25 patients, described in this paper, were published previously by Romanelli et al.[36]

was seen in 62 patients and multifocal or generalised epileptiform activity in 58. Results on ictal EEG recording were available for 80 patients; focal seizure onset was seen in 43 patients and seizure onset could not be restricted to one region in 37 patients. Invasive EEG recording was performed in 47 patients; focal epileptiform activity was seen in 24 and multifocal in 23 patients.

MEG recordings were performed in five patients; multiple interictal spike zones were found in one patient.
CT and/ or MRI findings were available for 111 patients, structural abnormalities were considered (predominantly) focal in 48 patients and multiple tubers (none appearing the dominant one) were found in 63 patients.
Ictal SPECT scans were performed in 9 patients, showing focal abnormalities in 7 and multifocal in 2 patients.
AMT PET scans were performed in 22 patients, showing one region of increased uptake in 10 and multiple regions in 12 patients.
Surgical specimens were available for 91 patients. The commonest diagnosis was tuber tissue (84 cases). Other findings included diffuse gliosis, hamartoma and focal cortical dysplasia.
Focal resections were carried out in 71 patients; 90 patients underwent a lobectomy (74), or multilobar resection (16). Hemispherectomy was performed in 9 patients and corpus callosotomy in 7 patients. The duration of follow-up was available for 162 patients ranging from 1 month to 47 years.

Surgical seizure outcome

Seizure freedom (classification 1) was achieved in 101 patients (57%) (mean follow up 3.7 years, range 0.1- 47 years). Seizure frequency was improved by > 90% in 32 patients (18%) (mean follow up 4.2 years, range 0.5- 20 years) and by > 50% in 27 patients (15%) (mean follow-up 3.5 years, range 0.5- 9 years). In 17 patients (10%) seizure frequency did not improve by > 50% (mean follow-up 2.4 years, range 0- 8 years). The duration of follow up did not differ between the seizure free patients and the patients with recurrent seizures (mean difference 0.09, 95% CI -1.4 to 1.6, data not shown in table)

Factors predicting prognosis

Table 2 shows the distribution of the analysed factors between patients with and patients without recurrent seizures as well as the statistical significance of these factors as analysed in univariable analysis. Factors related to recurrent seizures were: the presence of tonic seizures (RR 1.7; 95 % CI 1.2- 2.4), and moderate or severe intellectual disability (or IQ <70) (RR 1.8; 95% CI 1.2- 2.8). In addition, patients with multifocal abnormality on SPECT examination more often had recurrent seizures (RR 7.0; 95% CI 1.1- 43) and all patients undergoing corpus callosotomy had recurrent seizures (corpus callosotomy versus resective surgery RR 2.5; 95% CI 2.1- 3.0). Restricting the analysis to patients with follow–up of at least one year (133 patients) revealed similar results. Risk factors of seizure recurrence were tonic seizures (RR 2.3, 95% CI 1.1-2.7, data not shown in table) and moderate or severe intellectual disability (RR 2.0; 95% CI 1.2- 3.3). Restricting the analysis to 170 patients who underwent resective epilepsy

surgery only (as the main goal of corpus callosotomy may not be seizure freedom) did not reveal different results. Relationships between age at seizure onset, duration of epilepsy, age at time of surgery, other seizure types, interictal EEG, ictal EEG, invasive EEG recording, MRI and PET findings and seizure outcome after surgery were not found. The only factor related to seizure reduction > 90% (outcome classification 1 and 2; data not shown in *Table 2*) was moderate or severe intellectual disability (RR 4.8, 95% CI 1.4- 15.8). Again, recurrent seizures were more frequent after corpus callosotomy (RR 2.4, 95% CI 1.2- 4.9), although reduction of seizure frequency of >90% was achieved in 3 of these 7 patients.
Multivariable analysis appeared not to be feasible, due to lack of combined data on variables that had a statistically significant relationship with outcome on univariable analysis.

Table 2 Risk factors of recurrent seizures (outcome classification 2, 3 and 4). Risk ratios or mean differences with corresponding 95 % CI of factors related to seizure recurrence in a univariable analysis

Risk factor	**means for patients with and without recurrent seizures**			
	recurrent seizures		**mean difference**	**95% CI**
	yes	**no**		
age at seizure onset (years)	1.0	1.7	0.7	-1.9- 1.5
duration of epilepsy (years)	8.9	7.4	-1.5	-4.9- 2.0
age at surgery (years)	9.1	8.2	-0.8	-3.6- 1.9
risk factor	**% of recurrent seizures for patients with and without characteristic**			
	characteristic present	**characteristic absent**	**RR**	**95 % CI**
GTCS	25/ 48 (52%)	29/ 66 (44%)	1.2	0.8- 1.7
IS	7/ 15 (47%)	47/ 99 (47%)	1.0	0.6- 1.8
TS	21/ 31 (68%)	33/ 83 (40%)	1.7	1.2- 2.4
CPS	29/ 67 (43%)	25/ 47 (53%)	0.8	0.6- 1.2
multiple seizure types	34/ 66 (52%)	33/ 86 (38%)	1.3	0.9- 1.9
mild ID (IQ< 90)	29/ 50 (58%)	5/ 12 (42%)	1.4	0.7- 2.8
moderate or severe ID (Q< 70)	18/ 24 (75%)	16/ 38 (42%)	1.8	1.2- 2.8
multifocal interictal EEG	29/ 58 (50%)	22/ 62 (35%)	1.4	0.9- 2.2
multiple ictal onset zones	12/ 37 (32%)	17/ 43 (40%)	0.8	0.5- 1.5
multifocal invasive recording	9/ 23 (39%)	7/ 24 (29%)	1.3	0.6- 3.0
multifocal CT/ MRI findings	32/ 63 (51%)	18/ 48 (38%)	1.4	0.9- 2.1
multifocal SPECT findings	2/2 (100%)	1/7 (14%)	7.0	1.1- 43.0
multifocal PET findings	1/ 12 (8%)	5/ 10 (50%)	0.2	0.0- 1.2
cc versus resective surgery	7/7 (100%)	70/ 170 (41%)	2.5	2.1- 3.0

RR= risk ratio, CI= confidence interval, GTCS= generalised tonic clonic seizures, IS= infantile spasms, TS= tonic seizures, CPS= complex partial seizures, ID= intellectual disability, cc= corpus callosotomy.

Discussion

This systematic review shows that epilepsy surgery was very successful in patients with drug-resistant epilepsy and tuberous sclerosis. Seizure freedom was achieved in 57%, and seizure frequency improved by > 90% in another 18% (together 75%) of patients. These seizure outcomes are lower than in patients with temporal lobe epilepsy surgery (66% seizure free), but higher than in patients with extra temporal lobe epilepsy surgery (27- 46% seizure free)[43]. Two risk factors of recurrent seizures were identified: tonic seizures and moderate or severe intellectual disability. In addition, the presence of multifocal SPECT abnormality, and a corpus callosotomy, in comparison to resective surgery, were related to recurrent seizures. Prudence may be called for the reliability of these factor as SPECT examination and corpus callosotomy were only performed in a very small number of patients. The aim of a callosotomy is often not complete seizure control, but prevention of spreading of epileptiform activity and subsequently prevention of generalised invalidating seizures. However, it is important to notice that there has not been any report on seizure freedom in TSC patients as a result of this procedure.

Many authors[15,21-23,25-28,30,35,36] have shown that epilepsy surgery in TSC can be performed safely and effectively as is underlined in this review. Seizure freedom has been related to localised and concordant EEG and MRI findings in one study of in 17 TSC patients.[30] This review is the first to show that co-existence of multiple seizure types with early onset, multiple cortical tubers and multifocal or generalised epileptogenicity is not necessarily associated with a poor seizure outcome.

This review has several limitations. The first major limitation is intrinsic on data pooling, prompting the use of oversimplification and forced dichotomisation of many of the putative prognostic factors. Second, additional data were only achieved in a minority of cases and important variables may have been underrepresented. In comparison to other reviews on epilepsy surgery the number of patients is small which indicates that epilepsy surgery has long been limited to a selection of TSC patients. In recent years epilepsy surgery in TSC has become more challenging and more aggressive strategies have been applied.[41] The increasing number of published studies on epilepsy surgery in TSC patients may lead to the identification of statistically significant relationships between, for example, multifocal EEG findings and recurrent seizures in the near future. These relationships are not found in here. Further, we cannot know the importance of factors other than those examined in this review. The final limitation is the variable length of follow-up across studies. However, results had not changed after restriction of the analysis to patients in whom a follow-up of at least one year was obtained.

Before epilepsy surgery can be performed assessment of functional localisation of the primary epileptogenic tuber is mandatory. Studies addressing the surplus value of MEG in patients

with TSC showed that epileptiform activity as recorded with MEG was more focal than recorded with video EEG[8] and closer to tubers than recorded with EEG.[10] MEG results could not be related to recurrent seizures in this review because MEG was only performed in five patients. The potential of DWI may be valuable in the differentiation between epileptogenic and inert cerebral tubers in patients with TSC. A higher appearent diffusion coefficient (ADC) has been identified in epileptogenic tubers as compared to non- epileptogenic tubers in patients with TSC.[12] Recent studies explored the surplus value of AMT PET in the differentiation between epileptogenic and inert tubers.[15,16] A significant relation between higher tuber AMT uptake and epileptiform activity in the same lobe was found interictally. The value of fluorodeoxyglucose (FDG) and flumazenil in identifying the epileptogenic zone is limited in patients with tuberous sclerosis and was therefore not analysed in this study. SPECT, with use of signal subtraction techniques, co- registered with MRI, can have an important role in defining the epileptogenic tuber as well.[14]

Cortical tubers are reported to have a non-static behaviour[44] and an age dependent tendency to become epileptogenic.[4] However, until now little evidence is found to support the epileptogenic potential of all tubers. Some studies found tubers to be electrically silent whereas others found frequent epileptiform activity associated with the tuber or the region surrounding the tuber.[15,28] More so, in some patients epileptiform activity could not be localised to tubers[45] and recent TSC animal models revealed spontaneous seizures in association with astrocyte specific disruption but in the absence of tubers.[46] In any case, even in the presence of multiple tubers epileptogenic activity can often be localised to one or two regions, and electro-encephalographic patterns can be consistent over time.[22,47]

Seizure control can be achieved in carefully selected patients by resection of the epileptogenic tuber(s) and associated epileptogenic region(s). Although tonic seizures and moderate or severe intellectual disability were found to be associated with recurrent seizures these factors were also present in seizure free patients. In our opinion these results suggest that epilepsy surgery should be considered in all TSC patients with drug resistent epilepsy.

