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ORIGINAL ARTICLE

Mutational analysis of *GABRG2* in a Japanese cohort with childhood epilepsies

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A few mutations in the gene encoding the gamma 2 subunit, *GABRG2*, of the gamma-aminobutyric acid receptor type A have been reported in various types of epilepsy. The aim of this study is to investigate the role of *GABRG2* in the pathogenesis of childhood epilepsy in a large Japanese cohort. Genetic analysis of *GABRG2* was performed on 140 Japanese patients with various childhood epilepsies largely including Dravet syndrome and genetic epilepsy with febrile seizures plus. The mutational analysis identified one novel missense mutation of *GABRG2* (c.236A>G: p.N40S) in a patient with generalized tonic-clonic seizures (GTCS). The mutation was heterozygous and replacing a highly conserved Asn residue with a Ser. The affected amino acid was located at residue 40 of the mature *GABRG2* protein, which was near the first one of two high-affinity benzodiazepine-binding domains of the γ2 subunit (Lys-41-Trp-82). This mutation in such an important position may hamper the function of the channel and contribute to the case's pathogenesis of GTCS.
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Keywords: epilepsy; GABRG2; genetics; mutation

INTRODUCTION

Epilepsy is a group of heterogeneous disorders characterized by paroxysms resulting from bioelectric hyperexcitation of neuronal networks of the brain. Recently, it has been well recognized that dysfunctions of ion channels expressed in the brain contribute to such hyperexcitation and hence closely associate with the pathogenesis of epilepsy. Accordingly, to date, a number of mutations of the genes encoding ion channels have been identified in various types of epilepsy.

Gamma-aminobutyric acid receptor type A ($GABA_A$ receptor) is one such ion channel where mutations have been identified in several epilepsy phenotypes. $GABA_A$ receptor, which is a ligand-gated chloride ion channel, serves as a major component of the neuronal inhibitory system in adult brain. It is considered that majority of the receptors in the brain function as a pentamer consisting of α1, β2 and γ2 subunits.

Mutations of the genes encoding the subunits of $GABA_A$ receptors were so far found in the genes encoding α1 (*GABRA1*), δ (*GABRD*) and γ2 subunits (*GABRG2*).[1–4] A mutation of *GABRA1* was found in autosomal dominant juvenile myoclonic epilepsy, a rare inherited idiopathic epilepsy phenotype.[2] Several variants of *GABRD* were not reported as causes of epilepsy but suggested to associate with susceptibility to genetic epilepsy with febrile seizures plus (GEFS+).[3] In contrast, mutations in *GABRG2* have been reported as causes of a wide spectrum of epilepsies, from Dravet syndrome to milder conditions such as childhood absence epilepsy, GEFS+ and FS+.[1,4–8]

Although major progress has been made in tying *GABRG2* to some categories of epilepsy (that is, GEFS+, childhood absence epilepsy and Dravet syndrome), its role in most kinds of childhood epilepsy such as benign epilepsy of childhood with centrotemporal spikes, generalized tonic-clonic seizures (GTCS), partial epilepsy and so on remains poorly understood. Accordingly, this study further investigates the role of *GABRG2* in the pathogenesis of childhood epilepsy.

MATERIALS AND METHODS

Patients

Our study included 140 pediatric patients who had been diagnosed with epilepsy at various departments of neurology in regional tertiary pediatric hospitals (Table 1). The epilepsy phenotypes included Dravet syndrome, GEFS+, GTCS, partial epilepsy, juvenile myoclonic epilepsy, benign familial neonatal seizures, childhood absence epilepsy, benign epilepsy of childhood with centrotemporal spikes, epilepsy with continuous spikes and waves during slow sleep and progressive myoclonus epilepsy (PME). We also recruited 48 healthy Japanese volunteers as the ethnic matched control group. Each patient

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Table 1 Clinical and genetic characteristics of the patients

		GABRG2 variants				
Diagnosis	*Number*	*c.236 A>G*	*c.315 C>T*	*c.588 C>T*	*c.1254C>T*	*IVS1+18del T*
Dravet syndrome	55	0	33	38	2	1
GEFS+, FS+	36	0	14	27	0	0
BECCT	2	0	1	2	0	0
BFNC	7	0	3	5	0	0
CAE	5	0	2	3	1	0
CSWS	1	0	1	1	0	0
GTCS	6	1	3	4	0	0
JME	9	0	5	9	0	0
PME	1	0	1	0	0	0
PS	12	0	6	11	0	0
WEST syndrome	6	0	5	5	0	0
Control	48	0	22	39	0	0

Abbreviations: BECCT, benign epilepsy of childhood with centrotemporal spikes; BFNC, benign familial neonatal convulsions; CAE, childhood absence epilepsy; CSWS, epilepsy with continuous spikes and waves during slow sleep; FS+, febrile seizures plus; GEFS+, genetic epilepsy with febrile seizures plus; GTCS, generalized tonic-clonic seizures; JME, juvenile myoclonic epilepsy; PME, progressive myoclonus epilepsy; PS, partial epilepsy.

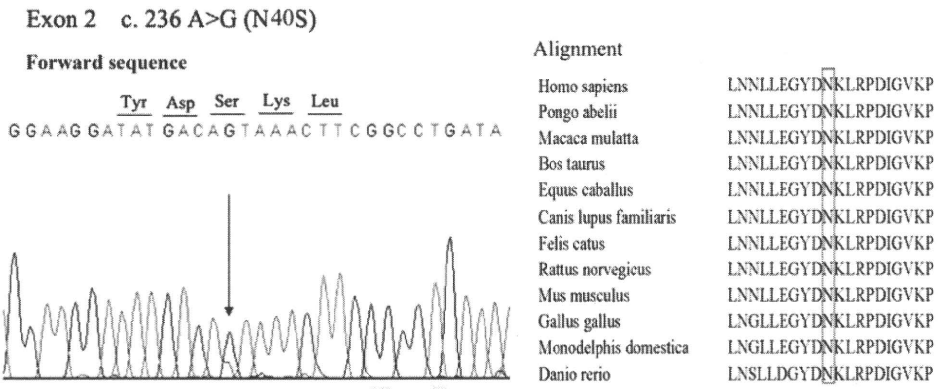

Figure 1 *GABRG2* mutation and alignment of affected amino acid. Arrow indicates where mutation occurs. Rectangular box represents the corresponding amino acid to the amino acid where the mutation occurs, that is highly conserved throughout different species.

or parent/guardian signed an informed consent form approved by the Ethics Review Committee of Fukuoka University or similar committees of the participating institutions.

Genetic analysis

Using QIAamp DNA Blood kit (Qiagen, Hilden, Germany), genomic DNAs were prepared from ethylenediaminetetraacetic acid-treated whole blood samples. Genetic abnormalities were sought within all 10 exons of *GABRG2* and their flanking intronic splice sites by a direct sequencing method with an automatic sequencer as described earlier.[9,10] Details of the PCR conditions and the primers used are available on request. Reference sequences of mRNA were based on information available from RefSeq, accession numbers: Human *GABRG2*, NM_198904.

Statistical analysis

To determine whether the polymorphisms were involved in the pathogenesis of epilepsy, we determined the genotype and allele frequency of the polymorphisms in our patients and in the control population. Data analysis was performed by Fisher's exact test using an SPSS software package (version 13.0). All *P*-values were two-tailed. The significance level was considered to be 5%.

RESULTS

Mutational analysis for the 140 patients revealed one novel missense mutation (c.236A>G: p.N40S, Figure 1) in one of six patients with GTCS. The mutation was heterozygous and not found in other patients of the cohort or in the 48 ethnic matched control individuals. The DNA of the parents of the patient was not available. The patient was a girl whose first seizure occurred when she was 15-years old, and after that re-occurred once a month. She exhibited the typical seizure and electroencephalographic phenotype and susceptibility to antiepileptic drugs of GTCS supporting that the diagnosis of GTCS. She had no mental retardation or other neurological disorder. Family history of febrile seizures or epilepsy was not found in parents of the patient. The Asn residue at the position of 40, which was replaced with Ser by the mutation, is a highly conserved amino acid throughout many species (Figure 1).

