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Genetic analysis of *tuberous-sclerosis genes 1* and *2* in nonlesional focal epilepsy

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ABSTRACT

Germline mutations of *TSC1* (harmartin) and *TSC2* (tuberin) are known to cause tuberous sclerosis (TSC), an autosomal dominant disorder with severe neurological and systemic manifestations. In addition, increasing data indicate aberrant patterns of allelic variants in patients with lesion-associated epilepsy, but absence of other stigmata of TSC. Animal models of TSC suggested that mutations in the *TSC2* gene, even in absence of manifest neuropathological changes, induce aberrant neuronal activity. On this basis, we have carried out a mutational analysis of *TSC1* and *TSC2* in patients with pharmarcoresistant focal epilepsy without evidence of epileptogenic lesions on neuroradiological and histopathological examination ($n = 10$). SSCP analysis revealed an allelic variant of *TSC2* to be significantly increased (exon 41: 50.0% vs controls 14%, $P = 0.0132$), which previously was reported to be increased in gangliogliomas and mineralized focal cortical dysplasia as well. Our data suggest allelic imbalances of *TSC2* in nonlesional focal epileptic tissue.

1. Introduction

Germline mutations of the *TSC1* (harmartin) and *TSC2* (tuberin) genes have been identified as underlying causes of tuberous sclerosis (TSC) [1,2]. Hamartin and tuberin constitute a tumor suppressor complex with a central role in the PI3K–mTOR pathway [3]. Although patients with epileptogenic lesions such as cortical malformations and glioneuronal tumors do not present stigmata associated with tuberous sclerosis, several studies have revealed aberrant patterns of allelic variants in the TSC1 and TSC2 genes of patients with focal cortical dysplasia (FCD) or gangliogliomas [4–6].

Data from animal models of TSC reveal that mutations in the *TSC2* gene, even in the absence of neuropathological changes, can induce cognitive deficits and alterations of synaptic plasticity [7]. Patients without evidence of epileptogenic lesions on either magnetic resonance imaging (MRI-negative patients) or histopathological examination (histo-negative patients) form a distinct and rather small but clinically important and difficult-to-manage subgroup in the heterogeneous sample of patients with pharmacoresistant focal epilepsy [8]. The main findings of a recent study analyzing a cohort of approximately 1200 patients who underwent comprehensive presurgical assessment at Bonn-Neurocenter were that MRI-negative patients are less often operated than patients with MRI-visible lesions and that histo-negative patients with epilepsy less frequently achieve a seizure-free outcome [8]. The pathogenetic basis of histo-negative epileptogenic foci in patients with pharmacoresistant epilepsy is virtually enigmatic. Here, we describe our mutational analysis of *TSC1* and *TSC2* of 10 MRI- and histo-negative patients.

2. Materials and methods

2.1. Presurgical assessment

As part of our standard practice, patients underwent 1.5- or 3-T brain MRI, neuropsychological tests, and video/EEG monitoring using conventional scalp EEG recordings (10–20 system) or intracranial recordings [9]. The presurgical neuropsychological evaluation focused primarily on executive functions and memory functions as previously described [10]. The psychometric test results were standardized according to normative data from 488 healthy subjects who all underwent the same test battery. Details are provided in the Supplementary Material (see Appendix).

2.2. Surgical specimens

Biopsy samples were gained from patients with chronic pharmacoresistant epilepsy who underwent surgical treatment at the University of Bonn Medical Center, Germany ($n = 20$). In each patient surgical removal of the epileptogenic focus was deemed essential to

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achieve seizure control according to established guidelines described in detail before [9]. All patients included in our study gave written and informed consent for further scientific investigations. All procedures were carried out in accordance with the Declaration of Helsinki and approved by the local ethics committee. Surgical specimens were fixed in formaldehyde overnight and embedded in paraffin. Macroscopic and histopathological examinations were performed by experienced neuropathologists at the University of Bonn. The microscopic examination of the specimens from the group with MRI-negative epilepsy ($n=10$) yielded nonspecific findings (Table 1). Although in some cases heterotopic neurons in white matter or blurring of the gray–white matter junction was present, no lesions related to the clinicopathological spectrum of focal cortical dysplasias, as recently reclassified by Blümcke and co-workers, were observed [11]. Hippocampal tissue from patients with neuroradiological and neuropathological evidence of hippocampal sclerosis served as the "epilepsy control group" ($n=10$) (patient parameters are provided in Supplemental Table 1—see Appendix).

2.3. DNA isolation, polymerase chain reaction, single-strand conformation polymorphism and sequence analyses

Starting from formalin-fixed (overnight fixation in biopsy samples, 2- to 4-week fixation interval in autopsy samples) and paraffin-embedded tissue sections we performed DNA extraction using the QIAamp DNA Minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA isolated from 100 blood samples and 8 hippocampal autopsy samples of Caucasians without central nervous system diseases served as nonepileptic brain tissue controls (Supplemental Table 2—see Appendix). Polymerase chain reaction (PCR) was carried out as described before in an automated thermocycler (Uno Block, Biometra, Göttingen, Germany) [5]. Single-strand conformation polymorphism (SSCP) and sequence analyses of PCR products were performed according to standard protocols described before [5]. Further details are given in the Supplementary Material (see Appendix).