In addition to seizure control, deterioration of cognitive functioning may be prevented,[48] behaviour and socialisation improved, ultimately resulting in a better quality of life for both the patient and their caregivers. The necessity of novel functional localisation techniques in identifying the primary epileptogenic tuber needs to be explored further in the near future. Finally, molecular analysis of resected epileptogenic tubers may give insight in the underlying mechanism of epileptogenesis in tuberous sclerosis and ultimately enhance and broaden our understanding of the pathophysiology of epilepsy in general.

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9

Epilepsy surgery in tuberous sclerosis: The Dutch experience

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On behalf of the Dutch Collaborative Epilepsy Surgery Programme

Abstract

We report the pre-surgical identification of epileptogenic tubers and post-surgical outcome of 25 patients with tuberous sclerosis in The Netherlands. The pre-surgical evaluation of the Dutch Comprehensive Epilepsy Surgery Programme comprised a detailed seizure history, interictal and ictal video EEG registrations, 3D FLAIR MRI scans, neuropsychological testing and if possible magneto-encephalography.

Epilepsy surgery was performed in six patients. At follow-up, four patients were seizure free and seizure frequencey had not changed in two patients. Improved development and behaviour was perceived by the parents of two patients. Epilepsy surgery was not performed in 19 patients because seizures were not captured, ictal onset zones could not be localised or were multiple, interictal EEG, video EEG and MEG results were not concordant, or seizure burden had diminished during consensus decision. A higher cognition index was found in the surgical patients compared with the non-surgical patients.

Epilepsy surgery can be performed safely and successfully in patients with tuberous sclerosis in whom semiology, interictal EEG, ictal EEG, MEG and the location of tubers are concordant. In other cases the risk of surgery should be weighed against the chance of seizure relief and, in case of children, subsequent impact on neurodevelopment.

Introduction

Cortical tubers, the hallmark of tuberous sclerosis complex (TSC) are associated with a variety of neurological symptoms including epilepsy and mental retardation. Epilepsy is often the presenting symptom and seizures occur in the majority of patients.[1] The manifestation of epilepsy is age-related with the highest incidence in the first year of life. Ten to thirty percent of children with infantile spasms (IS) are diagnosed with TSC. Partial seizures are the main seizure type in older patients with TSC, but these can also occur in children with TSC-related IS. Only few studies have reported long-term follow-up in TSC.[2-5] Although a good short-term outcome has been described, relapses are frequent and long-term outcome is unfavourable with respect to the severity of epilepsy and mental status.[6]

Adequate treatment of the seizures is mandatory. Despite ongoing development of new anti-epileptic drugs, epilepsy in TSC is often medically intractable.[7] Other treatment options are the ketogenic diet, vagal nerve stimulation and epilepsy surgery.

Successful epilepsy surgery in patients with TSC was published for the first time in 1964.[8] Subsequently, many authors have reported good seizure outcome after epilepsy surgery in selected series of patients with TSC.[9-20] We performed a systematic review of the published cases from observational studies of epilepsy surgery in patients with TSC. Studies were included if they at least reported quantitative seizure outcome and description of the type of surgery. Seizure freedom has been achieved in 101 of the 177 patients (57%) and seizure frequency has been reduced by >90% in another 32 patients (18%) (total of good seizure outcome in 75% of patients) (*chapter eight*).[21] Source localisation and advanced neuro-imaging techniques have improved the demarcation of the epileptogenic zone and its relation to tubers enabling epilepsy surgery in an increasing number of patients with TSC.

In this study we report on pre-operative identification of the epileptogenic tuber in patients with TSC and drug-resistant epilepsy, on surgical removal of the presumed epileptogenic tuber and on outcome of this treatment in terms of both seizure frequency and cognitive function.

Methods

Between 1999 and 2006, 25 patients with the diagnosis of TSC,[22] confirmed by mutation analyses in 21 patients (10 *TSC1* and 11 *TSC2*), were examined at the outpatient departments of Paediatric Neurology (Sylvia Tòth Centre) and Neurology, UMC Utrecht, The Netherlands, and referred to the Dutch Comprehensive Epilepsy Surgery Programme.Pre-operative evaluation included: detailed (seizure) history and physical examination, cerebral MR images, high-resolution (HR) EEG, seizure recording with long-term video EEG, and a comprehensive neuropsychological assessment. Additionally, magneto-encephalography (MEG) recording

was performed if cooperation was expected. When appropriate, a Wada test, for evaluation of language and memory networks, was performed at second stage.

MR images were processed on a Philips Gyroscan ACS-NT 1.5 T whole body system (Philips Medical Systems, Best, The Netherlands). All patients underwent a transaxial or coronal Fluid Attenuated Inversion Recovery (FLAIR) and a transaxial T1 scan. Slice thickness was 1.5 mm. without interslice gap. Scan parameters were: repetition time (TR) = 11000ms, echo time (TE) = 125 ms, inversion time (TI) = 2600 ms. Tubers were automatically segmented with use of a K-Nearest Neighbour segmentation technique.[23,24] Both the number of tubers and the proportion of brain volume occupied by tubers (tuber/brain proportion; TBP) was assessed.

HR EEG recording (85 channel; BioSemi Mark-6, Brainstar system 4.0) was performed at a sampling rate of 1024 Hz, using a cap containing Sn electrodes (ElectroCap Inc.). The electrodes of the HR EEGs were positioned according to the 10% system accepted by the American EEG society. Electrode positions were registered with a magnetic tracking device (Polhemus, Colchester, VT, USA). In addition, three anatomical marker points (e.g. nasal and pre-auricular) and head-shape were measured, which allowed matching with MRI and MEG markers.

Video EEG was planned in all patients with 21-channel recordings and electrode positions according to the 10-20 system. Registration of at least two seizures from each seizure type was required for continuation of the pre-surgical programme.

Neuropsychological tests were selected from a fixed battery according to the widely varying age and abilities of the patients. Every patient underwent at least a test of intelligence or of mental development.[25] The other tests covered major domains of cognition, i.e. intelligence, vocabulary, memory and learning, graphical construction, executive functioning, attention, speed and fine motor functions. Administration and scoring were according to the test manuals. The results (quotient scores, centiles or standard scores) were converted to a general scale with the values 1 (extremely below average), 2 (below average), 3 (average) and 4 (above average). In case of cut-off scores, scale values 1 and 3 were used. We assigned a 'cognition index' to each patient by adding the scale values and dividing the sum by the number of tests (maximum of 10). The cognition index could vary between 1.00 and 4.00.

MEG, using 151 axial gradiometers arranged as a helmet (Omega 151, CTF system Inc., Port Coquitlam, British Columbia, Canada), was recorded at a sampling rate of 625 Hz, inside a magnetically shielded chamber (Vacuumschmelze GmbH, Hanau, Germany). Head position with respect to the helmet and, after each recording session, electrode positions and head-shape were recorded with four magnetic localising coils. The signals were (software) bandpass filtered between 0.7 and 70 Hz.

When performing the *Wada test*, effectiveness of the amobarbital injection was EEG-controlled. Language processing was screened by asking the patient to name five visually presented everyday objects such as a spoon and a toothbrush, to describe the 'Cookie theft' picture[27] and to execute four items from the 'Token test'.[28] Results were qualitatively judged. With respect

to memory screening, the patient was asked to look at a picture of an everyday subject, a photograph of a person, a playing card, a card with the name of a country, and a card with a Dutch saying for recall 15 minutes later. A passing score of 60% recall was required.

Results of the above-mentioned investigations were evaluated in the Dutch Collaborative Epilepsy Surgery Programme. Patients in whom seizure semiology, interictal EEG recording, localisation of the ictal onset zone, and localisation of the target tuber were concordant were considered eligible for epilepsy surgery. Image-guided surgery was performed with Stealth Station (Medtronic). With the results of the video EEG recording and, if available, the MEG results, the target tuber was identified. This epileptogenic tuber was demarcated on T2-weighted images. Electrocorticography (ECoG) tailored the resection.
Outcome was evaluated by taking the history and with neurological examination at 3, 6 and 12 months and at the end of follow-up (most recent visit to outpatient clinic). A post-operative MRI was performed at 3 months and neuropsychological examinations followed at 6, 12 and 24 months. Post-operative seizure outcome was scored on basis of the Engel classification.[29]

Results

Table 1 summarises demographic and pre-surgical illness variables of the 25 TSC patients.
Seizure semiology: Median age at seizure onset was 0.6 years, range 0.1 to 14 years. At onset 14 patients had infantile spasms (IS), six had complex partial seizures (CPS), four had secondary generalised tonic-clonic seizures (sGTCS), and one had tonic seizures (TS). At time of examination 19 patients had CPS (the sole seizure type in 12), seven had TS (the sole type in two), six had sGTCS (the sole type in two), two had myoclonic seizures (MS) and one patient had atonic seizures. Seizure frequency was daily in 13 patients, weekly in 9 patients and less frequent in three patients.
MRI scans: Multiple tubers were found in all patients. The median number of tubers was 27 (range 8- 58), the median TBP was 1.2% (range 0.2% to 5.1%) as assessed in 22 patients.
Interictal EEG: In one patient interictal epileptiform activity was absent. The interictal epileptiform activity could be localised to one region in seven patients and was multifocal in the other 17, involving both hemispheres in 13 patients.
Video EEG recording: One patient feared the surgical procedure and discontinued the presurgical programme before ictal EEG recording. In 9 patients one region of seizure origin was found. Seizures were not captured in five patients. Multiple regions of seizure onset were found in two patients. Seizure origin could not be localised in eight patients.
Cognition index was assessed in 24 patients. The cognition index was above average in only one patient, average in three, below average in six and extremely below average in 13 patients.