Three exonic variants were found on exon 3 (rs11135176, n=74), exon 5 (rs211037, n=105) and exon 10 (c.1254 C/T: p.D379D, n=3), and one intronic variant (IVS1+18delT, n=1) was found (Figure 2; Table 1). Variants of rs11135176 and rs211037 were also found in the control population. None of the variants led to any amino-acid changes in the protein sequence. Fisher's exact test showed no

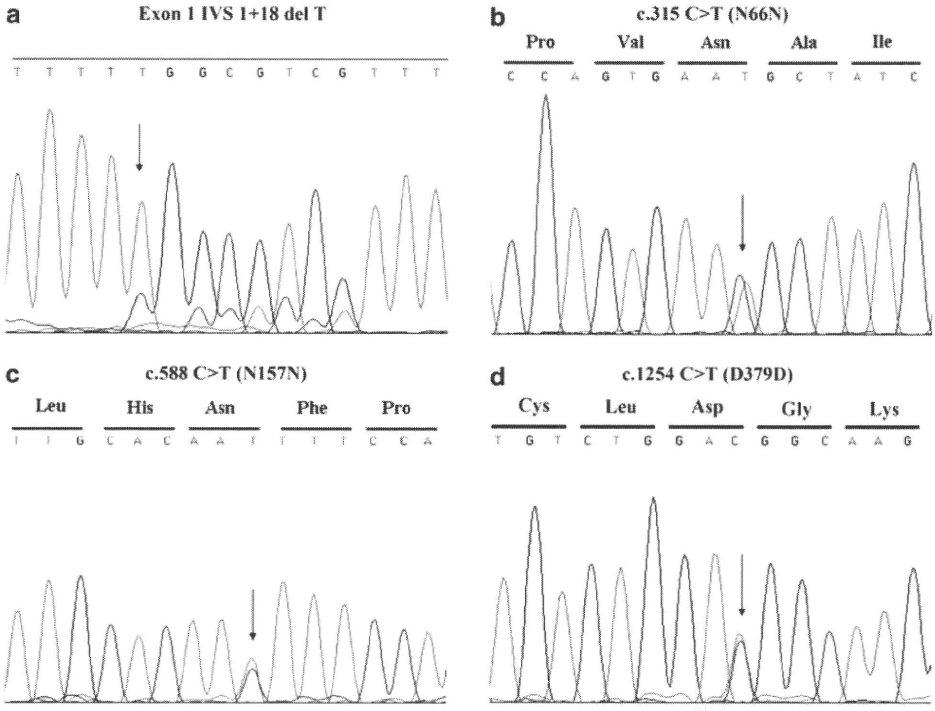

Figure 2 Four *GABRG2* variants are identified. Arrows indicate where variants occur. Along these variants there are two known SNPs (rs211037 and rs11135176) and one new SNP (c.1254 C>T).

Table 2 Distribution of polymorphisms in the *GABRG2* gene in patients and controls

		Genotype count (frequency)				*Allele count (frequency)*		
cDNA nucleotide	*Population*	*CC*	*CT*	*TT*	P-*value*[a]	*C*	*T*	P-*value*[b]
c.315C>T	Patients	66 (0.47)	61 (0.44)	13 (0.09)	0.49	193 (0.69)	87 (0.31)	0.30
	Control	26 (0.54)	20 (0.42)	2 (0.04)		72 (0.75)	24 (0.25)	
c.588C>T	Patients	35 (0.25)	67 (0.48)	38 (0.27)	0.69	137 (0.49)	143 (0.51)	0.41
	Control	9 (0.19)	24 (0.50)	15 (0.31)		42 (0.44)	54 (0.56)	
c.1254C>T	Patients	137 (0.98)	3 (0.02)	0 (0.00)	0.57	277 (0.99)	3 (0.01)	0.57
	Control	48 (1.00)	0 (0.00)	0 (0.00)		96 (1.00)	0 (0.00)	

[a]Genotype counts.
[b]Allele counts are compared between patients and control population by Fisher's exact test.

significant differences ($P>0.05$) in the genotype and allele frequency between the two groups (Table 2).

DISCUSSION

This study reports one novel missense mutation of *GABRG2* (c.236 A>G: p.N40S) in a female with the typical GTCS phenotype. The mutation was heterozygous and not found in 48 ethnic matched control samples. No other relevant genetic variations were found in the 140 patients. To our knowledge, this is the first report of *GABRG2* mutation in GTCS providing compelling evidence of the involvement of *GABRG2* in epilepsy.

$GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the central nervous system, and several anti-epileptic drugs including benzodiazepines, barbiturates and neurosteroids act by enhancing $GABA_A$ receptor currents. These inhibitory receptors are pentamers formed by assembly of multiple subunit subtypes and the $\alpha1\beta2\gamma2$ receptor is the most abundant receptor isoform. The $\gamma2$ subunit contributes to receptors involved in both phasic and tonic inhibition and underlies the benzodiazepine sensitivity of both modes of inhibition.[11] It is also critical for receptor trafficking, clustering and synaptic maintenance.[12,13] Up to date, at least seven mutations of *GABRG2* including missense mutations, nonsense mutations and splice-site mutation have been associated with a broad spectrum of epilepsies.[1,4–8,14]

The N40S mutation identified in this study affects a highly conserved Asn at residue 40 of the mature $\gamma2$ subunit, thus, the mutation is adjacent to the first one of the two high-affinity benzodiazepine-binding domains of the $\gamma2$ subunit (Lys-41-Trp-82 in the mature $\gamma2$ subunit). Wallace *et al.*[15] had suggested that R43Q mutation in the benzodiazepine-binding site can attenuate benzodiazepine sensitivity of $GABA_A$ receptor. Another study on this mutation was shown to accelerate deactivation of the receptor.[16] These lines of evidence support that the N40S mutation should attenuate the $GABA_A$ receptor functions whereby increasing the intracortical

excitability of the brain. This notion accords with the recent findings with the *GABRG2* mutations in the N-terminal domain.[17]

In addition, Kang and Macdonald[18] suggest that with heterozygous expression, the R43Q mutation may result in impaired receptor trafficking and increased retention of the receptor in intracellular compartments, including the ER. This reduced cell surface expression would result in decreased inhibitory $GABA_A$ receptor current *in vivo*, and consequently, an increase in neuronal excitability and epilepsy. Several subsequent studies have identified the retention of mutant receptors in the ER[14] and shown that *GABRG2* mutations have reduced trafficking either to the membrane surface with relatively normal function[18,19] or to the surface with impaired function.[15,20] It is believed that the main electrophysiological deficit of $GABA_A$ receptor resulting from the mutation is due to intracellular trafficking abnormality of channel molecules. Thus, the N40S mutation may result in an aberrant trafficking of the $GABA_A$ receptor. We anticipate that this mutation N40S may contribute to the patient's pathogenesis of GTCS.

Recently, Wang *et al.*[21] reported that the *GABRG2* polymorphism rs211014A allele was higher in the febrile seizures group ($P<0.005$), suggesting that *GABRG2* polymorphisms may predict susceptibility of febrile seizures. Another study[22] found that children with the *GABRG2* (SNP211037)-C allele had a higher incidence of idiopathic generalized epilepsies, indicating that the *GABRG2* (SNP211037)-C allele is a candidate genetic marker for idiopathic generalized epilepsies. In this study, three exonic variants and one intronic variant are identified. Among these variants, c.1254C/T is a novel polymorphism. None of these variants leads to any amino-acid changes in the protein sequence. However, in this study, there are no significant differences for these polymorphisms in genotype and allele frequency between patients and control population, suggesting that they are not involved in the etiology of Japanese childhood epilepsy.

In summary, one novel missense mutation of *GABRG2* (N40S) was identified in a patient with GTCS. To our knowledge, there have been no previous reports of mutation of *GABRG2* in GTCS. This finding indicates that mutation *GABRG2* can underlie the pathogenesis of GTCS and thus reinforces the involvement of $GABA_A$ receptors in epilepsy. However, the contribution of mutations of *GABRG2 per se* to the pathogenesis of epilepsy remains unclear because we do not have sufficient patients. Further studies will involve gathering more cases especially GTCS, progressive myoclonus epilepsy patients and performing the *GABRG2* analysis.

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Review article

Epilepsies and epileptic syndromes starting in the neonatal period

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Abstract

As seizures in the neonatal period have generally been identified only by direct clinical observation, there is frequently a lack of objectivity as to whether seizures are categorized as epilepsies or non-epilepsies. A major characteristic of neonatal seizures is electro-clinical dissociation and some electro-graphic seizures do not produce clinical symptoms. It is difficult to correctly identify real epilepsies or epileptic syndromes in the neonatal period without ictal electroencephalogram (EEG). Some epileptic syndromes starting in the neonatal period such as early myoclonic encephalopathy, Ohtahara syndrome, or migrating partial seizures in infancy are categorized as malignant epilepsies. A suppression-burst EEG pattern (SBP) is usually seen in neonates with serious brain damage, malignant epileptic syndromes or other neurological conditions. However SBP has not been consistently defined in the literature. We review malignant epilepsies and benign familial and non-familial neonatal seizures starting in the neonatal period and propose the characteristics of SBP in Ohtahara syndrome. Epileptic encephalopathies with SBP in the neonatal period are known to evolve into relatively few types of epileptic syndromes. We emphasize the importance of ictal EEG for diagnosis and treatment of malignant epilepsies and epileptic syndromes in the neonatal period.

Keywords: Neonatal seizures; Epilepsy; Epileptic syndromes; Ictal EEG; Suppression-burst EEG pattern

1. Introduction

Epilepsies and epileptic syndromes in the neonatal period are usually age-related and characterized by a variety of behavioral seizure manifestations, distinctive electroencephalogram (EEG) patterns, and the natural history of neonatal seizures has good or poor outcomes that often start in early life. The underlying etiologies of neonatal seizures are diverse including the genetic basis of the disorders. Neonatal seizures have generally been identified in most NICUs only by direct clinical observation. There is usually a lack of objectivity as to whether seizures are categorized as epilepsies or non-epilepsies. A common and important feature of neonatal seizures is electro-clinical dissociation, with some electro-graphic seizures showing no clinical symptoms [1]. Recent studies have revealed that ictal EEG recording is essential for the identification of true neonatal seizures of cortical origin [2]. This paper is divided into three parts. The first part deals with the problems of diagnosis and classification of neonatal seizures. The second part concerns epilepsies and epileptic syndromes starting in the neonatal period. The last part relates a variety of suppression-burst EEG patterns seen in patients with malignant epileptic syndromes such as Ohtahara syndrome and early myoclonic encephalopathy or other neurological conditions.