2.4. Statistical analysis

The two-tailed Fisher's exact test was applied to define the significance of each sequence alteration in the pathological specimens compared with controls. The cutoff for significance was set at a two-sided $P<0.05$.

3. Results

According to the inclusion criteria the 10 patients with MRI-negative epilepsy showed no specific epileptogenic lesion on microscopic examination and were classified as having histo-negative epilepsy (Fig. 1). All exons of the *TSC1* and *TSC2* genes were screened for sequence alterations by SSCP analysis. Frequencies of allelic variants were compared with those obtained from 100 unaffected control individuals. The GenBank AF013168 served as reference. Sequence alterations were discovered in six patients. In the *TSC1* gene two nucleotide polymorphisms were observed that had been reported previously [12].

One of these polymorphisms located in exon 14/intron 13 was detected in two patients. It was identified as a combination of a silent base exchange (A to G) at position 1556 in exon 14 (Glu, codon 445) and a noncoding base exchange (C to G) at position 1555–55 in intron 13. Its frequency was not significantly increased (20.0%) compared with that of controls (15.0%).

The second polymorphism occurring within our reference group in *TSC1* was located in exon 22. It did not reveal a significant frequency (10.0%, controls: 25.0%), and presented as a base exchange C to T at nucleotide 3050 (Ala, codon 943).

On the contrary we found a significantly increased silent polymorphism in the *TSC2* gene, which encodes a G-to-C exchange at nucleotide 5346 in exon 41 (50.0% vs controls 14%, $P=0.0132$) (Fig. 2). Former studies reported this polymorphism to be significantly increased in gangliogliomas and mineralized focal cortical dysplasia with balloon cells, as well [5] (Fig. 3).

An additional base exchange was found in intron 31 that was also described previously [13]. It is characterized by a C-to-G base transition at nucleotide 3902–56, and did not reveal a significantly increased frequency (20.0%) compared with controls (11.0%).

Sequence analysis of exon 41 (*TSC2*) in patients with temporal lobe epilepsy on the basis of hippocampal sclerosis did not show allelic variants/polymorphisms to be present here. We observed the same result in a series of autopsy controls (Supplemental Tables 1 and 2—see Appendix).

Neuropsychological evaluation revealed below-average performance regarding executive functions as well as memory functions in every MRI- and histo-negative patient in at least one test (Supplemental Table 3—see Appendix).

4. Discussion

Here we report on a mutational analysis of *TSC1* and *TSC2* in patients with therapy-refractory epilepsy in whom specific epilepsy-associated lesions were not located on either MRI or histopathological examination. Those patients represent an important subgroup within the group of patients with focal epilepsy and have a rather poor clinical outcome [8]. Intriguingly, our present genetic analysis of biopsy specimens of the clinically assumed epileptogenic focus

Table 1
Individual parameters of the patients with nonlesional epilepsy.

Patient ID/sex	Age at epilepsy onset/sugery	Seizure type	Resection	Outcome	Neuropathological diagnosis	Sequence alterations			
						TSC 1		TSC 2	
						e14	e22	i31	e41
1/F	31/39	CPS[a]	Standard AMTL-L	Seizure free	GWMA, neuronal heterotopias	+	–	+	+
2/F	30/60	CPS	Standard AMTL-R	Seizure free	Astrogliosis	–	–	–	–
3/M	10/30	CPS	Standard AMTL-L	Unchanged	GWMA	–	–	–	–
4/F	13/29	CPS, sGTCS	Standard AMTL-L	Unchanged	Astrogliosis	–	–	–	+
5/M	2/11	CPS	Standard AMTL-R	Unchanged	GWMA, neuronal heterotopias	–	–	–	–
6/M	4/11	SPS, CPS, sGTCS	Tailored AMTL-L	1 year seizure free, then relapse, new semiology	GWMA	–	–	+	+
7/M	13/30	SPS, CPS, sGTCS	Standard AMTL-R	1 year seizure free, then relapse	Astrogliosis	+	–	–	–
8/M	19/28	SPS, CPS, sGTCS	SAH-L	2.5 years seizure free, then relapse	Astrogliosis	–	+	–	+
9/M	10/24	CPS, sGTCS	Fronto- medial-R	Reduction, semiology unchanged	No pathological findings	–	–	–	+
10/M	6/34	SPS, CPS, sGTCS	Parieto-occipital-L	Unchanged	Astrogliosis, neuronal heterotopias	–	–	–	–

[a] CPS, complex partial seizures; SPS, simple partial seizures; sGTCS, secondarily generalized tonic–clonic seizures; AMTL, anteromedial temporal lobe resection; SAH, selective amygdalo-hippocampectomy; GWMA, gray–white matter abnormallities; e14, exon 14/intron 13; e22, exon 22; i31, intron 31, e41, exon 41.