Table 1 Presurgical evaluation of 25 patients with tuberous sclerosis and drug-resistant epilepsy

Patient/ age	age at onset (years)	seizure type at onset	seizure type at exam	seizure frequency	CI	interictal EEG	ictal EEG	Tuber no/ TBP (%)	MEG
1/ 19	0.6	IS	sGTCS	weekly	2.3	R posterior temporal, L frontotemporal	L frontotemporal	18/ 0.34	R parieto-temporal
2/ 10	1.2	TS	CPS, TS	daily	1.0	R parietal, R temporal, L temporal	no seizures	20/ 0.18	
3/ 10	0.9	sGTCS	CPS	weekly	2.0	R hemisphere, L posterior temporal	multiple onset zones	20/ 1.0	few spikes
4/ 27	9	sGTCS	CPS	weekly	3.4	L frontotemporal	L mesiotemporal	53/ 1.0	few spikes
5/ 4	0.1	CPS, TS	TS	daily	1.0	R frontocentral, L frontocentral, L temporal	L central	29/ 2.52	
6/ 24	0.6	IS	sGTCS, CPS	daily	2.0	L hemisphere	L central, R frontopolar	24/ 1.49	
7/ 17	5	CPS	CPS	weekly	3.8	R posterior temporal, R and L ant. Temporal	R temporal	19/ 0.34	R temporal
8/ 3	0.2	IS	TS, MS, CPS	daily	1.0	multifocal, R frontotemporal	R frontotemporal	27/ 1.69	
9/ 22	0.8	IS	sGTCS	monthly	1.4	L temporal, R frontoparietal	no seizures	35/ 1.24	few spikes
10/ 2.3	0.5	IS	CPS	daily	1.0	L posterior temporal, L and R parietal	no seizures	29/ 2.69	
11/ 30	0.4	IS	CPS	monthly	1.9	L centroparietal	not performed	58/ 1.68	few spikes
12/ 10	0.1	IS	TS, aTS, CPS, MS	daily	1.0	R hemisphere, L parietal	not localising		
13/ 5	0.4	IS	CPS	daily	1.0	L frontopolar/ temporal, R anterior temporal	not localising	32/ 1.48	
14/ 5	0.2	IS	CPS	daily	1.0	L parieto-occipital	L parietal	8/ 0.17	

Table 1 Continued

Patient/ age	age at onset (years)	seizure type at onset	seizure type at exam	seizure frequency	CI	interictal EEG	ictal EEG	Tuber no/ TBP (%)	MEG
15/ 3.5	0.5	CPS	CPS with myoclonias	daily	2.0	R parieto-occipital	R temporo-occipital	17/ 0.19	
16/ 9	0.3	IS	CPS	monthly	1.0	L parietal, L temporal	no seizures	34/ 5.14	
17/ 3	0.2	IS	TS	daily	1.0	R hemisphere	not localising	20/ 0.32	
18/ 23	12	sGTCS	CPS	daily	3.0	R frontopolar	R frontopolar		R frontal/ frontopolar
19/ 5	2.3	CPS	CPS	weekly		R posterior temporal	R posterior temporal	26/ 0.52	R posterior temporal
20/ 22	0.3	IS	CPS, sGTCS	daily	1.8	no epileptiform activity	not localising	24/ 1.49	
21/ 28	14	sGTCS	sGTCS, TS, CPS	weekly	1.0	L frontocentral	not localising		L frontal
22/ 36	0.9	CPS	CPS	weekly	2.4	R frontotemporal	R mesiotemporal	34/ 1.84	R fronto-temporal
23/ 34	0.8	IS	CPS, sGTCS	weekly	1.2	R and L temporal, parasaggital	multiple onset zones	30/ 1.08	L parietal
24/ 9	0.3	IS	CPS, MS	daily	1.0	R hemisphere, L temporal	L centroparietal, parasaggital	18/ 0.32	
25/ 3	1.5	CPS	CPS	weekly	3.0	R central, L temporal	no seizures	10/ 0.18	

CI= cognition index, MEG= magneto-encephalography, IS= infantile spasms, sGTCS= secondary generalised tonic clonic seizures, CPS= complex partial seizures, TS= tonic seizures, MS= myoclonic seizures, aTS= atonic seizures, R= right, L= left, ant.= anterior, TBP=tuber/brain proportion.

MEG recording was performed in 11 patients. In four patients too few spikes were identified and source localisation could not be obtained. Unifocal epileptiform activity was seen in six patients. Results were concordant with interictal and ictal EEG activity in five of these patients, identifying the target tuber.
A *Wada test* was performed bilaterally in patients 7 and 22, with the epileptogenic tuber in the right temporal lobe, because post-operative memory deficit was feared. Both showed language representation in the left hemisphere. With respect to memory, recall after amobarbital injection in the right carotid artery was 100% in patient 7 and 80% in patient 22, which rendered them suitable candidates for right temporal lobe surgery.

Epilepsy surgery was not performed in 19 patients (*Figure*), including the patient who discontinued the programme and the five patients in whom video EEG recording failed to capture seizures. In patients 1 and 23 results of interictal EEG, video EEG and MEG recording were not concordant. In five patients the ictal onset zone could not be localised (patients 12, 13, 17, 20 and 21). Video EEG recording revealed multiple ictal onset zones in patients 3, 6, and 24. Invasive recording was not expected to identify one epileptogenic tuber because eight of these patients had multiple seizure types clinically. In patients 4 and 5 surgery was postponed because seizure burden diminished after a change in medication. Deliberations have not yet been closed for patient 14. Six patients underwent epilepsy surgery (*Table 2*). Seizure semiology had been consistent over time in five of them, but had changed in patient 8. Further, interictal EEG, ictal EEG, and MEG recordings (performed in 4 of the surgical patients) and the location of one or a cluster of tubers were all concordant. Patients for whom surgery was considered an option differed from those for whom surgery was rejected in cognition index only (difference of means 0.8; 95 % confidence interval 0.03 to 1.6).

Figure Scheme showing consensus decision in 25 TSC patients evaluated for epilepsy surgery.

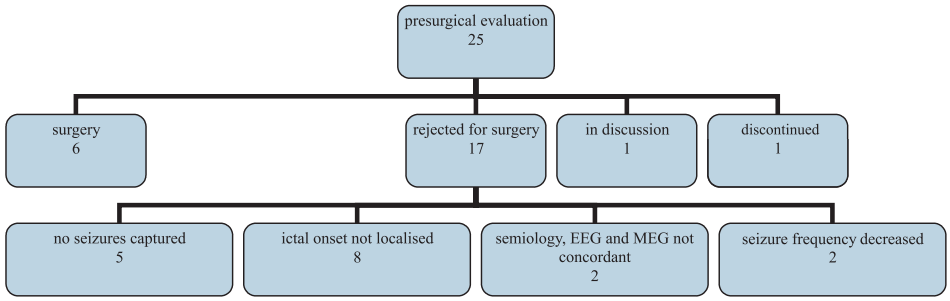

Table 2 Results of epilepsy surgery in six patients with tuberous sclerosis

Patient	age at surgery (years)	ECoG	surgery	PA	outcome	follow-up (months)
7	17	pre: continuous spiking pattern R, midtemporal neocortex and hippocampus, post: no epileptiform activity	R temporal lobectomy, R amygdalahippocampectomy	BC, DN	Engel 1a, CI: 3.8	38
8	3	pre: continuous spiking pattern R anterior temporal, burst and recruiting pattern posterior temporal, post: burst and continuous spikes posterior temporal	R temporal lobectomy, R amygdalahippocampectomy	BC	Engel 4, CI: 1.0	29
15	5	pre: continues spiking pattern over tuber region, post: few isolated spikes border resection	R parieto-occipital tuberectomy	GC, BC	Engel 1a, CI: 2.0	9
18	23	pre: continuous pattern R frontobasal, burst of spikes subfrontal, post: sporadic spikes R precentral	R frontopolar resection		Engel 4	
	24	pre: continuous spiking pattern border resection, extensive region of sporadic spikes R frontal, post: sporadic spikes R precentral	R frontal lobectomy	GC, BC	Engel 4, CI: 3.0	32
19	5	pre: recruiting pattern R parieto-temporal, post: sporadic spikes border resection	R posterior temporal resection	DN, BC	Engel 1a,CI: 2.0	71
22	36	pre: sporadic spikes R anterior temporal and hippocampus, post: sporadic spikes border resection	R temporal lobectomy, R amygdalahippocampectomy	BC, MTS	Engel 1b	10

ECoG= electrocorticography, PA= histopathology, R= right, pre= before resection, post= after resection, BC= ballooning cells, DN= dysmorphic neurons, GC= giant cells, MTS= mesial temporal sclerosis, CI= cognition index

Surgery (Table 2)

The identified epileptogenic tuber was located in the right hemisphere in all surgical candidates (in the temporal region in four, the frontal in one and the parieto-occipital region in one patient). All four patients with temporal lobe epilepsy had multiple tubers located in this region. ECoG tailored the resection, resulting in a temporal lobectomy in three (ECoG tailored the posterior resection border) a posterior temporal resection in one, a tuberectomy in the right parieto-occipital region in one and a right-sided frontal corticectomy in one patient. Intra-operative ECoG patterns consisted of a continuous spiking pattern, recruiting activity, bursts of spikes and a sporadic spiking pattern, according to the criteria published previously.[30] ECoG after resection showed bursts of spikes in the parieto-temporal region in patient 8 and sporadic spikes over the precentral gyrus in patient 18.

In all patients histopathological findings indicated a disorder of cortical lamination and showed the presence of ballooning cells in the surrounding white matter, consistent with the diagnosis of tuberous sclerosis. In patient 18 an asymptomatic giant cell astrocytoma was present as well and was partly resected.

At the end of follow-up (9 to 71 months) Engel classification 1 was achieved in four patients (67%), of whom three patients had Engel 1a. One of the seizure free patients had been taken off medication after two years of seizure freedom. The period of follow up is relatively short in two patients who are still on antiepileptic drugs. Seizure frequency improved in the first three months by > 50% in patient 8 but worsened again resulting in no improvement at the end of follow-up. A re-resection was considered, but postponed because no general agreement was reached which tuber should be considered epileptogenic.

Post-operative MRI showed incomplete tuber resection even after a re-resection for recurrent seizures in patient 18. After re-resection no improvement of seizure frequency was achieved.

The cognition index was assessed at 6, 12 and 24 months in patients 7, 8, 18 and 19, and at 6 months only in patients 15. Post-operative neuropsychological examination is planned in patient 22. The measured post-operative cognition index was stable over time in all patients, with the exception of patient 19. In this patient cognition index had improved slightly at 6 months (cognition index of 2), deteriorated at 12 months (cognition index of 1.3) and was back at the initial level post-operative level at 24 months (cognition index of 2). Parents of this patient perceived improvement of both development and behaviour post-operatively. Parents of patient 8 perceived improved development during the first three months (period of improved seizure control), but an arrest was perceived when seizure frequency was back at the pre-surgical level.

Post-operative complications consisted of quadrant hemianopia in patients 7, 19 and 22.

Discussion

In the last few decades a dozen series have reported favourable seizure outcome after epilepsy surgery in patients with TSC.[10-12,16-20,31-35] Seizure outcome in our series of patients (67% Engel classification 1) is comparable to that reported by others.

Epilepsy surgery is often not considered when bilateral epileptogenic zones are present or when a progressive epileptic encephalopathy is feared. Severe mental retardation is often regarded as another contraindication to epilepsy surgery because retardation is frequently associated with diffuse cerebral dysfunction. For those reasons, and because – often numerous – tubers are not always clearly demarcated and may be located in eloquent cortex, the option of epilepsy surgery has long been refused in the majority of patients with TSC and pharmacologically intractable epilepsy.