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2. The problems of diagnosis and classification of neonatal seizures

A major characteristic of neonatal seizures is electro-clinical dissociation and electro-graphic seizures are not consistently associated with clinical symptoms. Many of the abnormal movements identified by neonatologists as being neonatal seizures in NICUs are often not accompanied by paroxysmal discharges. These movements may reflect brainstem release phenomena, which are not true epileptic seizures of cortical origin. Although these movements are not epileptic, their presence usually reflects a severe underlying brain insult, with an increased risk of subsequent epilepsies.

It is difficult to correctly identify true epilepsies or epileptic syndromes in the neonatal period without ictal EEG recording. Most neonatal seizures are acute symptomatic and do not persist long [3,4]. Seizures of the newborn rarely lead to chronic epilepsy or epileptic syndromes. Clinical observation of seizure symptomatology, unaccompanied by EEG confirmation, has severe limitations [5]. Accordingly, EEG plays an important part in understanding the nature of paroxysmal movements in neonates and in differentiating epileptic seizures from non-epileptic events [6]. If we see abnormal seizure-like movements or behaviors which are suspected neonatal seizures (tonic, clonic, tonic clonic, myoclonic, pedaling, crawling, spasm, abnormal eye movements or sucking) or abnormal seizure-like events which are suspected neonatal seizures (apnea, vomiting, tachypnea, bradycardia or flushing), we need to record ictal EEGs to identify real neonatal seizures of cortical origin and classify them correctly. Major characteristics of ictal EEG findings in seizures are sudden rhythmic, repetitive, and stereotyped discharges lasting for at least 10 s on two or more EEG channels (Figs. 1 and 2) and more detailed explanation concerning the ictal EEG in the context of neonatal seizures is described in representative literature [7,8].

3. The epilepsies and epileptic syndromes starting in the neonatal period

3.1. Idiopathic neonatal seizures as chronic epilepsy

3.1.1. Benign familial neonatal seizures

Benign familial neonatal seizures (BFNS) is characterized by: onset within the first week of life, initial tonic phase with cyanosis followed by clonic movements of the whole body, family history of neonatal seizures, normal psychomotor development, normal interictal EEG, and favorable outcome (but absence of subsequent epilepsy is still under discussion). The first BFNS family with eight cases over three generations was reported by Rett and Teubel [9]. The probandus male patient developed an initial tonic phase with cyanosis followed by clonic movements of the whole body including the face and eye muscles on the third day of life and he had more seizure events on the following day. A brother born 16 months later had similar seizures. Several interictal EEGs were reported for these two patients and single EEGs for three other affected relatives. The authors noted familial history, the normal interictal EEGs and the favorable outcome. On the EEG, seizures started with a generalized flattening of the background activity followed by focal or generalized spikes and slow waves lasting as long as the clinical manifestations. A prolonged flattening of the EEG could follow the seizures. In the ictal EEGs of BFNS reported by Hirsch et al., the length of the flattening could vary from 5 to 9 s and the complete seizure lasted from 59 to 155 s. All these individuals had BFNS mapped in chromosome 20 [10]. No guideline has been proposed concerning the treatment of BFNS. Most patients were given phenobarbital (PB) for a duration of 2–6 months, rarely longer.

3.1.2. Benign idiopathic (non-familial) neonatal seizures

Benign idiopathic (non-familial) neonatal seizures (BINS) is characterized by: seizures occurring between day 1 and day 7 after birth, mainly clonic seizures and mostly partial and/or apneic, seizures that last for 1–3 min, usually normal neurological state, normal interictal EEG, and no family history. North et al. reported apneic seizures in 31% of their cases [11]. Clonic seizures were often lateralized, starting on one side then affecting the other side, and rarely of a generalized type. They lasted from 1–3 min. They were frequently repeated, leading to a status epilepticus. The mean duration of status epilepticus was about 20 h, but could be shorter. The neurological state of the infants is usually normal at the onset of seizures. Then infants become drowsy and hypotonic, the various anti-epileptic drugs given to stop seizures being partly responsible for this evolution. Drowsiness and hypotonia may last for several days after the end of status epilepticus. Then the infants recover a normal neurological state. Several anti-epileptic drugs have been used for BINS, such as PB, phenytoin, diazepam, or chloral hydrate. Treatment has not had a consistent effect on the duration of seizures. If there is no family history of neonatal seizures, a normal or moderately altered interictal EEGs, and no explanatory etiology, the diagnosis of BINS can be proposed, but it is necessary to follow these infants until at least 2 years of age, to assess the normality of their neurological development.

3.2. Symptomatic neonatal seizures as chronic epilepsy

3.2.1. Ohtahara syndrome

Early infantile epileptic encephalopathy with suppression bursts (OS) was first described by Ohtahara

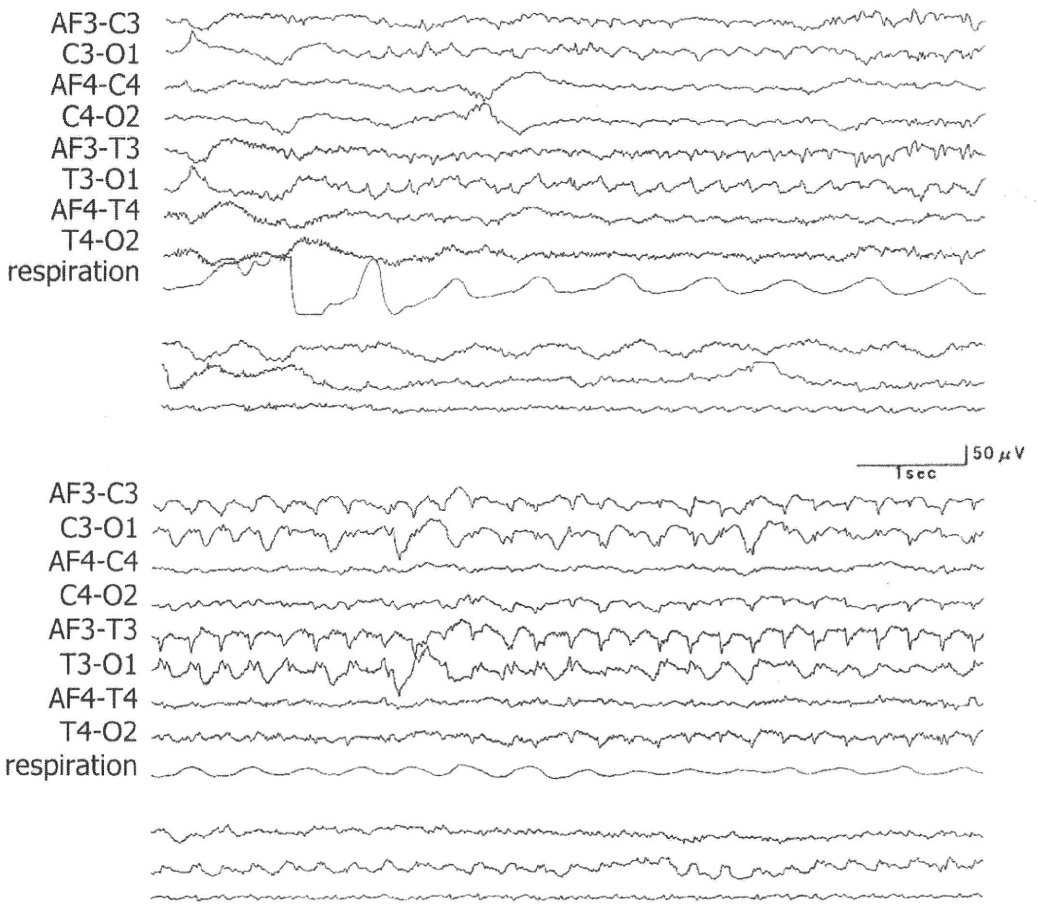

Fig. 1. Ictal EEG findings of neonatal seizures in a 3-day-old girl with hypoxic-ischemic encephalopathy.

and colleagues in 1976, and subsequently was presented as the earliest form of epileptic encephalopathy [12]. Seizures develop within the first 10 days of life in the majority of reported cases of OS, and may occur as early as the first hour after delivery. The seizure types in OS are variable. The most frequent type is spasms, which may be either generalized and symmetrical or lateralized. Spasms may occur singly or in clusters in both awake and asleep states. The duration of spasms is up to 10 s, and the interval between spasms within a cluster ranges from 9 to 15 s. Erratic myoclonus is not a feature of OS. Later in the course, there may be generalized tonic-clonic seizures [13]. Soon after the onset of seizures, the infants become inactive and hypotonic. Their psychomotor development is arrested, and they usually develop severe neurologic abnormalities such as spastic diplegia, hemiplegia, tetraplegia, ataxia, or dystonia. In the etiologies of OS, metabolic disorders were rarely observed. The syndrome is now believed to be mainly attributable to a cerebral malformation. Seventy-five percent of cases subsequently evolve to West syndrome (WS) between 2 and 6 months of age, and later a much smaller number progress to Lennox-Gastaut syndrome. Treatment of OS is usually disappointing. ACTH, zonisamide, levetiracetam or high dose PB may be useful in some cases [14].