Insights have changed and several studies have proven that, even in the presence of multiple bilateral tubers, the epileptogenic zone and one or two tuber regions consistently coincide, especially when partial seizures are the clinical manifestation.[9,16,21,31] Our results add that even a number of 30 tubers and more is no impediment for good seizure outcome, if one or two tubers can be resected. Shifting foci are often feared, but little evidence is available to support the hypothesis of an epileptogenic potential of every tuber, rather, consistent electroencephalographic patterns are found over time.[36]

Experience taught that surgical outcome is best for patients in whom semiology, EEG and MRI findings concordantly point to a surgically accessible location.[1] The favourable outcome in the admittedly small number of suitable patients makes every attempt to identify the primary epileptogenic zone worthwhile. Factors related to post-surgical recurrence of seizures were: tonic seizures, the presence of multiple regions of abnormal HMPAO uptake on SPECT imaging, a corpus callosotomy (in comparison to resective surgery), and moderate or severe intellectual disability (*chapter eight*).[21] The last factor is in agreement with the results in our centre as one of the patients with poor seizure outcome had severe intellectual disability.

Despite the considerable variation in duration of follow-up, many studies have shown that other tubers do not become epileptogenic after removal of the main epileptogenic region and associated tuber. This is in agreement with the hypothesis that after resection of a primary focus the secondary foci may be eliminated or silenced.[37] The assumption of a poor outcome in case of multiple seizure types with early onset, multiple cortical tubers and multifocal or generalised epileptogenicity is not (yet) empirically supported, which calls for the consideration of epilepsy surgery in all TSC patients with drug-resistant epilepsy.

In complex cases additional information may be obtained with MEG, subtraction SPECT, alpha [^{11}C] methyl-L-tryptophan (AMT) PET, or invasive EEG recording. A single localisation of epileptiform activity was detected more often by MEG than EEG. In addition, MEG sources were closer to presumed epileptogenic tubers than EEG sources.[38] AMT PET has proven to

distinguish between epileptogenic and non-epileptogenic tubers.[9,16] Above all, ECoG is essential to tailor the resection of the epileptogenic zone after the target tuber is removed. The excision should be extended in non-eloquent cortex if a continuous pattern of spikes, recorded with ECoG, persists after initial tuberectomy. Although our series of patients is too small to draw firm conclusions, sporadic spike activity in other regions than the border of the resection, and incomplete resection of the tuberal region(s) appear to be related to recurrent seizures. When multiple epileptogenic tubers are identified in distant brain regions epilepsy surgery has proven to be very successful in selected cases with the proposed technique of multistage surgery.[20] The perspective of future seizure recurrence should be weighed against that of a (temporary) relief of severe epilepsy, e.g. during a critical period of neurodevelopment. Although parents of two children in our series evaluated development and behaviour to have ameliorated, psychometric follow up indicated neither catch up nor - for that matter - decay.

In conclusion, we suggest that surgical treatment should be considered in every TSC patient with drug-resistant epilepsy. Although seizure freedom is an unrealistic aim in patients with severe cognitive disability, the chance of substantial seizure reduction renders surgery a compelling option if seizure semiology, interictal HR EEG, ictal EEG, and MRI findings are concordant. In complex patients, without clear identification of the epileptogenic tuber, more efforts with multimodal non-invasive techniques (MEG, AMT PET, subtraction SPECT) are necessary and worthwhile.

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Part IV

10

Differential localisation of hamartin and tuberin and increased S6 phosphorylation in a tuber

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** these authors contributed equally to this study*

Abstract

In a tuberous sclerosis patient with a mutation in the *TSC1* tumour suppressor gene, no second hit mutation was found in a resected cortical tuber. Tuber giant cells showed predominantly nuclear hamartin, cytosolic tuberin, and hyperphosphorylation of S6. Differential accumulation of hamartin and tuberin in separate cellular compartments of giant cells may prevent formation of the hamartin-tuberin complex, resulting in increased S6 phosphorylation. These data provide an alternative mechanism for tuberogenesis.

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterised by the presence of hamartomas in many different tissues. Cortical tubers are pathognomonic for TSC patients and are often associated with neurological symptoms, including epilepsy. Histopathological analysis of cortical tubers reveals disruption of the normal cortical lamination, and the presence of dysmorphic neurons and giant cells.[1]

TSC is caused by a mutation in either the *TSC1* gene on chromosome 9q34, or the *TSC2* gene on chromosome 16p13.[2,3] Tuberin, the protein product of the *TSC2* gene and hamartin, the *TSC1* gene product, interact to form a cytoplasmic complex that antagonises activation of p70 S6 kinase (S6K),[4] thereby inhibiting phosphorylation of the ribosomal protein S6, down-regulating translation, and reducing cell growth. Cells lacking either tuberin or hamartin show constitutive phosphorylation of S6.[5]

Mutations to either the *TSC1* or *TSC2* gene can prevent formation of the hamartin-tuberin complex and it has been suggested that dysfunction of the complex causes the development of TSC-associated lesions.[6] The tuberin-hamartin interaction stabilises both proteins and maintains the complex in the cytoplasm.[7]

Second hit phenomena have been proposed to explain the wide phenotypic variation in TSC. This is supported by the finding of loss of heterozygosity (LOH) at the *TSC1* or *TSC2* loci in TSC-associated angiomyolipomas, giant cell astrocytomas, and rhabdomyomas.[8] However, evidence for second hit mutations in cortical tubers has been hard to demonstrate,[9] and it is possible that a different pathogenic mechanism is responsible for the development of these particular lesions.

In this study we characterise a cortical tuber from a TSC patient with a germ-line mutation in the *TSC1* gene.

Patient and Methods

Patient

A six- year old female was born after an unevenful pregnancy and delivery. Psychomotor development was normal. The first seizure occurred at the age of two years. Seizures were defined as complex partial seizures consisting of lowered consciousness, deviation of eyes and head to the right and a tonic spasm of the left arm. Despite administration of several antiepileptic drugs, seizures were intractable with a frequency of five times a week. Magnetic resonance imaging (MRI) indicated the presence of multiple cortical tubers. The patient met the revised clinical diagnostic criteria of tuberous sclerosis showing associated skin abnormalities.

The epileptogenic tuber, identified with high-resolution neurophysiology recordings and 3D MR images, was resected at the age of six years, with consent from the patient's parents. Five years post-operatively the patient is still seizure free.

DNA analysis

A de novo germ-line mutation, 2295C-T (R692X), was identified in DNA isolated from peripheral blood cells. To determine whether the normal copy of the *TSC1* gene was inactivated in the tuber allele-specific oligonucleotide (ASO) hybridisation and sequence analysis of the 21 *TSC1* coding exons were conducted.

Immunoblotting

Western blots were incubated with antibodies against tuberin and hamartin, as described previously.[6] All other antibodies were from Cell Signaling Technology (Beverly, MA) and were used according to the manufacturer's recommendations. The hamartin antiserum recognises epitopes distal to the premature stop introduced by the 2295C-T substitution.

Immunohistochemistry

Paraffin sections (7µm) were microwaved (7 minutes, 750 Watt), and blocked with 0.5% non-fat dry milk powder in TENG-T (10mM Tris-HCl, 5mM EDTA, 150mM NaCl, 0.25% gelatin, 0.05% Tween-20; pH 8.0). Hamartin and tuberin antisera (1:250), and phospho-specific and total S6 antibodies (1: 50) were applied overnight at room temperature. Second layer antiserum (1: 250) and rabbit peroxidase anti-peroxidase immunocomplex (1: 1500) (both from Nordic, Tilburg, NL) were applied in phosphate buffered saline, containing 2% normal human serum (Jackson Immunoresearch, West Grove, PA), for 1.5 hours each. Immunoreactivity (IR) was visualised using 3,3'-diaminobenzidine tetrahydrochloride as chromogen. Primary antisera controls did not reveal any staining.

Results

The tuber showed a loss of cortical lamination, prominent astrogliosis, and the presence of abnormal, giant cells in both grey and white matter regions (*Figure 1A*). These features are characteristic for TSC.

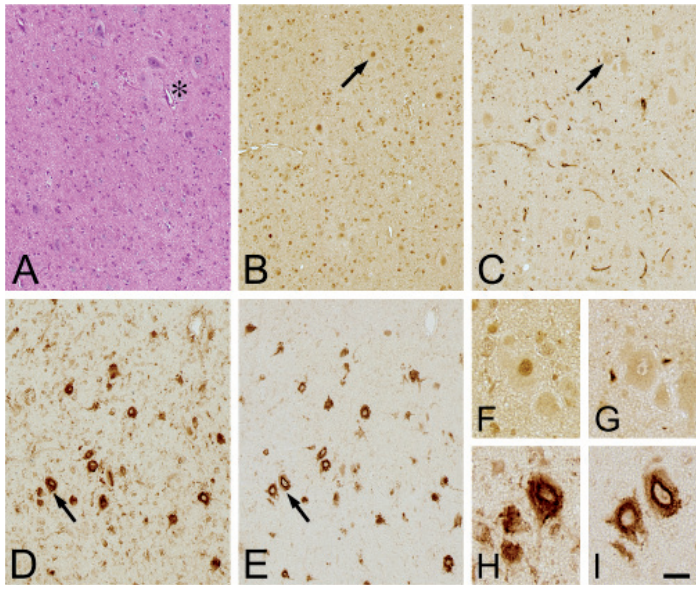
By ASO hybridisation, the normal and mutant *TSC1* alleles were detected at equal levels in tuber and leukocyte DNA (*Figure 2*). Sequence analysis of the tuber DNA identified the germ-line R692X (2295C-T) mutation and two additional heterozygous changes, M322T (1186T-C) and E445 (1556A-G), that are known *TSC1* polymorphisms (http://zk.bwh.harvard.edu/projects/tsc/). The heterozygosity across the *TSC1* gene in the tuber DNA provided additional evidence against

a large deletion or mitotic recombination event at the *TSC1* locus causing loss of the normal copy of the *TSC1* gene. In addition, there was no evidence for a non-germline inactivating *TSC1* mutation in the tuber.

Immunoblots did not reveal differences between the amount of either hamartin or tuberin in the tuber or adjacent normal cortex (*Figure 3*). However, there was an increase in the phosphorylation of S6 in the tuber tissue.

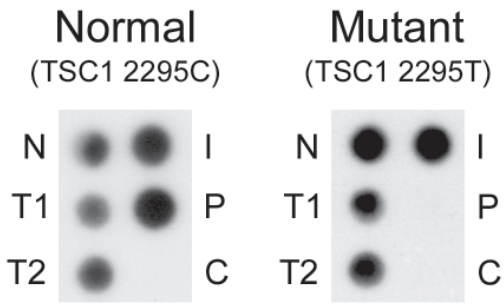
Consistent with the DNA and immunoblot results, most neural cells in whole tuber sections showed hamartin *Figure 1B*) as well as tuberin IR (*Figure 1C*). Giant cells showed a granular cytoplasmic staining for tuberin (*Figure 1G*), consistent with previous findings (M. Nellist unpublished observations). Surprisingly, hamartin IR was predominantly nuclear and most prominent in giant cells with a neuron-like appearance (*Figure 1F*). Nuclear staining for hamartin was also present in giant cells with astrocytic features and in (reactive) astrocytes. Strikingly, although all neural cells showed total S6 IR (*Figure 1D*, *H*), only the giant cells of the tuber showed IR for phosphorylated S6 (*Figure 1E*, *I*).

Figure 1



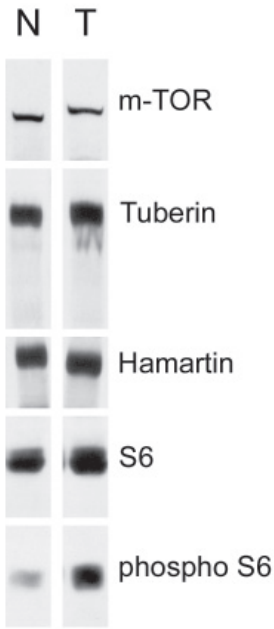
Histopathology (hematoxylin-eosin (H&E)) (A) and immunoreactivity (IR) for hamartin (B, F), tuberin (C, G), total S6 (D, I) and phosphorylated S6 (E, I) in whole tuber sections. (B, C) and (D, E) are adjacent serial sections. Note the predominant nuclear IR for hamartin (B,F) and the cytoplasmic IR for tuberin (C,G) in neuron-like giant cells of the tuber. Also note the prominent labeling of virtually all neural cells by the S6 antiserum (D, H), but only of the giant cells by the phospho-specific S6 antiserum (E, I). Asterisk in A marks a group of giant cells. Arrows in (B, C) and (D, E) point to a neuron-like giant cell common to these panels, which are shown magnified in (F, G) and (H, A). Scale bar in I = 100 µm for a; 75 µm for B through E; and 25 µm for (F through I).

Figure 2



Allele specific oligonucleotide hybridisation analysis of the *TSC1* R692X (2295 C-T) mutation. Exon 17 of the *TSC1* gene was amplified from DNA isolated from normal cortex of the index patient (N); the cortical tuber from the index patient (T1, T2); peripheral blood of the index patient (I); peripheral blood of the index patient's parent (P); and a negative control (no DNA) (C). Oligonucleotides specific for the normal (*TSC1* 2295C; left) and mutant (*TSC1* 2295T; right) alleles were hybridised against the amplified DNA. Only the index patient was positive for the mutant oligonucleotide. DNA from the tuber contained both copies of the *TSC1* gene and showed no evidence of loss of heterozygosity.

Figure 3



Immunoblot analysis of the tuber. Expression of mammalian target of rapamycin (m-TOR), tuberin, hamartin, and S6 in the tuber (T) and normal cortex (N) is shown. Equal amounts of total protein (20 ug) from the brain tissue homogenates were loaded in each lane. The phosphorylation of S6 at serine residue 235 was analysed with a phospho-specific antibody (Cell Signaling Technology).

Discussion

The mechanisms involved in the development of cortical tubers remain unclear. A two-hit model, similar to that proposed for other TSC-associated lesions, is one possibility.[8] Alternatively, haploinsufficiency at either the *TSC1* or *TSC2* locus could initiate tuber pathogenesis.
We characterised an epileptogenic tuber from a TSC patient with a germ-line *TSC1* mutation and found no evidence for a second, inactivating *TSC1* mutation in the tuber DNA. We cannot completely rule out an intragenic deletion or a mutation in a non-coding region of the *TSC1* gene. Our results indicate that, if a two-hit mechanism is responsible for tuber pathogenesis, then only a small proportion of the cells in the tuber are nullizygous for a functional *TSC1* allele. Immunoblotting did not detect any differences in the levels of expression of tuberin or hamartin between epileptogenic and normal cortex, suggesting that tuberogenesis is not due to reduced expression of hamartin or tuberin in the tuber as a whole. However, compared to adjacent normal cortex, the levels of phosphorylated S6 were increased in the tuber. Immunohistochemistry revealed strong staining for phosphorylated S6 in giant cells, consistent with the differences observed by immunoblotting. The giant cells showed cytoplasmic staining for tuberin, and, interestingly, nuclear staining for hamartin. The expression of tuberin and hamartin in separate cellular compartments would prevent formation of the hamartin-tuberin complex and thus explain the increased phosphorylation of S6 in the giant cells.
Our data support the notion that tubers are dynamic lesions[10] and suggest that the differential subcellular localisation of hamartin and tuberin may provide an alternative mechanism for tuber pathogenesis that does not require a second-hit mutation. Further studies in a larger patient population and including *TSC2* mutations are required to elucidate the mechanisms underlying tuberogenesis.

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11

General discussion

The studies reported in this thesis have focussed on two neurological manifestations of tuberous sclerosis complex (TSC): mental retardation and epilepsy. This thesis describes the prognostic significance of the genotype, the tuber status, and the epilepsy syndrome for cognitive function, and attempts to identify the epileptogenic tuber with high-resolution neurophysiological and neuroimaging techniques. In addition, outcome after epilepsy surgery is reported.

Cognitive function and its prognostic indicators in TSC

A diagnosis of TSC confronts patients and caregivers with a high risk of mental retardation. Estimates of the prevalence of mental retardation in patients with TSC have varied from 38 to 69%.[1] Several of the studies investigating cognitive function have used indirect measures, such as the level of schooling, and other studies have characterised patients as having either normal intelligence or profound retardation.[2] Patients of our sample (*chapters three and four*) had deficits in multiple cognitive domains and only 19% of the patients had a cognition index (a marker of cognition that incorporates more cognitive domains than the intelligence equivalent) in the average or above average range. Intelligence testing alone is therefore not sufficient and more thorough psychometric evaluations need to be performed.

In addition to scientific use, comprehensive assessment of cognitive function has at least two purposes. First, if cognitive problems have arisen, developmental, pedagogical, and educational assistance offered as soon as possible help to guide and inform patients and caregivers. Next, underlying causes of emerging cognitive difficulties may be identified and treated, if possible. To be able to prevent cognitive deterioration, factors that may have an impact on cognitive function need to be known.

A number of possible critical determinants of cognitive function in patients with TSC have been addressed in this thesis. First, the type of mutation has been related to the cognition index. At first glance, patients with a *TSC2* mutation had a lower cognition index than patients with a *TSC1* mutation (*chapter three*). However, the ranges of cognitive function in *TSC1* and *TSC2* patients overlapped considerably and knowledge of the mutation type in the individual patient is therefore not adequate to predict cognitive development with sufficient certainty. Second, a higher total tuber volume was related to a lower intelligence, and tended to be related to a lower cognition index (*chapter four*). The predictive validity of tuber status is strongly dependent on the method of tuber status assessment and the sample under investigation. Previous measurements of tuber status indicated that patients with a higher number of tubers have an increased risk of cognitive impairment,[3] a finding that was not supported by our results. This may be explained by the fact that these measurements were, to our opinion, less accurate and consistent. Although an early onset of epilepsy, a third pos-

sible determinant, was the only independent predictor of cognitive deficit in our study, the assumption that TSC patients without epilepsy have a normal cognition index could not be confirmed (*chapter four*). Larger sample sizes are needed for the identification of other possible mediating or moderating effects.
To date, there is no intervention available to change the genotype or tuber status. Further, treatment of early-onset seizures, especially infantile spasms is not optimal, although vigabatrin is effective in a selection of patients.[4] As individuals with less severe neurological involvement are also more likely to respond favourably to the treatment of seizures, it will be impossible to test the effect of anti-epileptic therapy on cognitive function outside randomised controlled clinical trials. However, designing such trials will prove difficult because of the large heterogeneity of the TSC population. Another issue that should be addressed in this context is the prevention of early-onset seizures. To be able to develop strategies preventing seizure development, the nature and timing of the epileptogenic trigger needs to be known. Until such strategies have proven effective, epilepsy surgery should be considered especially in cases where cognitive deterioration may be related to seizure burden.

Targeting and timing of epilepsy surgery

Before epilepsy surgery can be performed safely and accurately, identification of the epileptogenic tuber is mandatory. Given the complexity of epilepsy in patients with TSC and its precarious prognosis, intensive evaluation is essential. In selected series, surgery has proven successful in 75% of the patients (*chapter eight*). Still, some neurosurgeons are reluctant to perform epilepsy surgery in patients with TSC, partly because of fear of post-surgical shift of epileptogenicity to other tubers. Such fear of shifting foci may be unnecessary, as we found that, in a selection of patients, the localisation of interictal epileptiform activity is remarkably consistent over time (*chapter five*). Whether these consistent foci are concordant with the localisation of seizure onset (obtained with long-term video EEG recordings) is currently being analysed.
The aims of the studies described in *chapters six and seven* were multiple: 1) to analyse the differences between advanced neurophysiological and neuroimaging techniques (magnetoencephalography (MEG) and diffusion-weighted MRI (DWI), respectively) and the currently used techniques in the pre-operative evaluation, 2) to assess the usefulness of these techniques in the pre-operative evaluation, and 3) last but not least, to give patients who participated in the studies the best diagnostic work-up with the attempt to enable epilepsy surgery. Although we found that MEG sources were closer to presumed epileptogenic tubers than EEG sources (*chapter six*), and diffusion characteristics distinguished epileptogenic from non-epileptogenic tubers (*chapter seven*), the value of these methods in the identification of the epileptogenic

tuber could not be proven, because epilepsy surgery, the gold standard for awarding a tuber epileptogenic, was not performed in the majority of patients included in these studies. In order to increase insights in the value of these techniques, future research should be both sufficiently powered and include epilepsy surgery. Simultaneous EEG recording and functional MRI (fMRI) permits non-invasive investigation of the mechanisms and localisation of spike generation.[5] Experience with this technique in TSC is lacking, and this should be tested in future studies. Although all of the techniques above may add to the understanding of epileptogenicity in TSC, their ability to increase the rate of seizure-free outcome after epilepsy surgery has to be awaited. Given the large differences between TSC patients, the question can be raised whether a single source-localisation technique will be applicable in all patients. Currently, a pragmatic and empirical approach is most likely to be fruitful in the individual patient.

An important question is not only whether the individual patient will benefit from epilepsy surgery, but also how soon surgery should be performed in order to prevent developmental arrest or deterioration. Several studies have suggested that successful epilepsy surgery may lead to improvement of cognitive functioning.[6] As a consequence, early surgical treatment in patients with localised seizure onset is supported by many epileptologists. Patients with an early onset diffuse epileptic encephalopathy, such as West syndrome (WS), will probably be rejected. However, a focal origin has been reported in WS associated with TSC[7] and both the number of interictal EEG foci and the number of seizure types can decrease with time (*chapter five*). Thus, considering epilepsy surgery in these patients at a later point in time is justifiable. Further studies are needed to determine the optimal timing of epilepsy surgery.