3.2.2. A case of OS

The patient was a boy born after 40 weeks of gestation. His birth weight was 2968 g. His perinatal history was unremarkable. His elder half sibling had been diagnosed with OS. He also had a single spasm at 22 days of age. The frequency of spasms increased gradually. EEG at 36 days of age revealed a suppression-burst pattern. He was transferred to our hospital because of clusters of spasms. On admission, generalized hypotonia was recognized. Typical flexor spasms in clusters were observed. No external anomalies were seen except for microcephalus (<-2SDs). Blood examination, metabolic screen, and chromosomal analysis were unremarkable. Head CT and MRI did not reveal abnormal findings. EEG at 45 days of age demonstrated a suppression-burst pattern (Fig. 3). The suppression-burst patterns was constant during both awake and sleep states. He was diagnosed as having OS. TRH therapy was not effective, but his spasms were controlled after ACTH therapy. However, at 3 months of age, he developed focal seizures which were refractory to several

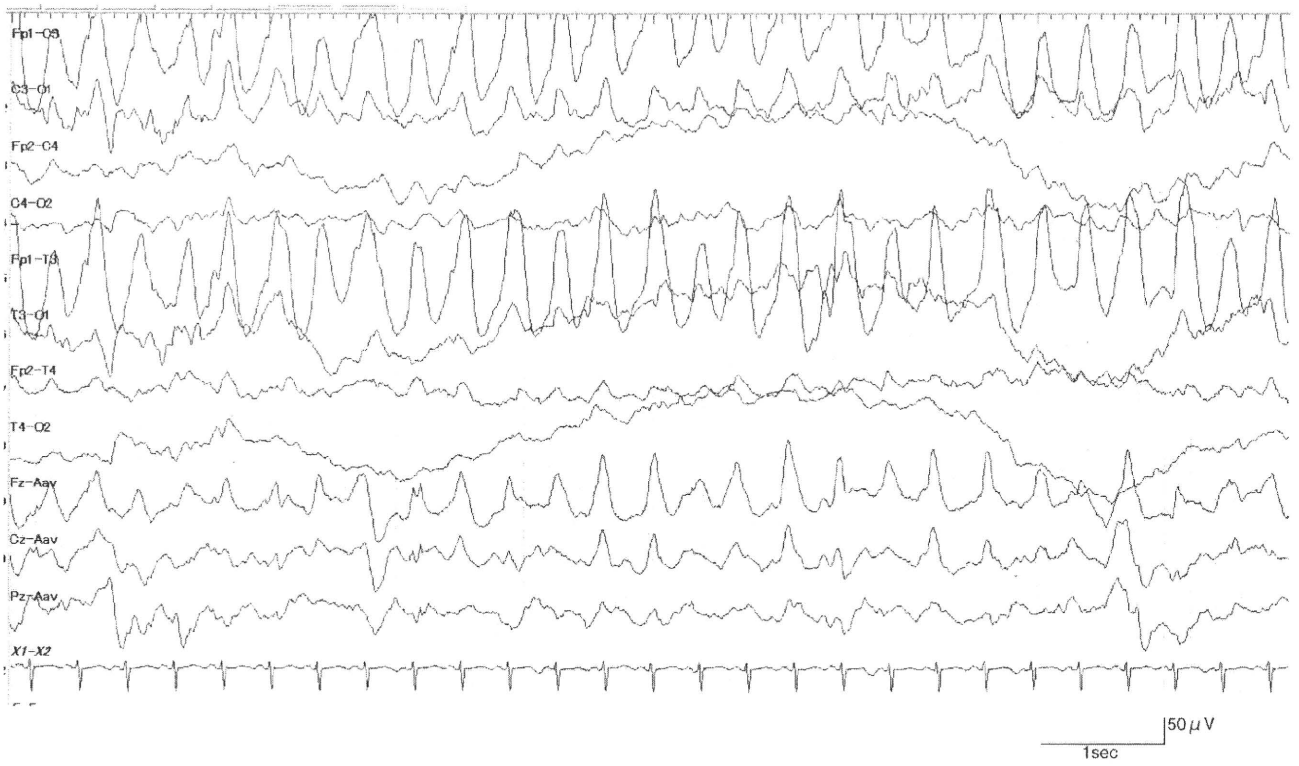

Fig. 2. Ictal EEG findings of neonatal seizures in a 4-day-old boy with hypoxic-ischemic encephalopathy.

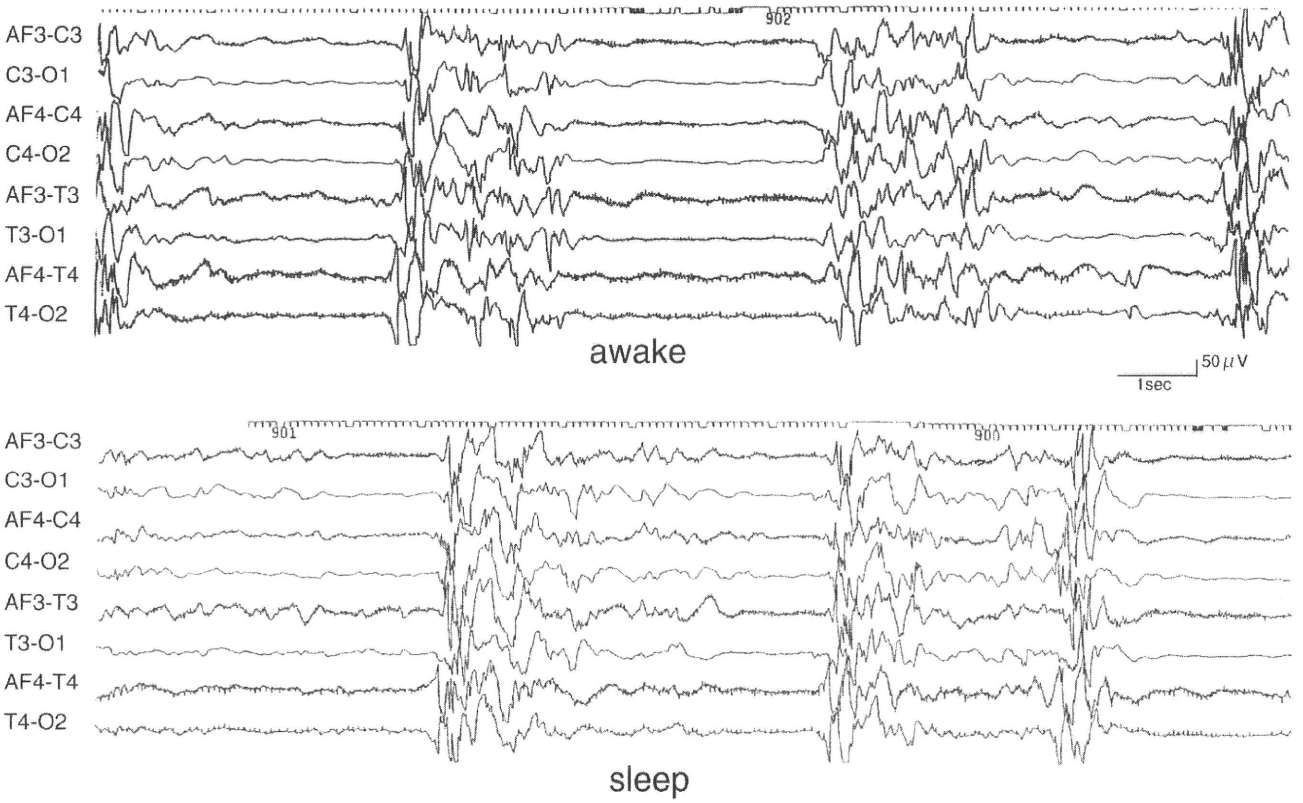

Fig. 3. EEG at 45 days of age demonstrated suppression-burst pattern during both awake and sleep states in a case of OS.

anti-epileptic drugs. His psychomotor development was severely delayed.

3.2.3. Early myoclonic encephalopathy (EME)

EME is associated with onset in early infancy, a suppression-burst EEG pattern, a variety of seizure types, and poor psychomotor outcome [15]. EME is very similar to OS, and was not regarded as a separate entity until 1989. The two entities are now classified in the group of epileptic encephalopathies. The prominent erratic myoclonia (usually non-epileptic) in EME is not present in OS and spasms and tonic seizures predominate in OS. Etiology is also different. Metabolic etiologies are predominant in EME, whereas malformative etiologies predominate in OS.

Major characteristics of EME include onset in early infancy, marked erratic myoclonia (usually non-epileptic), spasms and tonic seizures in later period, etiology is dominated by metabolic disorders, EEG demonstrates suppression-burst pattern mainly noted during sleep (Fig. 4), very poor prognosis, and the evolution of EME is less well documented. SBP in EME is similar to SBP in OS, but the burst phase is shorter with longer periods of suppression phase than OS, and SBP in EME persists in older children. The clinical course of EME is severe and anti-epileptic agents and corticosteroids or ACTH have not been effective.