Current approach to epilepsy surgery

Epilepsy surgery is reported increasingly effective in TSC and it appears logical that patients with consistent seizure semiology, unifocal interictal and ictal EEG abnormalities, and a corresponding tuber in a surgically accessible region will benefit most. However, a systematic review of the literature provided circumstantial evidence that the presence of multiple seizure types, multiple lesions on imaging, and multifocal EEG abnormalities are not associated with seizure recurrence (*chapter eight*). Because the pre-surgical evaluation techniques were highly heterogeneous between studies, one major shortcoming of this review was the limited number of patients in whom all variables were obtained. In addition, publication bias might have occurred as negative results may not have been reported.
Unfortunately, there are no studies available clarifying the origin of recurrent seizures in TSC. It is hard to imagine that distant independent foci became active shortly after surgery. On the contrary, it has been hypothesised that after resection of a primary focus, secondary dependent foci may be eliminated or silenced.[8] In our patients in whom seizures persisted despite surgery,

residual epileptogenic cortex had been left in situ, for instance in cases of proximity to eloquent cortex (*chapter nine*). Unfortunately, the possible intrinsic epileptogenicity of residual tissue can not be addressed pre-operatively in a non-invasive manner. Currently, we do not perform epilepsy surgery in TSC patients with a variety of seizure types in whom the ictal onset zone can not be defined to a single region or a corresponding tuber, or if the epileptogenic tuber is identified in eloquent cortex. However, given the results of the systematic literature study we may need to revise our strategy.

Considerations for future research

The estimated prevalence of TSC in the Netherlands is 1: 16.000. Of the 1000 Dutch TSC patients, only about 100 are described in this thesis. Despite the great achievements of surgical programmes in the last decades, epilepsy surgery has only been performed in 6 patients. Even if less strict criteria will be applied, it appears unlikely that surgery will be suitable in more than 10% of the patients. Although the benefit of surgery may be very large for the individual patient and study of resected tubers adds to the understanding of tuberogenesis (*chapter ten*), the benefit of surgery on a population basis will remain small. For this reason, future research needs to focus on other treatment strategies as well.

Seizure prevention

How a mutation in either the *TSC1* or the *TSC2* gene leads to tuberogenesis is not exactly known. This may in part be explained by the fact that tubers are composed of a mixture of different cell types, making it difficult to identify the subset of cells that has undergone a second mutational event.[9] Alternatively, hamartin and tuberin may accumulate in different cellular compartments (*chapter ten*), preventing the formation of the hamartin-tuberin complex, resulting in activation of mammalian target of rapamycin (mTOR) kinase.[10] The mTOR pathway is involved in a number of cell processes, among which cell growth control, nutrient uptake, and protein translation.[9]

In the absence of a well functioning hamartin-tuberin complex, mTOR inhibition may be a target of therapy. Rapamycin, an antifungal macrolide antibiotic, is known to inhibit mTOR. The potential benefit of rapamycin has been shown in rodent models, where the drug reduced the size of *TSC2*-related renal tumours.[11] Rapamycin was subsequently proposed as a candidate for the treatment of TSC in humans. Oral rapamycin has been shown to induce regression of astrocytomas associated with TSC,[12] and clinical trials of rapamycin for renal angiomyolipomas associated with TSC are underway. The question is whether rapamycin may also be of benefit for patients with other (neurological) manifestations of TSC. Although the formation of tubers starts as early as the first trimester of gestation, these are dynamic lesions

undergoing active proliferation throughout life.[13] Because of its cell cycle arresting properties and selective apoptosis induction, rapamycin may be able to affect tuber formation or even alter the threshold for abnormal firing and thereby that of seizures. This needs to be tested in randomised clinical trials.

Seizure control

Factors contributing to drug resistance do not distinguish TSC-associated lesions from other structural brain abnormalities. These factors include the early age at seizure onset, the epilepsy syndrome, the frequency of seizures, the response to treatment, and abnormal EEG findings, but none of these factors alone can explain multi-drug resistance.
Two hypotheses have been proposed to explain medical refractoriness of seizures: the drug target hypothesis and the multi-drug transporter hypothesis. Frequent seizures, as often observed in TSC patients, may reduce the pharmacosensitivity of anti-epileptic drug (AED) targets. Overexpression of various ATP-binding cassette (ABC) transporters has been found to be associated with decreased brain concentration of AEDs in patients with different types of multi-drug-resistant epilepsy. More specifically, it has been suggested that epileptogenic cortical tubers have a high risk for developing the refractory phenotype, with multi-drug resistance protein-1 (MDR-1) and multi-drug resistance associated protein (MRP-1) as the main protagonists and the major vault protein (MVP) playing a complementary role.[14] Further understanding of the mechanisms underlying multi-drug resistance in epilepsy in general and in TSC in particular, may allow new rational treatment strategies replacing the largely empirical approach. Such strategies may include the design of AEDs that are not a target of resistance mechanisms, and the development of agents that can inhibit the proteins responsible for drug resistance and can be used in conjunction with AEDs.

Conclusions

- Cognitive dysfunction in patients with TSC is a multifactorial condition. Of all contributing factors, early-onset seizures may be most amenable to treatment.
- The epileptogenic tuber may be identified with high accuracy by means of magnetoencephalography and diffusion-weighted MRI. However, the usefulness of these advanced source localisation techniques for the average TSC patient is probably limited and can only be tested in patients subjected to epilepsy surgery.
- Epilepsy surgery should be considered in all multidrug-resistant TSC patients.
- The majority of TSC patients will prove not eligible for epilepsy surgery. For this reason, other treatment options need to be explored.

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Summary

Tuberous sclerosis complex (TSC) is associated with a variety of neurological symptoms such as epilepsy, mental retardation and behavioural disorders. The occurrence of epilepsy is very common (80-90%) and seizures are often the presenting symptom. About half of the TSC patients with epilepsy do not respond sufficiently to anti-epileptic drugs. Epilepsy surgery may be considered in these patients but uncertainties exist about which patients are good surgical candidates.
The diversity of cognitive functioning in patients with TSC is very large. The type of mutation, the tuber status and the epilepsy classification are likely to influence cognitive functioning, but to date, this has not been investigated systematically.

The principal aim of this thesis was to identify epileptogenic tuber(s), enabling the selection of patients for epilepsy surgery. In addition, we analysed possible determinants of cognitive functioning in patients with TSC.
Chapter one introduces the disease and its neurological aspects and outlines the contents of this thesis. A brief overview of historical reports of the disease and a rendition of its founders is given in *chapter two*. In **Part I**, we made an attempt to distinguish patients with different *TSC1* mutations from patients with different *TSC2* mutations by means of neurological and cognitive phenotypes. The associations between tuber status, epilepsy variables and cognitive impairment have been investigated. In **Part II**, the additional value of advanced neurophysiological and neuroimaging techniques to identify the epileptogenic tuber has been explored. The outcome of epilepsy surgery in TSC world wide and in our centre is described in **Part III**. In **Part IV**, mechanisms underlying tuberogenesis and future perspectives of treatment of TSC patients with drug-resistant epilepsy are addressed.

Part I. Determinants of cognition

In *chapter three* we compared neurological and cognitive phenotypes of patients with different *TSC1* mutations with patients with different *TSC2* mutations on the basis of a comprehensive neuropsychological test battery, covering six cognitive domains, and objective tuber status assessment. We found a statistically significantly earlier age at seizure onset, lower cognition index, more tubers, and larger proportion of brain volume occupied by tubers (TBP) in patients with a *TSC2* mutation, but the overlap was considerable. We concluded that neurological and cognitive functioning can not be predicted in the individual with TSC based on their particular mutation type.
The main innovation in *chapter four* was the implementation of an automated tuber detection and segmentation technique to determine the associations between tuber status, epilepsy variables and cognitive functioning. The detrimental effect of a more severe tuber status was

hypothesised to be better defined by the TBP than by the number of tubers. A greater proportion of total brain volume occupied by tubers (TBP) was related to younger age at seizure onset, a lower intelligence equivalent and tended to be related to a lower cognition index. The number of tubers was not related to any of these factors, confirming our hypothesis. However, in a multivariable analysis, age at seizures was identified as the only independent predictor of cognitive functioning. To date there is no strategy available to prevent seizures, prompting adequate treatment as soon as seizures occur.

Part II. Presurgical evaluation

In *chapter five* we aimed to refute the assumption that epileptogenicity shifts from one tuber to another in 21 patients with TSC, at least a ten year history of epilepsy and more than three EEG recordings during that time. Three observers reviewed all EEGs and localised interictal epileptiform foci. In eight patients one or two regions of interictal epileptiform activity were found. In 13 patients three or more regions of epileptiform activity were detected. Patients with one or two foci were older at seizure onset, had less often infantile spasms or generalised tonic clonic seizures, and had a higher intelligence equivalent when compared to patients with three or more foci. The most consistent epileptiform activity was localised in the frontotemporal region. If these consistent foci are concordant with the localisation of seizure onset, which is currently being analysed, these patients may be eligible to epilepsy surgery.
Chapter six describes the additional value of magneto-encephalography (MEG) in the identification of the epileptogenic tuber. In 19 patients interictal epileptiform activity was selected offline by three observers, separately in high-resolution (HR) EEG and MEG recordings. MUSIC analysis for dipole orientations was performed for sources with at least moderate interobserver agreement. Sources were integrated in 3D FLAIR MR images and distances from the maximum of the dipole orientations of both EEG and MEG and the border of the closest tuber were calculated and compared. MEG sources were closer to presumed epileptogenic tubers (mean distance of 13.8 mm) than EEG sources (mean distance of 24.6 mm) and MEG more often revealed unifocal epileptiform activity. Therefore, MEG analysis was considered at least as accurate as EEG analysis in identifying the epileptogenic source in TSC.
In *Chapter seven* we investigated diffusion characteristics (with diffusion-weighted MRI (DWI)) of tubers. In four patients, apparent diffusion coefficient (ADC) maps were calculated in identified epileptogenic tubers, by means of simultaneous HR EEG and MEG recording, and compared with the ADC maps in non-epileptogenic tubers and in regions of normal appearing cortex. DWI proved a promising tool in differentiating epileptic from inert tubers in TSC, as trace ADC of the four epileptogenic tubers (mean 1099 mm/s, SD 35.0) was significantly higher than that of 18 non-epileptogenic tubers (mean 926 mm/s, SD 69.4). Furthermore, the trace

ADC of the non-epileptogenic tubers was higher than the trace ADC of 16 regions of normal appearing cortex (mean 784 mm/s, SD 61.7).
Both MEG and DWI were considered to contribute significantly to the selection of TSC patients for epilepsy surgery.

Part III. Epilepsy surgery in TSC

Chapter eight gives an overview of the current literature on seizure outcome after epilepsy surgery in patients with TSC and multi-drug-resistant epilepsy. Factors related to seizure recurrence in these patients are identified. Seizure freedom was achieved in 101 (57%) of the 177 included patients, from 25 studies. Seizure frequency was reduced by > 90% in another 32 patients (18%). Moderate or severe intellectual disability (defined as IQ <70) (RR 1.8; 95% CI 1.2- 2.8) and the presence of tonic seizures (RR 1.7; 95 % CI 1.2- 2.4) were related to seizure recurrence. In addition patients with multifocal abnormalities on ictal SPECT examination (RR 7.0; 95% CI 1.1- 43) and patients who underwent a corpus callosotomy (corpus callosotomy versus resective surgery RR 2.5; 95% CI 2.1- 3.0) had a higher risk of recurrent seizures, but group sizes were small.
In *chapter nine* we evaluated pre-surgical investigations in 25 Dutch TSC patients with multi-drug-resistant epilepsy in whom epilepsy surgery was considered. Suitable candidates were characterised by one region of seizure onset and sufficient seizure burden. Six patients underwent epilepsy surgery. Four of these patients had excellent outcome: Engel classification 1. Perceived improvement of cognition was found in two patients.

Part IV. Tuberogenesis

In *chapter ten* we report on the results of mutation analysis in a patient with a germ-line *TSC1* mutation and tuberous sclerosis who underwent epilepsy surgery. In the resected cortical tuber no second hit mutation was found, excluding loss of heterozygosity as the cause of tuber formation. Tuber giant cells showed predominantly nuclear hamartin, cytosolic tuberin, and concomitant hyperphosphorylation of S6. Differential accumulation of hamartin and tuberin in separate cellular compartments of giant cells may prevent formation of the hamartin-tuberin complex resulting in increased S6 phosphorylation, suggesting an alternative mechanism for tuberogenesis.

The limitations, implications for the individual with TSC, and future perspectives of other treatment options are discussed in *chapter eleven*.

Samenvatting

Tubereuze sclerose complex (TSC), ook wel de ziekte van Bourneville-Pringle genoemd, is een aandoening die zich manifesteert in verscheidene organen, waaronder de hersenen. TSC is een erfelijke aandoening met een autosomaal dominant overervingpatroon. De ziekte wordt veroorzaakt door mutaties in het *TSC1* gen op chromosoom 9q34 of in het *TSC2* gen op chromosoom 16p13. De genen coderen voor de eiwitten hamartine en tuberine die samen een eiwitcomplex vormen dat indirect een belangrijke rol speelt bij celgroei, stabilisatie van eiwitten en voedingsopname. Hoewel de ziekte bij iedereen met een *TSC1* of *TSC2* mutatie tot uiting komt, is de mate van de expressie erg variabel, ook binnen families.

De verschillende cerebrale laesies bij patiënten met TSC ontstaan door een stoornis van neuronale proliferatie, migratie en differentiatie. Deze laesies zijn subependymale noduli, heterotopieën en (sub)corticale tubers. Tubers zijn opgebouwd uit reuscellen en dysmorfe neuronen en verstoren de normale architectuur van de cortex.

De neurologische symptomen van TSC bestaan onder meer uit epilepsie, mentale retardatie en gedragsstoornissen. Epilepsie is het meest voorkomende symptoom (voorkomend bij 80-90% van de patiënten) en vaak ook de eerste uiting van de ziekte. Verschillende aanvalstypen komen voor. Bij patiënten die op zeer jonge leeftijd aanvallen krijgen, infantiele spasmen in het bijzonder, is het beloop vaak ongunstig. Epileptische aanvallen reageren bij 50% van alle TSC patiënten onvoldoende op medicijnen. Andere behandelingen zijn een ketogeen dieet, nervus vagus stimulatie en epilepsie chirurgie. Vooral deze laatste mogelijkheid wint de laatste decaden aan terrein. De omstandigheden voor epilepsie chirurgie zijn het meest gunstig als er sprake is van één enkele laesie (goed zichtbaar op een MRI) die een goede samenhang vertoont met de aanvalsbeschrijving en de resultaten van neurofysiologisch onderzoek. Tevens moet deze laesie niet in gebieden liggen waar belangrijke hersenfuncties als taal of motoriek gerepresenteerd zijn, i.e. de eloquente cortex. Bijna alle patiënten met TSC hebben echter verscheidene verspreide tubers en multifocale interictale EEG afwijkingen. Er wordt bovendien getwijfeld aan de consistentie van deze epileptogene bronnen en daarom is men vaak terughoudend met epilepsie chirurgie.

Het belangrijkste doel van de studies beschreven in dit proefschrift was bij patiënten met TSC en medicamenteus onbehandelbare epilepsie te onderzoeken of één epileptogene tuber vastgesteld kan worden en zo patiënten te selecteren die in aanmerking komen voor epilepsie chirurgie. *Hoofdstuk één* is de introductie van dit proefschrift. In *hoofdstuk twee* worden de historie van het ziektebeeld en haar grondleggers beschreven. In de studies van **deel I** van dit proefschrift wordt gezocht naar factoren, zoals het mutatie type, de tuber status zoals vastgesteld met behulp van een MRI scan en epilepsie variabelen, die een invloed hebben op het cognitieve functioneren van TSC patiënten. In de studies van **deel II** van het proefschrift wordt bewijs gevonden voor consistentie van epileptiforme activiteit. Tevens wordt de meerwaarde van niet-invasieve neurofysiologische technieken (hoge resolutie (HR) EEG, magneto-encephalografie (MEG) en beeldvormingtechnieken (diffusie-gewogen MRI (DWI) bestudeerd. In de studies van **deel III**

zijn de resultaten van epilepsie chirurgie beschreven. In de studie van **deel IV** wordt gezocht naar een alternatieve verklaring voor het ontstaan van tubers.

Deel I. Determinanten van cognitie

In *hoofdstuk drie* analyseerden wij de verschillen in neurologische en cognitieve verschijnselen tussen patiënten met een *TSC1* mutatie en die met een *TSC2* mutatie. Als groep hadden patiënten met een *TSC2* mutatie op jongere leeftijd epileptische aanvallen, een lagere cognitie index (een score voor het cognitieve functioneren die meer cognitieve domeinen bevat dan intelligentie niveau alleen), meer tubers en een grotere proportie van totale hersenvolume dat door tubers in beslag genomen wordt (TBP) (samen tuber status genoemd). De overlap tussen de groepen was echter groot, waaruit we de conclusie trokken dat het neurologische en cognitieve fenotype niet voorspeld kan worden op basis van het genotype alleen.
Hoofdstuk vier beschrijft de relaties tussen tuber status, epilepsie variabelen en het cognitieve functioneren. Wij maakten gebruik van een objectiveerbare maat voor het vaststellen van de tuber status en tevens van een uitgebreid gestandaardiseerd neuropsychologisch onderzoek waarin verschillende cognitieve domeinen werden getest. Een grotere TBP was gerelateerd aan een vroeger debuut van epileptische aanvallen en aan een lagere intelligentie equivalent (IE). Het aantal tubers was niet gerelateerd aan epilepsie variabelen of het cognitieve functioneren. Echter, na multivariate analyse bleek alleen de debuutleeftijd van epileptische aanvallen een onafhankelijke samenhang te vertonen met de cognitie index. Deze bevinding benadrukt het belang van verder onderzoek naar mogelijkheden ter preventie van epileptische aanvallen.

Deel II. Pre-chirurgische evaluatie

In *hoofdstuk vijf* hebben we de consistentie van interictale epileptogene bronnen onderzocht bij 21 TSC patiënten die langer dan 10 jaar epilepsie hadden en van wie er gedurende die tijd tenminste drie EEGs geregistreerd waren. Wij vonden 1 of 2 constante epileptiforme bronnen bij 8 patiënten en 3 of meer bronnen bij 13 patiënten. Patiënten met 1 of 2 bronnen hadden significant later aanvallen, minder vaak infantiele spasmen, minder vaak gegeneraliseerde tonisch-clonische aanvallen en een hogere IE. De meest constante bronnen waren rechts fronto-temporaal gelokaliseerd. Dit is een gebied dat redelijk goed toegankelijk is voor epilepsie chirurgie.
Hoofdstuk zes beschrijft de verschillen en overeenkomsten tussen HR EEG en MEG registraties bij 19 patiënten met TSC en epilepsie. De epileptiforme activiteit, geregistreerd met beide onderzoekstechnieken, werd beoordeeld door drie klinische neurofysiologen. Epileptiforme bronnen met een kappa-overeenkomst tussen beoordelaars van 0.4 of meer werden geïn-

tegreerd in een 3 dimensionale FLAIR MRI. De afstand van de epileptiforme bron tot de rand van de dichtstbijzijnde tuber werd gemeten en vergeleken tussen HR EEG en MEG. MEG bleek tenminste even goed als het HR EEG in het vaststellen van epileptogene bronnen. De MEG bronnen bleken bovendien dichter bij de tubers gelegen dan de EEG bronnen (13.8 mm versus 24.6 mm).
In *hoofdstuk zeven* werden de diffusie karakteristieken van tubers onderzocht met behulp van diffusie-gewogen MRI scans. De 'apparent diffusion coefficient' (ADC; kwantificatie van diffusie) van 4 epileptogene tubers werd vergeleken met de ADC van 18 niet-epileptogene tubers en 16 gebieden van normale hersenschors. De ADC van niet-epileptogene tubers was hoger dan die van normale hersenschors. Bovendien was de ADC van epileptogene tubers hoger dan die van niet-epileptogene tubers. Aan de hand van deze resultaten kan gesteld worden dat MEG en DWI bijdragen aan het selecteren van TSC patiënten voor epilepsie chirurgie.

Deel III. Epilepsie chirurgie bij patiënten met TSC

Een systematische literatuur studie betreffende de resultaten van epilepsie chirurgie bij patiënten met TSC en medicamenteus onbehandelbare epilepsie werd beschreven in *hoofdstuk acht*. Van de 177 patiënten, afkomstig uit 25 studies, waren er 101 (57%) aanvalsvrij na operatie. Bij nog eens 32 patiënten (18%) nam de aanvalsfrequentie aanzienlijk af (vermindering van > 90%). Patiënten bij wie aanvallen persisteerden ondanks operatie hadden vaker tonische aanvallen en vaker een matige tot ernstige ontwikkelingsachterstand (gedefinieerd als IQ< 70). Daarnaast hadden patiënten die een corpus callosotomie (doornemen van het corpus callosum) ondergingen en patiënten bij wie ictale SPECT scans multifocaal afwijkend waren (ten opzichte van patiënten met één SPECT focus) vaker recidiverende aanvallen, maar corpus callosotomie en SPECT onderzoek werden slechts bij een kleine groep patiënten verricht.
In *hoofdstuk negen* worden de resultaten van pre-chirurgisch onderzoek en van epilepsie chirurgie bij Nederlandse TSC patiënten gerapporteerd. Gedurende de afgelopen vijf jaren werden 25 patiënten aangemeld bij de Landelijke werkgroep functionele neurochirurgie. Eén aanvalsbegin was een vereiste voor continueren van het pre-chirurgische traject. Dit werd gevonden bij negen patiënten. Het enige significante verschil tussen de patiënten die wel geopereerd werden en de patiënten die niet voor een operatie in aanmerking kwamen, was het niveau van het cognitieve functioneren. Dit was hoger bij de geopereerde patiënten.
Op dit moment in de studie zijn er zes TSC patiënten geopereerd met goed resultaat: vier patiënten zijn aanvalsvrij en bij twee van de patiënten rapporteerden de ouders een vooruitgang in de ontwikkeling of in het gedrag van hun kind.