3.2.4. Migrating partial seizures in infancy (MPSI)

MPSI was first reported by Coppola in 1995 [16]. Major characteristics of MPSI are as follows: normal development before seizure onset, seizures onset before six months without identified etiology, migrating focal motor seizures at onset, nearly continuous and becoming intractable, intractability to conventional anti-epileptic drugs and corticosteroids, and profound delay in psychomotor development. Ictal EEG shows focal rhythmic spike activity shifting from one hemisphere to the other and progressively involving adjacent areas. MPSI usually begins between the first week and seven months of life. Although seizures continue to comprise focal motor features, they become frequent and polymorphous. The seizures tend to occur in clusters with major motor and cognitive deterioration lasting

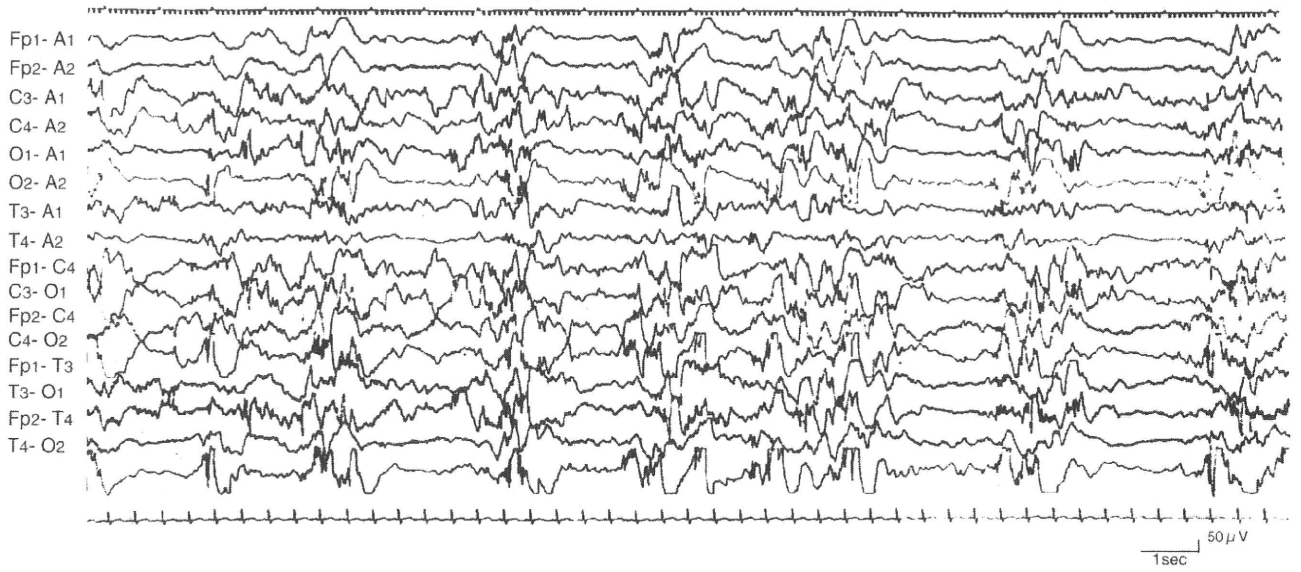

Fig. 4. A suppression-burst EEG pattern seen in a case of EME at 5 months of age.

Table 1
Variation of suppression-burst EEG pattern seen in various conditions.

	OS	EME	SSPE	Barbiturate coma	NS
EEG features	Seen in both during sleep and awake states	Enhances in sleep sate	Mostly seen in awake state	Seen in sleep state	Seen in both during sleep and awake states
	The burst phase is similar to hypsarrhythmia	Short burst phase with longer suppression phase	Short paroxysmal phase with longer suppression phase	Mostly suppression phase	Short paroxysmal phase with longer suppression phase
	Disappears within the first two or three months	Remains longer than OS	Appears in the specific period	During anesthetic state	Disappears within the first one month

OS: Ohtahara syndrome; EME: early myoclonic encephalopathy; SSPE: subacute sclerosing panencepalitis; NS: neonatal seizures with serious brain damage.

several weeks. This initial period lasts from 1 week to 3 months and improves, however it is difficult to judge whether improvement in seizure frequency and severity is due to the effect of anti-epileptic agents or the natural

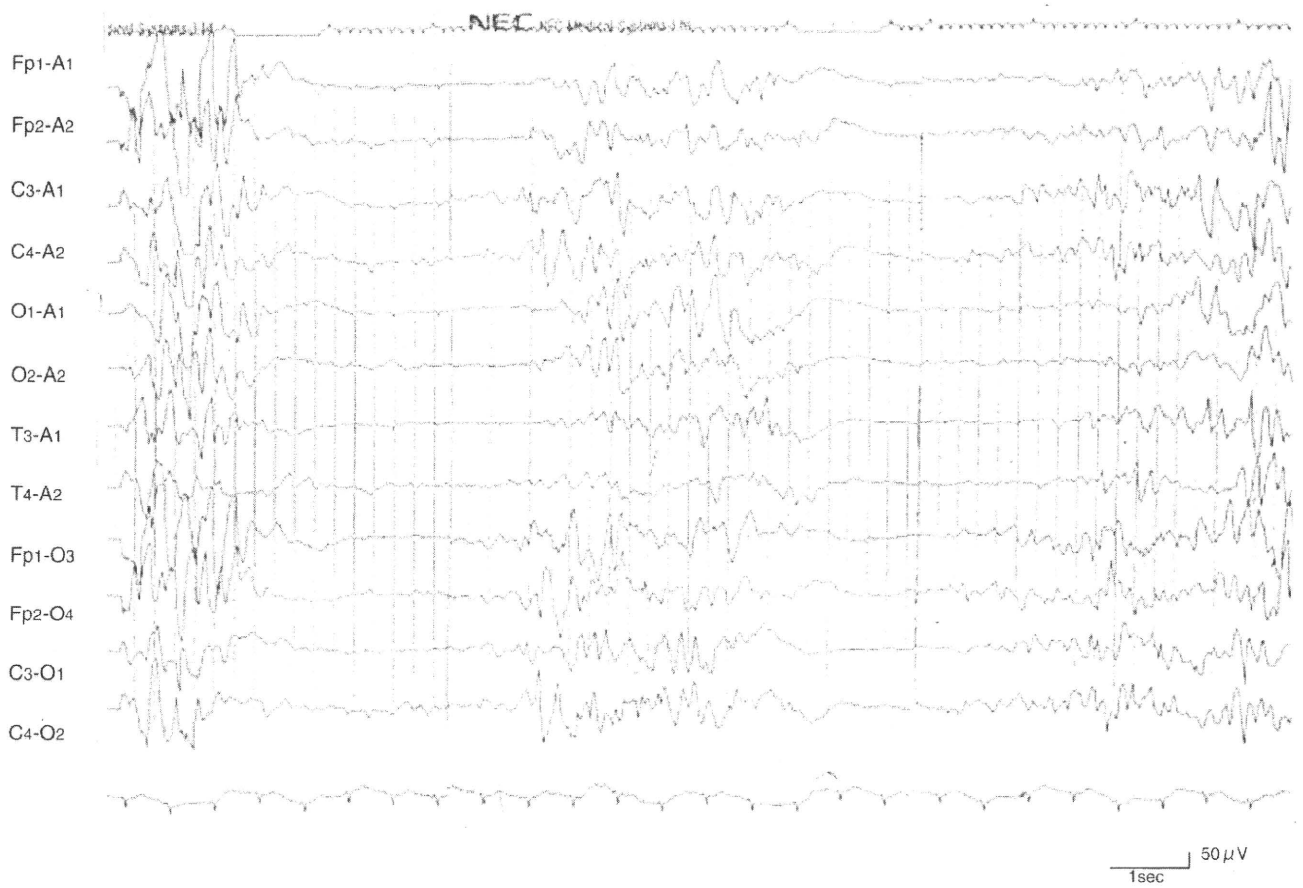

Fig. 5. A suppression-burst EEG pattern seen in a patient with serious brain damage caused by hypoxic ischemic encephalopathy at 7 days of age.

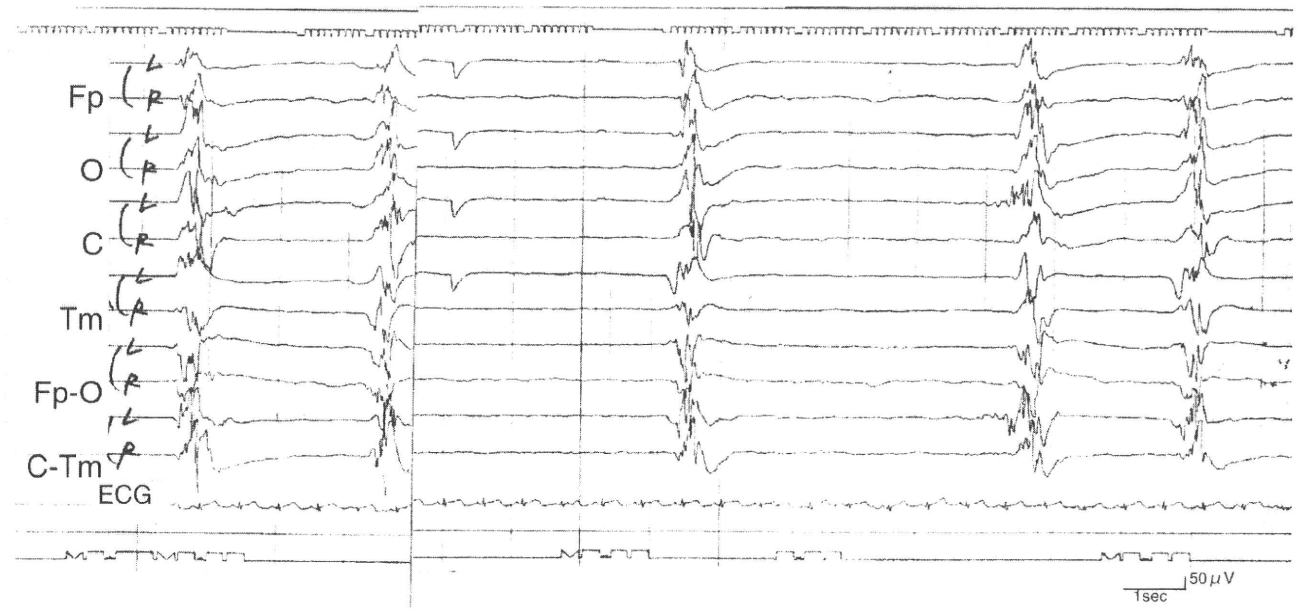

Fig. 6. A suppression-burst EEG pattern seen during barbiturate coma therapy in a 6-month-old boy.

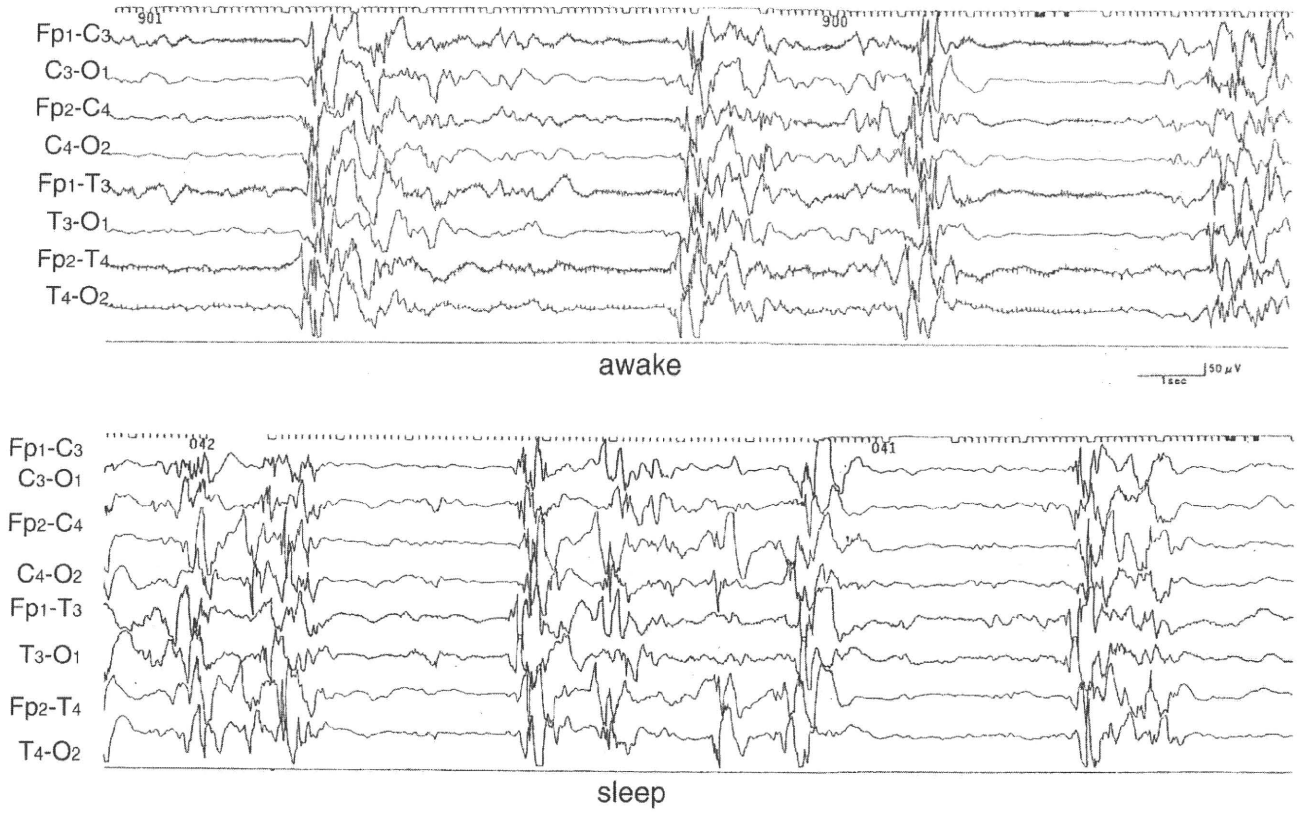

Fig. 7. Suppression-burst EEG pattern of OS in a 1-month-old boy should be seen during both sleep and awake states and should not change according to the sleep-wake cycle.

course of MPIS. Prevailing anti-epileptic agents, corticosteroid and ACTH are ineffective. In some cases, the combination of stiripentol with high-dose clonazepam or bromides was found to be effective [17]. Some patients die by the end of the first year of life, as a consequence of very frequent seizures with respiratory distress.

4. Variation of suppression-burst EEG patterns (SBPs) seen in various neurological conditions

There are several different EEG SBP in different conditions and we showed several representative types of EEG SBP (Table 1). The most characteristic EEG feature peculiar to the syndrome is SBP. SBP is seen in various conditions in the pediatric field, such as OS, EME, subacute sclerosing panencephalitis, serious brain damage in neonates (Fig. 5) or during barbiturate coma therapy (Fig. 6). Although this EEG pattern seen in OS or EME has been described in some literatures [18–20], there is no precise agreed definition. OS is a very rare syndrome and cases reported as OS in the literature may include doubtful cases. SBP seen in EEGs does not always indicate OS. We need to clarify the SBP in EEG seen in definite OS.

We propose the characteristics of SBP in OS as follows. The bursts must consist with high amplitude non-synchronized paroxysms like hypsarrhythmia and continue for 2–6 s. The suppression (low-amplitude) phase must show less than 10 μV or flat tracing and continue for 3–5 s. Suppression and burst phases must appear alternately and regularly every more than five seconds. SBP in OS should be seen in both during sleep and awake states and should not change according to the sleep-wake cycle (Fig. 7) and the burst phase is longer with shorter periods of suppression phase than EME. This pattern in OS usually disappears within the first two or three months. The SBP in OS should be distinguished from SBP seen in EME or other neurological conditions.

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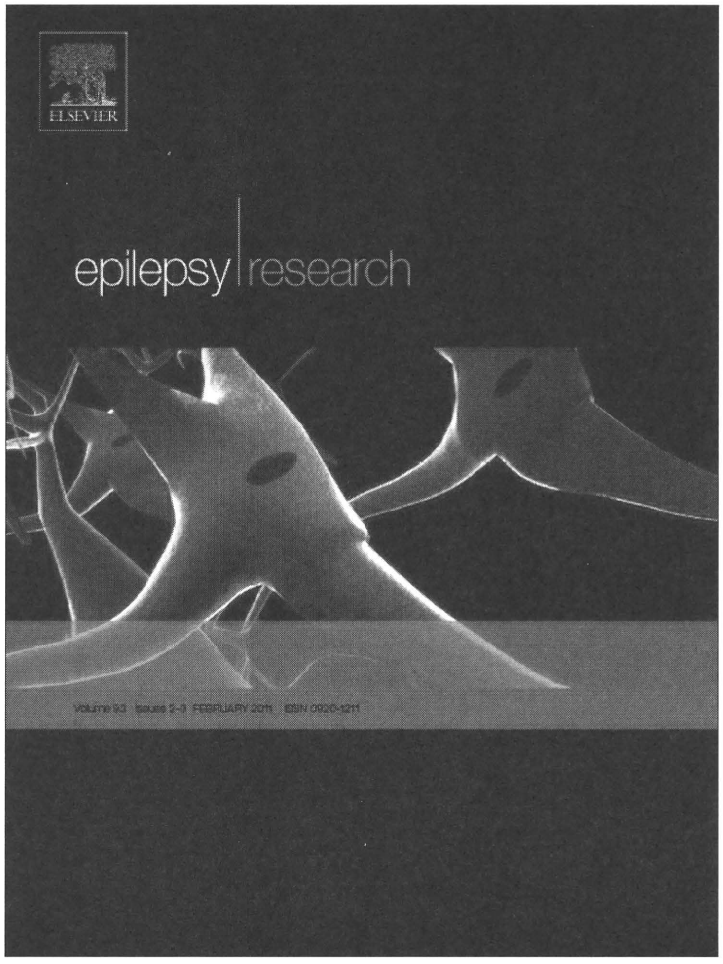

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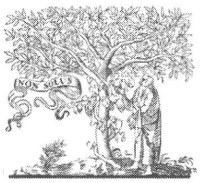

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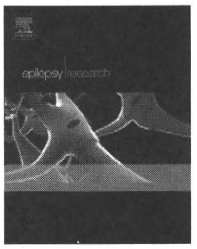

Callosotomy and subsequent surgery for children with refractory epilepsy

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Frontal lobe epilepsy;
Drop attacks;
Electroencephalography

Summary Callosotomy has been considered as a palliative therapy for refractory epilepsy patients with non-focal onset seizures. Residual partial seizures and unilateral epileptiform discharges on electroencephalography (EEG) are sometimes observed after callosotomy. These findings suggest that some patients may be candidates for subsequent resective surgery. Of 149 children who received a callosotomy, resective/disconnective surgery was subsequently performed in 19 patients. Most patients had generalized seizures and epileptiform discharges on EEG before callosotomy. Two-third had normal neuroimaging studies at initial presurgical evaluation. After callosotomy, ictal symptoms evolved into asymmetric features suggesting partial onset seizures. Post-callosotomy EEG showed completely lateralized or localized epileptiform discharges responsible for residual partial seizures in 16 of 19 patients. Fifteen patients underwent resective surgery of the unilateral frontal lobe, and the remaining received hemispherotomy or posterior quadrantectomy. After subsequent surgery, favorable seizure outcomes were obtained in 11 patients (57.9%). Favorable seizure outcomes may be achieved with callosotomy and subsequent surgery in selected patients who are not candidates for a conventional resective surgery at initial presurgical evaluation.