Deel IV. Genese van tubers

De resultaten van mutatie analyse in een door middel van epilepsie chirurgie verwijderde epileptogene tuber van een patiënt met een *TSC1* mutatie werden beschreven in *hoofdstuk tien*. Er werden geen aanwijzingen gevonden voor een "2de hit" (2de somatische mutatie) en verlies van heterozygositeit als verklaring voor het ontstaan van tubers werd hiermee uitgesloten. In reuscellen uit deze tuber bleek hamartine vooral in de kern aanwezig, terwijl tuberine in het cytoplasma werd aangetoond. Tevens werd er een teveel aan gefosforyleerd S6 gevonden in reuscellen. Deze bevinding levert een bijdrage aan de kennis van het onderliggende mechanisme betreffende het ontstaan van tubers, omdat het vóórkomen van hamartine en tuberine in verschillende celcompartimenten de vorming van het eiwitcomplex kan belemmeren. Dit kan aanleiding kan geven tot een toename van gefosforyleerd S6. Een teveel aan gefosforyleerd S6 leidt tot reuscelgroei.

Tot slot worden de implicaties van de resultaten van de in dit proefschrift beschreven studies bediscussieerd en worden mogelijkheden voor toekomstig onderzoek besproken in *hoofdstuk elf*.

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Dankwoord

Een patiëntgebonden onderzoek is niet haalbaar zonder de inzet van patiënten. Om te beginnen wil ik daarom alle **patiënten met tubereuze sclerose** die betrokken zijn geweest bij dit onderzoek en ook de **Stichting Tubereuze Sclerose Nederland** hartelijk danken.

Mijn eerste kennismaking met de aandoening tubereuze sclerose complex (TSC) en de vaak teleurstellende medicamenteuze behandeling vond plaats in 1998 tijdens een arts-assistentschap op de afdeling kinderneurologie in het UMC te Utrecht. Men ging zich afvragen of epilepsie chirurgie niet vaker overwogen zou moeten worden bij TSC patiënten met medicamenteus onbehandelbare epilepsie. Tegelijkertijd ontstond ook interesse in het toepassen van magneto-encephalografie ten behoeve van bronlokalisatie bij epilepsie patiënten. Tijdens een onderzoeksbespreking met Prof. Dr. O van Nieuwenhuizen en Prof. Dr. A.C. van Huffelen werd het doel van één van de hoofdstukken van dit proefschrift geformuleerd en stonden de namen van mijn promotoren op papier.

Prof. Dr. O. van Nieuwenhuizen, beste Onno, jij hebt dit onderzoek geïnitieerd en samen met professor Van Huffelen begeleid. Onze samenwerking verliep over toppen en door dalen. Als ik soms wanhopig werd van een onverwachte stemmingsomslag wuifde jij mijn verontwaardiging weg met een "never a dull moment". Dat bleef in ieder geval waar tot het einde. Jij hebt de ongelooflijke eigenschap om mij altijd weer aan het lachen te kunnen krijgen, zij het soms als een boer met kiespijn. Ook als ik me stellig voorgenomen had voet bij stuk te houden, wat jij al kon horen aan mijn tred door de gang, bereikten we meestal wel een compromis. Omdat het niet snel saai zal worden verheug ik me erop de samenwerking na 1 juli 2007 voort te zetten. Veel dank voor je enorme betrokkenheid.

Prof. Dr. A.C. van Huffelen, vele sessies zat ik met u achter het beeldscherm om meer dan 100 EEGs van TSC patiënten opnieuw te beoordelen. Deze sessies werden nooit stilzwijgend doorgebracht en zo ben ik alles te weten gekomen over uw opvoeding, uw leermeesters en uw gezin. Het ontcijferen van uw handgeschreven commentaar op mijn manuscripten was vaak een uitdaging. Als dit gelukt was bleek het manuscript in alle gevallen verbeterd, waarvoor veel dank.

Bij de lokalisatie van epileptogene bronnen was **Dr. G. Huiskamp** van grote waarde. Beste Geertjan, jij bent de enige die sneller e-mails beantwoordt dan professor Van Huffelen. Als ik weer eens een plaatje nodig had voor een presentatie werd per ommegaande aan dat verzoek voldaan, ook als ik e-mailde vanuit Melbourne en het in Utrecht midden in de nacht was. Veel dank ook voor de mooie dipool op het kaft (bewerkt door **Roy Sanders**).

Goede beeldvorming is een vereiste voor bronlokalisatie. Wij hadden als doel een geautomatiseerde objectieve en reproduceerbare methode te gebruiken om tubers te segmenteren. Het programma hiervoor moest echter wel eerst geschreven worden. Hierbij kregen we hulp in het Image Science Institute van (inmiddels) **Prof. Dr. W.J. Niessen**, opgevolgd door **Dr. K.L. Vincken** en **Dr. P. Anbeek**. Beste Koen, drie jaren en vele uren werkte je aan mogelijke segmentatie methoden die in de prullenbak belandden. Gelukkig was je daarna nog onverminderd enthousiast om je met Nelly te storten op het voor tubers toepasbaar maken van de KNN segmentatie techniek. Je enthousiasme was aanstekelijk.

Zowel in de patiëntenzorg als de wetenschap spelen vragen over erfelijkheid van TSC een belangrijke rol. Enkele studies zijn tot stand gekomen na bemiddeling door **Prof. Dr. D. Lindhout** en een buitengewoon prettige samenwerking met **Dr. M. Nellist**, **Dr. D. Halley** en **Dr. A. van den Ouweland** van de afdeling medische genetica van het Erasmus Medisch Centrum te Rotterdam. Beste Mark, je onterechte bescheidenheid overschaduwt nog net niet je enorme kennis van zaken. Je Britse komaf was zeer handig bij het corrigeren van de Engelstalige manuscripten.

In de loop van de jaren is de afdeling neuropsychologie in het WKZ overspoeld met verzoeken om neuropsychologisch onderzoek bij patiënten met TSC. **Drs. O. Braams** heeft, onder supervisie van **Dr. A. Jennekens-Schinkel** al deze onderzoeken uitgewerkt en de resultaten geïnterpreteerd. Beste Olga, dank voor dit vele werk. Beste Aag, je precisie, rijke taalgebruik en kritische houding waren erg leerzaam. Ik ben blij dat je, om met je eigen woorden te spreken, je tijd soms door mij liet stelen.

Bij het analyseren van de laatste resultaten en het schrijven van hoofdstuk acht was het advies van een zeer ervaren epidemioloog hard nodig. Gelukkig vonden wij **Prof. Dr. A. Algra** bereid om creatief met zijn spaarzame tijd om te springen. Beste Ale, je pragmatische aanpak spreekt mij enorm aan en maakt zelfs de lastigste statistiek begrijpelijk.

In de jaren van deze studie werden in totaal meer dan honderd patiënten met TSC onderzocht. Dit was niet mogelijk geweest zonder de verwijzingen door **Dr. B.A. Zonnenberg** en de **Nederlandse kinderneurologen**.

Na kritische beoordeling door de **landelijke werkgroep functionele neurochirurgie** werden zes patiënten met TSC geopereerd (en werden er 19 voor operatie afgewezen). De neurochirurgen **Dr. P.C. van Rijen** en **Drs. P. Gosselaar** en klinisch neurofysioloog **Dr. F.S.S Leijten** hebben zorg gedragen voor een goed behandelingsresultaat.

In de afgelopen jaren was mijn doel, naast promoveren, om neuroloog te worden, met als aandachtsgebied de kinderneurologie. Als lid van de visitatiecommissie van de Nederlandse Vereniging voor Neurologie heb ik in diverse neurologische keukens kunnen kijken. Zonder andere klinieken tekort te willen doen heb ik de ruimte voor keuzestages en het goede opleidingsklimaat in Utrecht altijd zeer gewaardeerd. Hiervoor wil ik **Prof. Dr. J. van Gijn** en **Prof. Dr. J.H.J. Wokke** zeer bedanken.

Zonder de vele gezellige en collegiale **arts-assistenten neurologie** was het werkplezier zeker minder groot geweest. **Jeannette**, jammer dat je 5 maanden eerder dan ik je opleiding hebt afgerond. Omdat we beiden zo van pragmatisch en efficiënt werken houden, vonden we vaak tijd voor koffie en konden ons dan samen opwinden over het wel en wee in het UMC. **Dieta**, gezellig dat we mijn laatste 6 maanden weer samen in de kliniek zitten. Ook daarna verwacht ik nog vaak een telefoontje of e-mail waarin je begint met "jij hebt vast wel ...; kan je mij dat even sturen?"

Vele goede vriendinnen, vrienden en familie hebben misschien weinig bijgedragen aan dit proefschrift maar spelen een belangrijke rol in mijn leven. **Floor** en **Marieke**, het "stuck in a moment you can't get out of" ligt inmiddels ver achter ons, maar jullie luisterend oor, relativeringsvermogen (als het mij betreft in ieder geval) en uitstekende gevoel voor humor blijven voor mij van veel waarde. **Anne Marie**, ik kan me geen beter logeeradres voorstellen.

Als jongste in een gezin met twee grote broers, **Arnoud** en **Maurits**, had ik het waarschijnlijk het gemakkelijkste. Jullie hebben je zolang als ik mij herinner met mijn opvoeding bemoeid en zorgden ervoor dat ik niet al te meisjesachtig en bovenal niet al te verwend werd, hoewel anderen daar misschien anders over denken. Bovendien gingen jullie mij voor als promovendus of medisch specialist.

Ik vond al snel dat ik zelf prima kon bepalen wat het beste voor mij was, maar ben erachter gekomen dat mijn **ouders** in het verleden wel eens de wijste zijn geweest. Jullie stimuleerden mij steeds het maximale te bereiken (hoewel ik zelf een zes vaak goed genoeg vond).

Lieve **Bart**, wat een fantastisch vooruitzicht al die jaren die wij nog, samen met **Justus** en hopelijk ook vaker met **Boris**, gaan leven.