Introduction

Callosotomy is considered a palliative therapy for refractory epilepsy without a resectable epileptic focus (Asadi-Pooya et al., 2008). Benefits have been shown for patients with disabling drop attacks, particularly children (Maehara and Shimizu, 2001; Cross et al., 2006; Sunaga et al., 2009). This procedure has also been used in patients with symptomatic or cryptogenic generalized epilepsy including infantile spasms and Lennox–Gastaut syndrome (Pinard et al., 1999; Cukiert et al., 2006). However, postoperative residual partial seizures are often observed even in patients without generalized seizures (Gates et al., 1987; Spencer et al., 1991). These residual partial seizures may occur more frequently postoperatively (Spencer et al., 1984), but are often milder with a more gradual onset (Baba et al., 1996; Sunaga et al., 2009). In addition, electroencephalography (EEG) often reveals localized/lateralized epileptiform dis-

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charges after callosotomy (Spencer et al., 1993; Oguni et al., 1994; Baba et al., 1996; Matsuzaka et al., 1999). These improvements are associated with functions of the corpus callosum in epilepsy, i.e., propagation of focal onset seizures (Spencer et al., 1993; Tanaka et al., 2001) and synchrony and facilitation of epileptogenic activities between hemispheres (Wada and Komai, 1985; Ono et al., 2002; Matsuo et al., 2003). Although there has been controversy whether post-callosotomy residual partial seizures should be regarded as fragments of preoperatively observed generalized seizures or newly developed seizures (Spencer et al., 1984; Gates et al., 1987), those findings may suggest the existence of predominant epileptogenicity in the unilateral hemisphere (Ono et al., 2009).

The above concepts indicate that callosotomy is a diagnostic as well as palliative therapy, and selected patients who undergo this procedure may be candidates for subsequent resective surgery. So far, there have been some case presentations and small studies of this stepwise surgery reporting successful cases (Clarke et al., 2007; Nakayama et al., 2009) or poor results (Silverberg et al., 2010). However, no larger studies have been conducted. The authors' experiences with this stepwise procedure of callosotomy and subsequent resective surgery are discussed in this report.

Methods

In the Nagasaki Epilepsy Surgery Program, EEG-video monitoring, magnetic resonance imaging (MRI), interictal single photon emission computed tomography (SPECT), and developmental or cognitive tests have been routinely performed as an initial presurgical evaluation for children with refractory epilepsy. Patients with presumed partial epilepsy additionally received ictal SPECT and/or positron emission tomography (PET) if required. Then, resective or hemispheric/multilobar surgery (hemispherotomy or multilobar disconnective surgery) was planned for patients with partial onset epilepsy in whom a resectable focus was identified. Wada test and intracranial EEG (IEEG) study including functional mapping were also performed if possible and needed.

On the other hand, callosotomy was considered when resective surgery was not deemed applicable through the preoperative survey. Typical criterion for consideration of corpus callosotomy were (1) semiology of seizures (e.g., generalized atonic, tonic, tonic–clonic, and myoclonic seizures that were often expressed as drop attacks, epileptic spasms, atypical absence, and complex partial seizures (CPSs) which presumably originated from frontal lobe); (2) no responsible focal abnormality on MRI; (3) bilaterally synchronous or multiple interictal epileptiform discharges; and (4) bilaterally diffuse onset of ictal discharges (e.g., electro-decremental patterns and fast activities on EEGs) (Hanson et al., 2002). Although recent papers suggested the effect of callosotomy on seizures in patient with idiopathic generalized epilepsy (Jenssen et al., 2006; Cukiert et al., 2009), such patients have not been included in our surgical series as yet. Extension of callosal bisection was mainly determined by patient age and by distribution of epileptiform discharges on EEG. Patients less than 10 years old were usually submitted to total callosotomy. This was because total callosotomy was more effective than the partial callosotomy in children with drop attacks (Maehara and Shimizu, 2001), and disconnection syndrome was not obvious in children less than 10 years old (Sauerwein and Lassonde, 1997).

Postoperative clinical evaluations, including EEG-video monitoring, were usually scheduled at 1, 6, and 12 months, and then yearly after callosotomy. Depending on seizure status during the follow-up period, medical treatments were occasionally adjusted. In patients with stereotypical asymmetric seizures or with complex partial seizures suggesting a unilateral epileptogenic focus, examinations were repeated as with the initial presurgical evaluation and subsequent resective surgery was planned if applicable. Subsequent surgery was usually excluded for patients manifesting either multiple types of seizures or bilateral epileptiform activity.

Between 1987 and 2009, 149 children less than 15 years old received a callosotomy through our epilepsy surgery program. Of these, subsequent resective or hemispheric/multilobar surgery was performed in 19 patients (12.8%). Medical records and EEGs of these 19 patients were reviewed. The most recent seizure frequency at the final follow-up point was scored 6–95 months after the final surgery, and postoperative outcome was categorized with Engel's classification (Engel et al., 1993). Classes I and II outcomes were defined as favorable outcomes.

Correlations of some clinical measures or categories with the latest seizure frequency (including favorable or unfavorable outcomes) were statistically tested with Fisher's exact test and the Mann–Whitney *U* test if needed. *P* values of less than 0.05 were considered statistically significant. Multivariate analysis was not feasible due to the lack of sufficient sample size.

Our pediatric epilepsy surgery program and study were approved by National Nagasaki Medical Center Institutional Review Board.

Results

Pre-callosotomy status

Details concerning pre-callosotomy status are summarized in Table 1. Initial seizures were generalized tonic seizures in 10 patients, epileptic spasms in 7, atonic seizures in 3, CPSs in 2 and secondarily generalized tonic–clonic seizure (SG) in 3 patients. These seizures had poor lateralizing signs such as asymmetric postures, clonic movements of unilateral limbs, auras or starting from partial motor seizures. Drop attacks due to the above seizures were also seen in 8 patients. Seizures had occurred daily in all patients. Developmental delay or cognitive impairment was revealed in all patients. MRI demonstrated hemispheric cerebral atrophy after meningitis in 2, focal atrophy in the frontal lobe after head injury in 1, cortical dysplasia existing in the bilateral posterior cortex in 1, tuberous sclerosis with multiple and bilateral cortical lesions in 3, and no abnormality in 12 patients. Three patients with unilateral cerebral atrophy had mild or moderate hemiplegia, but they could walk independently. Interictal SPECT exhibited asymmetry in 8 patients: unilateral hypoperfusion in 5 patients (3 with atrophic lesions), and multiple hypo- or hyper-perfused areas in 3 patients with tuberous sclerosis. Bilaterally synchronous epileptiform discharges were observed on EEG in all patients except for 3 patients with hypsarrhythmia or multifocal epileptiform discharges. In 3 patients with unilateral cerebral atrophy, the amplitude of EEG discharges was inversely dominant in the non-affected hemisphere. Ictal EEGs were not focal but instead had a bilaterally diffuse onset pattern in all 19 patients.

Callosotomy was performed to prevent drop attacks and other generalized seizures because a resectable focus could not be identified through preoperative evaluations. Postoperative courses were uneventful, and permanent disconnection syndrome was not observed in any patients.

Table 1 Patients' status before callosotomy.

PN	Gender	Age at callosotomy	Seizures	MRI	SPECT	IIDs on EEG	Type of callosotomy
1	M	5 m.o.	SP	NL	Symmetric	HYPS	TC
2	F	9 m.o.	TS	BL (TSC)	Asymmetric	MF	TC
3	F	1 y.o.	CPS, TS, SP	BL (TSC)	Asymmetric	MF	TC
4	F	1 y.o.	SP	NL	Symmetric	BS	AC-PC
5	F	1 y.o.	SP	BL (CD)	Asymmetric	HYPS	TC
6	M	1 y.o.	SP	NL	Symmetric	BS	TC
7	F	3 y.o.	SP (DA)	NL	Symmetric	BS	AC
8	F	3 y.o.	SP (DA)	NL	Symmetric	BS	TC
9	M	3 y.o.	AT (DA)	NL	Symmetric	BS	TC
10	F	4 y.o.	TS (DA)	NL	Symmetric	BS	AC-PC
11	M	5 y.o.	TS (DA)	HAL	Asymmetric	BS-CP	TC
12	M	6 y.o.	TS, SG	NL	Symmetric	BS	AC
13	F	7 y.o.	TS (DA)	HAL	Asymmetric	BS-CP	TC
14	M	7 y.o.	AT (DA)	NL	Symmetric	BS	TC
15	M	11 y.o.	TS	NL	Symmetric	BS	AC
16	M	11 y.o.	CPS, SG	FAL	Asymmetric	BS-CP	AC
17	M	11 y.o.	TS	BL (TSC)	Asymmetric	BS	AC
18	F	12 y.o.	AT, TS (DA)	NL	Asymmetric	BS	AC
19	M	13 y.o.	TS, SG	NL	Symmetric	BS	AC

PN, patient number; M, male; F, female; m.o., month-old; y.o, year-old; SP, epileptic spasms; TS, tonic seizures; DA, drop attacks; AT, atonic seizures; SG, secondary generalization; CPSs, complex partial seizures; NL, no lesion; BL, bilateral lesions; TSC, tuberous sclerosis complex; CD, cortical dysplasia; HAL, hemispheric atrophic lesion; FAL, focal atrophic lesion in the frontal lobe; IIDs, interictal epileptiform discharges; HYPS, hypsarrhythmia; BS, bilaterally synchronous IIDs; MF, multi focal IIDs; BS-CP, bilaterally synchronous but contralaterally predominant IIDs; TC, one-staged total callosotomy; AC, anterior callosotomy; PC, posterior callosotomy.

Table 2 Patients' status after callosotomy.

PN	Seizures	SPECT or PET	IIDs on EEG	Subsequent surgery (interval, month)	IOZ on IEEG	Follow-up (month)	Seizure outcome
1	ASP (H&L)	Asymmetric	UH	Hemispherotomy (6)		41	I
2	ATS (H&L)	Asymmetric	MF	RSF (3)		10	III
3	CPS, ATS (H&L)	Asymmetric	MF	RSF (6)		6	I
4	ASP (H&L)	Asymmetric	UF	IEEG/RSF (62)	Extended	41	III
5	ASP (H)	Asymmetric	UP	PQ (10)		21	II
6	ASP (H&L)	Asymmetric	UF	RSF (6)		9	I
7	Subclinical	Asymmetric	UF	IEEG/RSF (55)	Localized	31	I
8	ASP (H&L)	Asymmetric	UF	IEEG/RSF (12)	Extended	34	I
9	AHD (H)	Asymmetric	UF	RSF (33)		10	II
10	ATS (H&L)	Asymmetric	UF	IEEG/RSF (21)	Extended	72	III
11	ASP (H&L)	Asymmetric	UH	Hemispherotomy (11)		59	I
12	ATS (H&L), SG	Symmetric	BF-IP	IEEG/RSF (38)	Extended	46	III
13	ATS (L)	Asymmetric	UH	Hemispherotomy (19)		41	I
14	AHD(H)	Asymmetric	UF	IEEG/RSF (11)	Localized	34	I
15	ATS (H&L)	Asymmetric	UF	IEEG/RSF (95)	Extended	62	III
16	CPS	Asymmetric	UF	IEEG/RSF (13)	Localized	95	I
17	ATS (H&L)	Asymmetric	UF	IEEG/RSF (6)	Extended	21	III
18	ATS (H&L)	Asymmetric	BF-IP	IEEG/RSF (22)	Extended	69	III
19	ATS (H&L), SG	Symmetric	BF-IP	IEEG/RSF (8)	Extended	71	III

PN, patient number; ASP asymmetric epileptic spasms; ATS, asymmetric tonic seizures; AHD, asymmetric head drops; H&L, both head and limbs were ictally involved; H or L, head or limbs were ictally involved, respectively; SG, secondary generalization; CPSs, complex partial seizures; IIDs, interictal epileptiform discharges; UH, unilateral hemispheric IIDs; MF, multi-focal IIDs; UF, unilateral frontal IIDs; UP, unilateral posterior IIDs; BF-IP, bilateral frontal but ipsilaterally predominant IIDs; interval, interval between callosotomy and subsequent surgery; IEEG, intracranial EEG; RSF, resective surgery of the unilateral frontal lobe; PQ, posterior quadrantectomy; IOZ, ictal onset zone; extended, extended to the motor cortex/parietal lobe; localized, localized to the resectable frontal lobe; seizure outcome, Engel's classification.

Post-callosotomy status

Clinical details concerning post-callosotomy status are summarized in Table 2. Clinical seizures were more or less reduced in frequency but still persisted (almost daily) after callosotomy in 18 patients. However, post-callosotomy residual seizures were transformed into milder seizures and were suspected to be unilateral in onset. Residual seizures were classified as asymmetric tonic seizures in 9 patients, asymmetric epileptic spasms in 6, asymmetric head drops in 2, and CPS without SG in 2 patients. Asymmetric tonic seizures were characterized by sustained posturing of unilateral limbs contralateral to the epileptic focus with or without head/body version. Asymmetric epileptic spasms briefly involved contralateral limbs and usually coincided with neck flexion. Asymmetric head drops were characterized by atonic head falling with ipsilateral deviation. Drop attacks were suppressed in 6 of 8 patients after callosotomy. Two patients had SG with improved duration and severity of seizures (patients 12 and 19). One patient had no obvious clinical seizures, but EEG showed frequent focal ictal discharges in the right frontal region (subclinical discharges, patient 7). In 9 patients, post-callosotomy interictal SPECT or PET revealed new ipsilateral hypo-perfusion/metabolism. Post-callosotomy interictal EEG demonstrated epileptiform discharges unilaterally localized in the frontal region in 10 patients, in the posterior region in 1, diffuse hemispheric discharges in 3, bilateral frontal discharges in 3 and bilaterally multifocal (frontal and temporal) discharges in 2 patients. Ictal EEGs exclusively suggested ipsilateral ictal onset in 17 (frontal region in 13 and hemispheric in 4) patients. Two patients had bilaterally independent frontal seizures (asymmetric tonic seizures and SG), but they predominantly occurred unilaterally. Based on these findings, subsequent resective surgery of the unilateral frontal lobe (RSF, $n=15$) or hemispheric/multilobar disconnective surgery (HMDS, $n=4$) were planned. Intervals between callosotomy and the second surgery ranged from 3 to 95 months. This range was due to additional medication prescribed as therapy for control of residual partial seizures. The reproducibility of seizures and EEG was also confirmed with repeated monitoring by EEG-video for most of the patients.

Resective surgery of the frontal lobe (RSF)

Eleven of the 15 RSF patients underwent IEEG-video monitoring using implanted subdural electrodes prior to RSF. The ictal onset zone (IOZ) was detected in the frontal lobe in all 11. However, IOZ simultaneously or immediately involved the primary motor cortex, and occasionally the parietal lobe in 9 (extended IOZ). RSF was performed based on ictal IEEG findings. However, functional or anatomical motor- and language-related areas were preserved even if they were encompassed by the IOZ. Resection of the prefrontal cortex guided by intraoperative EEG was performed in the remaining 4 patients because of younger age and clearly localized EEG abnormalities. Two of the 4 patients had an apparent cortical lesion of tuberous sclerosis within the resected area. Postoperative histological examination revealed microdysgenesis in 7 patients with normal MRI.

One patient (patient 11) still had secondary generalized seizures after RSF, and posterior callosotomy was additionally performed 2 years later in order to prevent secondary generalization.

Concerning surgically related complications, epidural empyema requiring surgical evacuation occurred in one patient (patient 17), but no morbidity remained. Postoperative hydrocephalus was seen in another patient (patient 3), and ventricle peritoneal shunting was required 1 month later.

Hemispheric/multilobar disconnective surgery (HMDS)

Hemispherotomy was performed in 3 patients and posterior quadrantectomy (Daniel et al., 2007) was performed in 1 patient without IEEG-video monitoring. It was determined by neurological conditions and findings from MRI and scalp EEG. Postoperative hydrocephalus was seen in one patient (patient 10), and ventricle peritoneal shunting was required 6 months later.

Postsurgical evaluation and statistical analysis

At the latest evaluation after the final surgery, freedom from seizures (class I) was accomplished in 9 patients (47.4%), and favorable outcomes (classes I and II) were achieved in 11 patients (57.9%). Anticonvulsants were continued after final surgery in all but one patient (patient 7). In patients who did not achieve favorable seizure control, residual seizures were similar to those post-callosotomy, such as asymmetric tonic seizures and asymmetric epileptic spasms. Independent seizures from the bilateral hemisphere continued in two patients (patients 12 and 19).

Pre-callosotomy factors, such as age at epilepsy onset, duration of epilepsy (not shown in table), age at callosotomy, presence of lesion(s) on MRI and asymmetric perfusion on SPECT, were not correlated with favorable outcomes. With respect to post-callosotomy factors, HMDS yielded more favorable outcomes than RSF, though this difference did not reach statistical significance (HMDS vs. RSF, 100% vs. 46.7%; $P=0.1$). Latency between callosotomy and subsequent surgery and asymmetric finding on PET/SPECT after callosotomy were not significantly correlated with seizure outcome. In patients with submitted RSF, ictal involvement of contralateral limbs and extended IOZ on IEEG were associated with unfavorable outcomes (classes III and IV) ($P=0.026$ and $P=0.024$, respectively).

Discussion

Seizure outcome comparing with conventional resective surgery

In this pediatric series, after stepwise surgery consisting of callosotomy and resective surgery, more than half of the patients had no or rare seizures. This result is comparable with previous reports of conventional resective surgery. Favorable surgical outcomes have been reported in 45—85% of patients with extratemporal lobe epilepsy (Wyllie et al.,